Pub #	P1-382
Session Information	POSTER SESSION: BASIC - Gonadotroph Biology (1:30 PM-3:30 PM)
Title	GNRH-Induced PACAP and PAC1 Receptor Expression in Pituitary Gonadotrophs: A Possible Role in the Regulation of Gonadotropin Subunit Gene Expression
Author String	H Kanasaki, IN Purwana, A Oride, T Mijiddorj, K Miyazaki Shimane University School of Medicine, Izumo, Japan
Body	We examined the expression of Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) and the PACAP type 1 receptor (PAC1-R) mRNA following gonadotropin- releasing hormone (GnRH) stimulation using the gonadotroph cell line, LβT2. GnRH stimulation increased PACAP and PAC1-R mRNA expression in a static culture. Increase in the cell surface density of the PAC1-R following transfection with PAC1-R expression vectors significantly increased gonadotropin LHβ and FSHβ subunit promoter activities following 100 nM PACAP stimulation. In addition, increasing concentrations of PACAP stimulation augmented the promoter activities for both LHβ and FSHβ in PAC-1R overexpressing cells. In the cells with PAC1-R, the effect of GnRH was further potentiated in the presence of PACAP from 5.31±0.93-fold to 9.89±0.38-fold for LHb and for FSHβ subunit; from 2.58±0.31 fold by GnRH alone to 10.90±2.79 fold with PACAP. The combination treatment with GnRH and PACAP did not augment the ERK phosphorylation induced by GnRH alone. PACAP expectedly increased cAMP accumulation and this effect was significantly attenuated in the presence of GnRH. PACAP gene expression was more prominent following lower frequency GnRH pulses (every 120 min) in a perifused culture. Our results suggest that PACAP and PAC1-R are produced locally within the gonadotrophs following GnRH stimulation. They subsequently affect the gonadotrophs in an autocrine manner and modulate the GnRH pulse-dependent specific regulation of gonadotropin subunits. Nothing to Disclose: HK, INP, AO, TM, KM

Pub #	P1-383
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Improved Pituitary Specificity of Cre Recombinase Transgenic Mice by Recombineering of a Mouse αGSU Bacterial Artificial Chromosome
Author String	MI Perez Millan, SA Camper, SW Davis University of Michigan, Ann Arbor, MI
Body	The Cre-loxP system is a powerful tool to study the effects of gene deletion, especially when tissue-specific and/or cell-specific deletion of a gene is required. In order to study the pituitary-specific effects of gene deletion, we generated transgenic mice that express the cre recombinase under the transcriptional control of the mouse glycoprotein hormone α-subunit (αGSU or *Cga*) gene. The αGSU protein is expressed in adult pituitary gonadotropes and thyrotropes and heterodimerizes with separate β-subunits of the glycoprotein hormones, LH, FSH and TSH, to give the biologically active heterodimeric hormones. αGSU is also expressed in the pituitary primordium, Rathke's pouch, extra-ocular mesenchyme, and olfactory epithelium. A sequence of 4.6 kb of the mouse αGSU gene promoter and enhancer targets the gonadotropes and thyrotropes as well as the other hormone-producing cells of the adult anterior pituitary gland (1). These sequences are sufficient to confer developmentally regulated and hormone-responsive gene expression in the pituitary gland and have been used successfully to create pituitary-specific deletions of several genes (2, 3, 4). Ectopic cre activity is observed in the skeletal and cardiac muscle of the 4.6 kb αGSU transgenic mice, however, limiting the usefulness of this transgene for some purposes. To generate αGSU-cre mice that more accurately recapitulate endogenous αGSU gene expression, we used homologous recombination in *E. coli* to introduce cre coding sequences into a 228 kb bacterial artificial chromosome containing the mouse αGSU gene. To determine the specificity and ability of these new αGSU-cre transgenic mice to induce loxP mediated recombination we bred them with genetically engineered reporter mice that express the lacZ gene (R26R) only after cre-mediated recombination occurs. αGSU-cre;R26R double transgenic mice exhibited robust lacZ expression in the thyrotropes and gonadotropes, where αGSU is expressed. Little or no expression was observed in other tissues where the previous αGSU-cre showed ectopic expression, including the skeletal and cardiac muscle, brain, kidney, lungs, testis, ovaries, tail and liver. Therefore, we have generated a valuable transgenic mouse strain to induce specific mutations of floxed genes, for examining gene function in the pituitary gland. (1) Cushman et al. Genesis 28:164, 2000 (2) Kendall et al., Mol Endo 8:1420, 1994 (3) Charles et al., Mol Endo 20:1366, 2006. (4) Zhao et al., Development 128:147, 2001. Sources of Research Support: NICHD 34283 awarded to SAC. Nothing to Disclose: MIPM, SAC, SWD

Pub #	P1-384
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	The Role of the Forkhead Transcription Factor, FOXM1, in Pituitary Gland Development
Author String	AG Ploegman, BS Ellsworth Southern Illinois School of Medicine, Carbondale, IL
Body	The central goal of our studies is to understand the molecular mechanisms that govern pituitary gland development. We have found that the forkhead transcription factor, FOXM1, is expressed in the developing mouse pituitary. FOXM1 promotes genes that push the cell into mitosis through G1/S phase and G2/M phase checkpoints by regulating the expression of targets such as Cyclin D, Cyclin B and Cdc25b. Also, FOXM1 has been linked to cell migration, via proteins MMP-2 and MMP-9, which are matrix metalloproteinases. We observed that FOXM1 co-localizes with proliferation markers such as BrdU in wild-type mouse embryos at ages e12.5 through e16.5 as well as with as a subset of cells that express Ki67 at ages e14.5 and e16.5, but not with the cell cycle inhibitors p27Kip1 and p57Kip2. This identifies FOXM1 expression in proliferating, non-differentiated pituitary cells. To assess the role of FOXM1 during pituitary gland development, we have acquired *Foxm1b* knockout mice. We are particularly interested in FOXM1's effect on pituitary morphology and function. To visualize pituitary morphology, we performed hematoxylin and eosin staining on *Foxm1b* null and wild-type embryos at ages e10.5, e12.5, e14.5, and e16.5, and determined that the pituitary does not appear to be hypoplastic. To investigate pituitary function we are using immunohistochemistry to label pituitary hormones. We found that FOXM1 is not required for production of ACTH. These studies should elucidate the importance of FOXM1 in the developing pituitary. Nothing to Disclose: AGP, BSE

Pub #	P1-385
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Conditional Deletion of *Sox2* Leads to Pituitary Hypoplasia and Abnormal Terminal Differentiation of the Pit1 Cell Lineage
Author String	SA Jayakody, CL Andoniadou, M Signore, C Gaston-Massuet, L Pevny, M Dattani, J-P Martinez-Barbera UCL Institute of Child Health, London, UK; University of North Carolina, NC; UCL Institute of Child Health and Great Ormond Street Children's Hospital, London, UK
Body	*Sox2* is a SOXB1-HMG box transcription factor that is expressed predominantly in the developing CNS and placodes, where it plays a crucial role during embryogenesis. Recently, mutations in human *SOX2* have been associated with hypopituitarism, demonstrating the requirement of *SOX2* for normal development and function of the hypothalamic-pituitary axis in humans. Indeed, expression analysis in mouse and human embryos has revealed that *Sox2* is present in the pituitary gland from early stages of development to postnatal life, where it is thought to play an important role in the progenitor/stem cells resident in the adult pituitary. However, at present, the molecular or cellular functions of *Sox2* in the pituitary gland are poorly understood a *Sox2* null mutants die before implantation. To further our understanding of *Sox2/SOX2* during normal development and in disease, we utilised the *Hesx1-Cre* knock-in mouse line to genetically ablate *Sox2* from the developing pituitary. *Hesx1*$^{Cre/+}$*;Sox2*$^{fl/fl}$ mice die perinatally and analysis of mutant embryos reveal severe hypoplasia of the anterior pituitary, with normal development of the posterior and intermediate lobes. Numbers of somatotrophs and thyrotrophs are greatly diminished whereas ACTH-expressing cells (corticotrophs and melanotrophs) appeared unaffected. In keeping with this finding, *Pit1* expression is almost absent and Prop1 is only weakly expressed in Rathkes' pouch (RP). Early induction of RP in *Hesx1*$^{Cre/+}$*;Sox2*$^{fl/fl}$ and expression of *Fgf8* and *Bmp4* in the ventral diencephalon is comparable with wild-type control embryos. Collectively, these observations demonstrate that *Sox2* within RP has a crucial function in normal proliferation of progenitors, and in terminal differentiation of the *Pit1* lineage. Overall, the *Hesx1*$^{Cre/+}$*;Sox2*$^{fl/fl}$ mouse provides a suitable model for understanding the pathogenesis of *SOX2* mutations in humans. Sources of Research Support: Wellcome Trust and Child Health Research Appeal Trust (CHRAT) studentship. Nothing to Disclose: SAJ, CLA, MS, CG-M, LP, MD, J-PM-B

Pub #	P1-386
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	S100b-Expressing Folliculo-Stellate Cells Are Found in SOX2-Positive Population in the Anterior Pituitary Lobe and Show Multiple Differentiation Capacities in the Defined Culture Conditions
Author String	M Osuna, H Yako, S Yoshida, Y Sonobe, K Inoue, T Kato, Y Kato Meiji University, Kawasaki, Japan; Meiji University, Kawasaki, Japan; Saitama University, Saitama, Japan; Meiji University, Kawasaki, Japan; The Japan Society for the Promotion of Science, Chiyoda-ku, Japan
Body	The anterior pituitary lobe produces and secretes six hormones (GH, PRL, TSH, ACTH, FSH and LH) which regulate physiological conditions such as growth, lactation, metabolism and reproduction. These hormones ar made by five types of endocrine cells, while folliculo-stellate (FS) cell does not produce any hormones. Non-endocrine FS cells express S100b known as a characteristic marker and play a role in supporting endocrine cells. In addition, FS cells have been also presumed to be a candidate of an organ stem cell in the anterior pituitary lobe. Hence, this study addressed to examine whether FS cells have capacity to differentiate into endocrine cells. Immunostaining of the pituitary gland using antibodies against a stem cell marker protein, SOX2, and a pituitary specific transcription factor, PROP1, showed that S100-positive cells are composed of three types of cells in the coexistence with SOX2 and/or PROP1 (S100 only, S100/SOX2 and S100/SOX2/PROP1). Transgenic rats expressing EGFP under control of the S100b gene promoter (S100bTg rat) were used for following primary cultured cell study. Firstly, the cultured anterior lobe cells were immuno-stained against anti-SOX2 antibody. More than 80% of SOX2-positive cell population was overlapped with S100b-positive FS cell population. Secondly, the differentiation capacity of S100b-positive FS cells were tested in two types of the defined culture conditions. One was including B27 supplement, T3, bFGF and EGF in serum-free medium. In this culture condition, the culture showed only a part of the S100b-GFP positive cells differentiated into skeletal muscle cells. Another cultivation in the serum-free medium supplemented with retinoic acid and bFGF showed differentiation from a part of S100b-positive FS cells into Pit1 positive (a specific transcription factor regulating GH, PRL and TSH) cell or GH-producing cell. Our results demonstrated that S100b-positive FS cells are heterogeneous but some population of them have a capacity to differentiate into other characteristic cells including pituitary hormone-producing cells. Nothing to Disclose: MO, HY, SY, YS, KI, TK, YK

Pub #	P1-387
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Estrogens Regulate Somatotroph Hormonal Production Directly through Estrogen Receptor Alpha
Author String	D Avtanski, E Pine-Twaddell, R Kineman, F Wondisford, S Radovick Johns Hopkins University School of Medicine, Baltimore, MD; University of Illinois at Chicago, Chicago, IL
Body	**Introduction:** Sex steroids are regulators of growth hormone (GH) and prolactin (PRL) synthesis and action. Among the sex steroids, estrogens play a major role in regulation of somatotroph function. Our previous experiments have shown that estradiol (E_2) directly stimulates GH and PRL gene expression in somatotroph-like cells *in vitro*. In addition, E_2 administration to wild-type (WT) mice results in an increase in GH mRNA and GH secretion. **Aim:** To evaluate the role of ERα in somatotrophs by deletion of ERα in somatotroph-like cells *in vitro* and generation of a genetically modified mouse model bearing a somatotroph-specific deletion of ERα. **Results:** Western blot analyses performed from GH_3 cell extract treated with 10^{-8}M E_2 demonstrate an increase in GH at 18 hours of incubation. ERα siRNA was transfected into GH_3 cells and the experiments repeated. Functional [ldquo]knock-down[rdquo] of ERα was documented and E_2 was unable to stimulate GH protein expression. In the same experiment, the levels of POU1F1 protein correlated with the GH levels. To provide further evidence for the effect of E_2 on somatotroph gene expression, we crossed a female mouse expressing a transgene containing Cre recombinase downstream from the GH promoter (rGHpCre) to male mice containing loxP sites flanking exon 3 of the ESR1 (ESR1 flox/flox mouse). This promoter fragment has been previously shown to confer high levels of hormonally regulated expression in the somatotroph. Cre recombinase expression in somatotrophs was expected to result in excision of the floxed exon of ERα in somatotroph cells. The results from the qRT-PCR analyses demonstrated that compared to WT, somatotroph-ERα-KO mice had 30% lower expression of GH and POU1F1 mRNA and 40% lower expression of PRL mRNA. **Conclusion:** Thus, E_2 regulates somatotroph GH gene expression through ERα to increase GH mRNA. Deletion of ERα from somatotrophs *in vitro* and *in vivo* demonstrates its critical role in GH regulation. Expression of POU1F1, the primary transcriptional regulator of GH gene expression, decreased after ablation of ERα, suggests a role in mediating the GH response to E_2 signaling. Nothing to Disclose: DA, EP-T, RK, FW, SR

Pub #	P1-388
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	The Notch Signaling Pathway Regulates Hypothalamic Neuron Placement and Axon Targeting to the Pituitar
Author String	PK Aujla, LT Raetzman University of Illinois at Urbana Champaign, Urbana, IL
Body	The failure of the hypothalamus and pituitary to form coordinately during embryonic development can lead to hypopituitarism, with infertility and growth abnormalities as possible consequences. The hypothalamic magnocellular neurons that synthesize arginine vasopressin (AVP) and oxytocin (OT), found in the paraventricular (PVN) and supraoptic nucleus (SON), send their terminal axons to the posterior pituitary to regulate homeostasis. We hypothesized that the Notch signaling pathway is necessary to specify hypothalamic magnocellular neurons and to project their axons to the pituitary. The Notch signaling effector gene *Hes1* is present in the developing pituitary and hypothalamus. *Hes1* null mice survive until embryonic day 18.5 (e18.5) and show reduction in the size of the posterior pituitary, which contains the terminal axons of AVP neurons. We found that at e16.5, AVP neurons are formed and specified in the SON and PVN, but are abnormally placed in and around these nuclei, suggesting that Notch signaling within the hypothalamus may be necessary for migration of AVP neurons. There is continued misplacement of AVP-positive cell body location within the PVN and SON at e18.5 in *Hes1* null embryos, and abnormal axonal projections to the pituitary at e18.5. The alternations in cell body location, axon pathfinding to and termination in the pituitaries of *Hes1* null mice indicate that Notch signaling facilitates migration of hypothalamic neurons and guidance of hypothalamic axons to the pituitary. Future work will utilize transgenic mice with loss and gain of Notch signaling function specifically within the developing hypothalamus to address how Notch action in the hypothalamus may affect the formation of the hypothalamic-pituitary axis. Sources of Research Support: NIH Grant R01 DK076647 and NIH Grant T32 HD007333. Nothing to Disclose: PKA, LTR

Pub #	P1-389
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Mutations in the Sonic Hedgehog Signaling Pathway in Patients with Hypopituitary Phenotypes
Author String	LC Gregory, EA Webb, L Panagiotakopoulos, MT Dattani UCL Institute of Child Health (ICH), London, UK
Body	The Gli-family of zinc-finger transcription factors regulates the sonic hedgehog (Shh) signalling pathway which is critical for normal CNS development. *Gli2* is essential for early pituitary and ventral forebrain development in mice, and mutations have been described in humans with holoprosencephaly (HPE), isolated hypopituitarism (HP) and cranial/midline facial abnormalities. HPE describes the failure of the forebrain to divide into distinct halves, which can result in defects in the face and brain structure and function. Mutations in *SHH* have been associated with pleiotropic phenotypes including HPE but not HP (the decreased secretion of one or more of the pituitary hormones), despite murine studies which implicate SHH in early hypothalamo-pituitary development. We aimed to establish whether disorders of hypothalamo-pituitary development were associated with mutations in *SHH*, *GLI2*, the highly conserved Shh Brain Enhancer 2 (*SBE2*) located 460kB upstream of *SHH* and regulating its expression, and Growth-arrest specific 1 (*GAS1*), a membrane bound glycoprotein that has an antagonistic effect on *Shh* signalling, and which induces HPE associated phenotypes in homozygous mice. We screened 100 hypopituitary patients for *GLI2* mutations by direct sequencing analysis. A novel heterozygous mutation at a highly conserved residue in the zinc finger DNA-binding domain (c.1552G>T, pE518K), was identified in a female patient with evolving CPHD (GH, TSH deficiencies), a small anterior pituitary and an absent posterior pituitary. A non-synonymous coding change; c.2159G>A p.R720H, was identified in a patient with a short neck, cleft palate and hypogonadotrophic hypogonadism. No mutations were identified in SBE2 in 346 patients with septo-optic dysplasia (SOD); nor were mutations in *GAS1* identified in 44 patients with holoprosencephaly (HPE). We identified a novel mutation in *SHH* in two siblings (c.1295T>A, p.I431T) with variable midline craniofacial phenotypes (Sibling 1 presented with cleft lip and palate, hypoplasia of the corpus callosum and developmental delay; sibling 2 had a solitary median maxillary central incisor as the only clinical feature). Our data suggest that mutations in *SHH*, *GAS1* and *SBE2* are not associated with hypopituitarism, although *GLI2* is an important candidate for various hypopituitarism disorders. Nothing to Disclose: LCG, EAW, LP, MTD

Pub #	P1-390
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Molecular Complexes Involved in the Differential Action of PITX2 on Its Somatolactotroph Targets
Author String	M-H Quentien, J-P Herman, A Enjalbert, T Brue Faculté de Médecine Secteur Nord, Université de la Méditerranée, Marseille, France; Assistance Publique-H [ocirc]pitaux de Marseille, H[ocirc]pital de la Timone, Marseille, France
Body	PITX2 mutations have been found in association with Axenfeld-Rieger syndrome (ARS) characterized, among other features, by eye, teeth, and face abnormalities, and sometimes by pituitary hormone deficits. PITX2 has previously been described by us and others to interact with the pituitary-specific POU homeodomain factor POU1F1 (human ortholog of PIT-1) to achieve cell-specific expression of prolactin (PRL) and growth hormone (GH) in pituitary somatolactotroph cells. We have investigated the functional properties of several PITX2 mutants reported in ARS patients relative to the regulation of these genes. The Y167X mutation on PITX2 isoform b (or Y121X on the shorter isoform a) displayed a markedly enhanced activation of the hPRL and hPIT-1 promoters, but not of the hGH promoter (1). This PITX2 mutation is the first described to result in a differential effect on the activation of its different physiological targets, PRL on one hand, and GH on the other hand. PRL and GH can be coexpressed in a same cell type, but the precise molecular nature of regulation of both genes remains obscure. PITX2 may differentially interact with differen transcription factors or cofactors when bound to the PRL and to the GH promoters. We have examined this hypothesis via the use of different PITX2 constructs (2) and a proteomic study. PITX2 constructs allowed us to map regions of interaction of this transcription factor with cofactors responsible for the differential activation of PRL and GH. These results form the basis for the identification of the PITX2 protein complex necessary for the differential GH or PRL expression. These complexes bound to different PITX2 constructs will be isolated to allow their subsequent identification and comparison by mass spectrometry. (1) Quentien MH et al., J Mol Endocrinol 2010 ; 9-19 (2) Berry FN et al., J Biol Chem 2006 ; 10098-104 Nothing to Disclose: M-HQ, J-PH, AE, TB

Pub #	P1-391
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Silencing of the Imprinted *DLK1-MEG3* Locus in Human Clinically Non-Functioning Pituitary Adenomas
Author String	P Cheunsuchon, Y Zhou, X Zhang, H Lee, W Chen, Y Nakayama, KA Rice, ET Hedley-Whyte, B Swearingen, A Klibanski Massachusetts General Hospital and Harvard Medical School, Boston, MA; Massachusetts General Hospital and Harvard Medical School, Boston, MA; Massachusetts General Hospital and Harvard Medical School, Boston, MA; Massachusetts General Hospital and Harvard Medical School, Boston, MA
Body	Human pituitary tumors are clinically categorized as functioning or non-functioning adenomas (NFAs). NFAs do not secrete excess functional hormones. These tumors can grow very large in size causing neurological and visual symptoms due to mass effect. The molecular pathogenesis of NFAs is largely unknown. We identified a novel non-coding RNA gene called *maternally expressed gene 3* (*MEG3*), which is highly expressed in the normal human pituitary. However, its expression is lost in NFAs of gonadotroph origin. We demonstrated that MEG3 activates p53 and inhibits cell proliferation *in vitro*. These data suggest that loss of *MEG3* gene expression plays a role in the pathogenesis of human NFAs. The *MEG3* gene belongs to the imprinted *DLK1-MEG3* locus consisting of multiple paternally expressed protein-coding genes and maternally expressed non-coding RNA genes. Loss of imprinting in this locus has been linked to human cancers. However, the status of this locus in human pituitary adenomas has not been reported. Therefore, we investigated 1) gene expression from the *DLK1-MEG3* locus in human NFAs and 2) the functional relevance of these genes in pituitary tumors. Expression of 24 paternally and maternally expressed genes from the *DLK1-MEG3* locus were examined in 44 human pituitary adenomas (25 NFAs, 7 ACTH-, 7 GH- and 5 PRL-secreting adenomas) and 10 normal human pituitaries using quantitative real-time RT-PCR. We found that 18 genes in the *DLK1-MEG3* locus were significantly down regulated in human NFAs, including 13 miRNAs. Their average expression level was 9.1±7.2% of the normal pituitary. In ACTH- and PRL-secreting adenomas, 12 and 7 genes were significantly down regulated respectively. Compared to their expression levels in normal pituitaries, the average expression levels of these genes in ACTH- and PRL-secreting tumors were 33.2±17.2% and 41.7±12.2%, respectively. In contrast, no gene was significantly down-regulated in GH-secreting tumors. The effect on cell proliferation of five miRNAs whose expression was lost in NFAs was also investigated by flow cytometry analysis. We found that one miRNA induced cell cycle arrest at the G2/M phase in PDFS cells derived from a human NFA. Our data indicate that the DLK1-MEG3 locus is silenced in NFAs. The growth suppression by miRNAs in PDFS cells is consistent with the hypothesis that the DLK1-MEG3 locus may play a tumor suppressor role in human NFAs. Sources of Research Support: Grants from the National Institutes of Health (A. Klibanski, R01DK40947), the Guthart Family Foundation and the Jarislowsky Foundation. Nothing to Disclose: PC, YZ, XZ, HL, WC, YN, KAR, ETH-W, BS, AK

Pub #	P1-392
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Defining the Existence of Peripheral Memory in Non-Neuronal Systems Using Pituitary Endocrine Cell Networks
Author String	DJ Hodson, M Schaeffer, N Romano, P Fontanaud, C Lafont, J Birkenstock, F Molino, H Christian, J Lockey D Carmignac, M Fernandez-Fuente, P Le Tissier, P Mollard Institute of Functional Genomics, CNRS UMR5203, Montpellier, France; University of Oxford, Oxford, UK; National Institute of Medical Research, London, UK

Body

The possession of long-term memory is typically attributed to the brain. Following stimulus, the strength and number of connections within and between neuronal ensembles is permanently altered, experience-dependently up-regulating function. Despite the physiological privilege conferred by this, it is not known whether populations of cells belonging to non-neuronal tissues possess features of memory. Using the mammalian pituitary gland as a model system comprised of organised cell populations which recurrently respond to demand, we set out to investigate the existence of peripheral memory. Lactation, a defined demand associated with high levels of prolactin (PRL) release from lactotrophs, was used to perturb system dynamics in a controlled manner.

By applying 2-photon Ca^{2+}-imaging to pituitary slices taken from mice expressing rPRL-promoter driven DsRed, lactotroph population connectivity was mapped. In virgins, the majority of connections were hosted by a few node cells, efficiently linking the lactotroph population and driving basal PRL release. Lactation was met with the emergence of multiple well-connected nodes which paced coordinated Ca^{2+}-spike firing, dramatically increasing PRL secretion. Remarkably, 21 days following weaning, a lactating-like wiring pattern remained despite reversion of PRL secretion to basal levels. The observed memory was not due to acute remodelling events since it was still evident 3 months post-weaning. Moreover, memories were encoded by suckling and not pregnancy as animals subjected to reduced lactational demand possessed identical connection distributions to virgins. Online 3D-reconstructions of the lactotroph population subjected to Ca^{2+}-imaging revealed the existence of a highly plastic lactotroph network, homotypically linked by gap junctions, thus providing a structural basis for storage of wiring patterns. Subsequently, the functional relevance of the memory was questioned by inducing a second lactation. In multiparas, evolved network behaviour in the form of synchronised cell activity generated even higher levels of PRL release compared to the first lactation. Functional upregulation was reliant on retrieval of memories encoded by suckling in primiparas since evolved network behaviour was absent in animals exposed to reduced demand during the first lactation but normal demand during the second lactation. In summary, memory is not confined to the CNS but also appears to be a property of peripheral tissues.

Sources of Research Support: Grants from the Agence Nationale de la Recherche (ANR Pit-Net), Institut National de la Santé et de la Recherche Médicale (INSERM), Centre National de la Recherche Scientifique (CNRS), the Universities of Montpellier 1 and 2, National Biophotonics and Imaging Platform (Ireland) (NBIPI), Fondation pour la Recherche Medicale (FRM), Réseau National des Génopoles, Institut Fédératif de Recherches No. 3, Région Languedoc Roussillon and the Medical Research Council (MRC). DH was supported by a personal fellowship from the FRM.

Nothing to Disclose: DJH, MS, NR, PF, CL, JB, FM, HC, JL, DC, MF-F, PLT, PM

Pub #	P1-393
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Inhibitory Effects of Anti-VEGF Strategies in Experimental Dopamine-Resistant Prolactinomas
Author String	GM Luque, MI Perez-Millan, AM Ornstein, C Cristina, D Becu-Villalobos IBYME-CONICET, Buenos Aires, Argentina; Universidad Nacional del Noroeste de la Provincia de Buenos Aires, Junin, Argentina
Body	Prolactin secreting adenomas are the most frequent type among pituitary tumors, and pharmacological therapy with dopamine agonists remains the mainstay of treatment. But some are resistant and a decrease in the number or function of dopamine receptor type 2 (D2R) has been described in these cases. D2R knockout (Drd2-/-) mice have chronic hyperprolactinemia, pituitary hyperplasia, and provide an experimental model for dopamine agonist resistant prolactinomas. We previously described that disruption of D2Rs increases pituitary VEGF expression. We therefore designed two strategies of antiangiogenesis using prolactinomas generated in Drd2-/- female mice: 1) direct intra adenoma VEGF-TRAP injection for three weeks (into sc transplanted pituitaries from Drd2-/- mice), and systemic VEGF neutralization with the specific antibody Mab G6-31 in female Drd2-/- 6 month-old mice. Both strategies resulted in substantial decrease of prolactin content and lactotrope area, and a reduction in tumor size was observed in in situ prolactinomas in the second model. There were significant decreases in vascularity, evaluated by CD31 vessel staining (decrease to 43.4 and 49.8 % in CD31 positive vascular area in transplants and in situ pituitaries, P= 0.004 and 0.009,respectively), and proliferation (PCNA staining) in response to both anti-VEGF treatments. These data demonstrate that the antiangiogenic approach was effective in inhibiting the growth of in situ dopamine resistant prolactinomas as well as in the transplanted adenomas. No differences in VEGF protein expression were observed after either anti-VEGF treatment. Our present results indicate that even though the role of angiogenesis in pituitary adenomas is contentious, VEGF might contribute to adequate temporal vascular supply and represent a complementary therapeutic target in aggressive dopamine agonist resistant prolactinomas. Nothing to Disclose: GML, MIP-M, AMO, CC, DB-V

Pub #	P1-394
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Kisspeptin-Induced Somatolactin α Secretion in Goldfish Pituitary Cells: Functional Role of cAMP/PKA-, PLC/PKC- and Ca^{2+}/Calmodulin-Dependent Cascades
Author String	Q Jiang, M He, WKW Ko, AOL Wong The University of Hong Kong, Hong Kong, China
Body	Recently, kisspeptin has been shown to induce growth hormone (GH) and prolactin (PRL) secretion in goldfish pituitary cells, indicating that the neuropeptide may have direct pituitary actions in teleost species. In fish models, somatolactin (SL), a member of GH/PRL family, is a pituitary hormone with pleiotropic functions, but its regulation by kisspeptin has not been previously examined. To investigate the functional role of kisspeptin in SL regulation, the SL isoform, namely SLα, was cloned in the goldfish. Together with the SLβ isoform reported previously, two forms of SL could be identified in the goldfish pituitary. As revealed by in situ hybridization and immunohistochemical staining, SLα and SLβ expression, both at the transcript level as well as protein level, were detected in two separate populations of pituitary cells located in the neurointermediate lobe (NIL) with SLα cells as the dominant form. Using RT-PCR, expression of kisspeptin receptor GPR54 was confirmed in immuno-identified SLα but not SLβ cells isolated by laser capture microdissection. In pituitary cell cultures prepared from goldfish NIL, kisspeptin treatment increased SLα release with no changes in SLα cell content and SLα mRNA levels. Consistent with the lack of GPR54 expression in SLβ cells, SLβ secretion, protein content and gene expression were not altered by kisspeptin stimulation. In parallel experiments, kisspeptin could also elevate cAMP production, up-regulate PKA and PKC activities, and trigger a rapid rise in intracellular Ca^{2+} levels in goldfish NIL cells. Using a pharmacological approach, cAMP/PKA and PLC/PKC pathways and subsequent activation of Ca^{2+}/calmodulin-dependent cascades were shown to be involved in SLα secretion induced by kisspeptin. Apparently, the Ca^{2+}-dependent cascades were triggered by extracellular Ca^{2+} entry via voltage-sensitive Ca^{2+} channels and mobilization of IP3-sensitive intracellular Ca^{2+} stores. Our results, as a whole, demonstrate for the first time that kisspeptin can act directly at the pituitary level to stimulate SLα release via complex network of post-receptor signaling mechanisms. Nothing to Disclose: QJ, MH, WKWK, AOLW

Pub #	P1-395
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Stimulatory Effect of Pituitary Adenylate-Cyclase Activating Polypeptide (PACAP) and Its PACAP Type I Receptor (PAC1R) on Prolactin Synthesis in Rat Pituitary Somatolactotroph GH3 Cells
Author String	A Oride, H Kanasaki, T Mijiddorj, I Purwana, K Miyazaki Shimane University School of Medicine, Izumo, Japan
Body	Pituitary adenylate cyclase-activating polypeptide (PACAP) is a multifunctional peptide which stimulates cAMP accumulation. In this present study, we investigated the role of PACAP and its receptor, PACAP type I receptor (PAC1R) on prolactin synthesis in pituitary somatolactotroph GH3 cells. PACAP increased prolactin promoter activity up to 1.3 ±0.1 -fold. This increase, while significant, was less than the increase resulting from thyrotropin-releasing hormone (TRH) stimulation. By transfection of a PAC1R expression vector to the cells, the response to PACAP on prolactin promoter activity was dramatically potentiated to a degree proportional to the amount of PAC1R transfected. In the PAC1R expressing GH3 cells, TRH and PACAP alone increased prolactin promoter up to 3.3±0.3 -fold and 4.9±0.2 -fold, respectively, and combined treatment with TRH and PACAP further increased prolactin promoters up to 6.8±0.6 -fold. PACAP binds both Gs- and Gq- coupled receptors and stimulates adenylate cyclase/cAMP and protein kinase C/extracellular signal-regulated kinase (ERK) signaling pathways. PACAP increased ERK phosphorylation in PAC1R expressing cells to the same degree as TRH. Combined treatment with TRH and PACAP had a synergistic effect on ERK activation. GH3 cells produce both prolactin and growth hormone. Stimulation of GH3 cells with TRH significantly increased the mRNA level of prolactin and attenuated growth hormone mRNA expression. PACAP increased both prolactin and growth hormone mRNA levels, particularly in PAC1R expressing cells. In addition, increasing amount of PAC1R in GH3 cells potentiated the action of TRH on prolactin promoter activity, as well as on ERK phosphorylation. Our current study demonstrates that PACAP and its PAC1R, functions as a stimulator of prolactin alone or with TRH in prolactin producing cells. Nothing to Disclose: AO, HK, TM, IP, KM

Pub #	P1-396
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Digital Expression Profiling of Tanycytes Suggests Molecular Mechanisms for Their Biological Function
Author String	LK Iyer, JA Ainsley, C Fekete, RM Lechan Tufts University School of Medicine, Boston, MA; Tufts Medical Center, Boston, MA; Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary; Tufts Medical Center, Boston, MA
Body	Tanycytes are specialized, elongated ependymal cells of glial origin that line the floor and ventrolateral walls of the third ventricle between the rostral and caudal limits of the hypothalamic median eminence. While much is known about the anatomy of these cells, their physiologic functions remain speculative and enigmatic. As a first step towards characterizing tanycytes, we carried out digital transcription profiling of mouse tanycytes by RNASeq analysis using samples isolated by laser capture microdissection. Brain and peripheral tissue samples served as controls. More than 38 million (31bp) Illumina sequence reads per sample were produced and mapped to the mouse trancriptome to produce gene expression estimates. A total of 22854 mouse genes had a non-zero expression in at least one of the samples. Statistically significant enrichment of the Ben Barres astrocyte gene singnature in the tanycyte data alone confirmed their astrocyte characteristics. The D2 and D3 isoforms of iodothyronine deiodinases, known to be responsible for deiodination of thyroid hormone into active and inactive metabolites, respectively, were highly expressed in tanycytes compared to other tissues, in keeping with an essential role of tanycytes in mediating the effects of thyroid hormone in the CNS. Enrichment of numerous inflammatory and cytokine pathways in tanycytes and their genes (eg CD5, IFNG, TLR2, IL5, IL12B, IL15, IL6 and CSF1) support the role of tanycytes as an important link between the peripheral immune system and their potential role in the regulation of local inflammation in the mediobasal hypothalamus. In addition, the presence of a number of neuroactive ligand receptors including GPR50, PRLHR, TSHR, GALR2, GPR156, and HTR6, indicate receptor-mediated regulation of these cells by neurotransmitters. The genes, BMP8A, FGF2, FGF4, FZD10 and members of the WNT gene family, components of the Human Embryonic Stem Cell Pluripotency, are also specifically enriched in tanycytes, supporting their role as neural stem cells. Finally, stringent criteria of basal expression level of at least 10 read counts per gene identified 294 genes to be specifically expressed in tanycytes, suggesting unique functions that will require further characterization. These data indicate that tanycytes are multifunctional cells and subserve functions well beyond their recognized roles as supporting and phagocytic cells. Sources of Research Support: NIDDK 078998. Nothing to Disclose: LKI, JAA, CF, RML

Pub #	P1-397
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Analysis of Promoter Region of a Novel Pituitary Transcription Factor *Prx2* in TtT/GF of Folliculo-Stellate Cell-Lineage
Author String	A Ishikawa, H Mitsuishi, T Susa, T Kato, Y Kato Meiji University, Kawasaki, Japan; Meiji University, Kawasaki, Japan; Meiji University, Kawasaki, Japan; Shizuoka University, Shizuoka, Japan; Japan Society for the Promotion of Science, Tiyodaku, Japan; Japan Society for the Promotion of Science, Tiyodaku, Japan
Body	We have previously cloned paired related homeobox protein 2 (*Prx2*) from the pituitary cDNA library and observed that PRX2 coexists with SOX2, PROP1 and S100b in the rat pituitary. SOX2 and PROP1 are markers of stem/progenitor cell in the pituitary. S100b is a marker of the folliculo-stellate cell, which is a candidate for the pituitary stem cell. These results suggested that *Prx2* is involved in the development and differentiation of the pituitary. At rat embryonic day 15.5 (E15.5), PRX2-positive cells were present in the primordial pituitary as well as in the surrounding part to be involved in pituitary vasculogenesis. Thus, PRX2-positive cells of different origins are present in the pituitary. We are currently investigating the cell-type specific regulatory mechanism for *Prx2* expression. First, we examined microarray and Real-time PCR analyses to identify *Prx2* expressing cells using non-endocrine cell lines derived from pituitary tumor and identified TtT/GF as a *Prx2* expression cell line. TtT/GF also expressed *Sox2, S100b* and *Vimentin* which is known as a marker of mesenchymal cell. Another finding was a high expression of *Sca1* which is reported to be characteristically present in the fraction of Side population (SP) of the pituitary defined as $Sca1^{high}$-SP. Genes associated with angiogenesis are highly expressed in the $Sca1^{high}$-SP [1]. Then, we analyzed promoter activity of the *Prx2* gene in TtT/GF cells by transfection assay using reporter vectors fused mouse *Prx2*-4993/+107 region and its mutants into the pSEAP2 Basic vector (-4993/+107-pSEAP). Transfection assay, however, did not show any promoter activity. These results indicated that other regions are required for the *Prx2* expression. Then, we searched for interspecifically conserved regions in the *Prx2* gene among mouse, cow and human, and found out 14 candidates. To examine a *cis*-acting activity, these regions were fused into the upstream of the -4993/+107-pSEAP and provided to transfection assay. As a result, one of the conserved regions located in the intron 1 showed an enhanced-promoter activity. This activity was not observed in Chinese hamster ovary cells. The region identified in this study may work in cell-type specific manner.Eventually, we could set up a tool to determine the promoter activity of *Prx2* gene and found one of the regulatory elements to control the *Prx2* gene expression. 1) Chen J et al. (2009) Stem Cells., 27, 1182-1195. Nothing to Disclose: AI, HM, TS, TK, YK

Pub #	P1-398
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	The Effects of Hypoxia-Inducible Factor on Growth Hormone in GH3 Cells
Author String	MR Haedo, M Fuertes, J Gerez, U Renner, J Stalla, GK Stalla, E Arzt FCEN, IFIBYNE-CONICET, University of Buenos Aires, Buenos Aires, Argentina; Max Planck Institute for Psychiatry, Munich, Germany
Body	The normal pituitary and pituitary tumors are characterized for having low oxygen concentration. The hypoxia inducible factor-1 (HIF-1) is a protein that is crucial in the adaptive response to hypoxia and is constituted by a HIF-1 alfa subunit, which is regulated by oxygen, and a HIF-1 beta subunit. Its presence has been reported in pituitary adenomas. However, the effect of HIF-1 on hormone secretion in pituitary adenomas has not been explored. We hypothesize that the low oxygen concentration of pituitary adenomas may affect hormone secretion, and thus affect patients suffering from this disease. To study whether low oxygen concentrations affect GH production and secretion, we used the GH3 lactosomatotrope cell line, which secretes growth hormone (GH) and prolactin, and performed GH secretion measurements and transfections with a GH promoter-luciferase construct. We have been able to demonstrate that 1% O2 slightly increases GH secretion in GH3 cells, and that HIF-1 is upregulated in these cells under these conditions. HIF-1 overexpression in GH3 cells increases GH promoter activity (224%, $p< 0,05$). Additionally, this increase in promoter activity is enhanced in the presence of cAMP (500 uM) (269%, $p<0,05$) and also in GH3 cells overexpressing Pit-1 (208% vs HIF-1 and 342% vs Pit-1), a transcription factor that is crucial for GH gene transcription. However, HIF-1 did not enhance GH promoter activity in the presence of other important transcription factors. Further studies using different lengths of the GH promoter proved that the above mentioned increases in promoter activity are maintained with a minimal promoter (-145 GH-LUC). We conclude that hypoxia affects growth hormone secretion, and that HIF-1 plays a role in this, in conjunction with the cAMP pathway. These results indicate an unprecedented regulation of the GH gene and may prove useful in basic and clinical studies, given the hypoxic nature of the pituitary and pituitary adenomas. Nothing to Disclose: MRH, MF, JG, UR, JS, GKS, EA

Pub #	P1-399
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	A Novel Human Ghrelin Variant Containing Intron 2 (In2-Ghrelin) Is Present in Normal and Tumoral Pituitaries: Potential Pathological Relevance
Author String	RM Luque, MD Gahete, J Cordoba-Chacon, L Lopez-Sanchez, L Jimenez-Reina, MA Japon, P Benito, A Leal-Cerro, MD Culler, RD Kineman, JP Castano University of Cordoba, Cordoba, Spain; University of Cordoba, Cordoba, Spain; Hospital Universitario Virgen del Rocio, Seville, Spain; Reina Sofia University Hospital, Cordoba, Spain; Virgen del Rocio University Hospital, Seville, Spain; Ipsen, Milford, MA; Jesse Brown Veterans Affairs Medical Center, Chicago, IL; University of Illinois at Chicago, Chicago, IL
Body	Ghrelin is a multifunctional hormone expressed in multiple tissues including the hypotalamo-pituitary unit, wherein it modulates diverse neuroendocrine secretions. We recently identified a ghrelin splice variant in mouse, In2-ghrelin, and showed that, compared native ghrelin, it is differentially distributed in various tissues (e.g. In2-ghrelin is far more abundant in pituitary), and is divergently regulated under extreme metabolic conditions, thereby supporting its putative pathophysiological relevance. Here, we have identified and cloned a new human In2-ghrelin variant counterpart, and have investigated its presence in normal and tumoral pituitary, as well as its involvement in relevant processes, including hormone secretion or cell proliferation. Like in native ghrelin, predicted human In2-ghrelin peptide sequence posseses two cleavage sites where it can be cleaved by convertases to generate two mature products. Since most known ghrelin actions, especially at the pituitary, require its acylation, and In2-ghrelin retains Ser3 acylation site, we synthesized these two predicted In2-ghrelin peptides including it acyl-modification. As observed in mice, human In2-ghrelin is expressed at higher levels than native ghrelin in the pituitary. Remarkably, mRNA of In2-ghrelin variant was markedly overexpressed in various types of pituitary tumors, including non-functioning pituitary adenomas (NFPA), corticotropinomas and somatotoprinomas. Of note, somatotropinomas presenting extension into sphenoid sinus expressed higher levels of In2-ghrelin. Functional studies revealed that both In2-ghrelin peptides are able to activate key intracellular signals (i.e. Ca2+) in cells derived from NFPA and ACTH-omas in vitro. This was associated with an increase of ACTH secretion in adenoma cells from Cushing's patients. Moreover, transfection with In2-ghrelin and/or treatment with In2-ghrelin peptides caused an increase in the proliferation rate of cell cultures derived from these three types of pituitary tumors. Taken together, our result provide primary evidence that In2-ghrelin is a potential novel element of the ghrelin family with a plausible pathophysiological role in human pituitary function, since it is expressed in normal pituitaries and overexpressed in adenomas, wherein it can regulate hormone secretion and/or tumoral cell proliferation. Sources of Research Support: FI06/00804 (to J.C.-C.), FPU-AP20052473 (to M.D.G.); BFU2010-19300 and CTS-5051 (to J.P.C.), R01DK030677 and Veterans Affairs Merit Award (to R.D.K.) and Grants RYC-2007-00186, BFU2008-01136/BFI (to R.M.L.). Nothing to Disclose: RML, MDG, JC-C, LL-S, LJ-R, MAJ, PB, AL-C, MDC, RDK, JPC

Pub #	P1-400
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Systemic Oxytocin Induces a Prolactin Secretory Rhythm Via the Pelvic Nerve in Ovariectomized Rats
Author String	CV Helena, R Cristancho-Gordo, AE Gonzalez-Iglesias, J Tabak, R Bertram, ME Freeman Florida State University, Tallahassee, FL; Florida State University, Tallahassee, FL
Body	We have shown that an intravenous (iv) injection of oxytocin (OT) initiates a rhythm of prolactin (PRL) secretion similar to that observed after cervical stimulation in ovariectomized (OVX) rats. We have also demonstrated that a peripheral target of OT is necessary for triggering the PRL rhythm, as an intracerebroventricular (icv) injection of OT failed to start the rhythm and the OT-induced rhythm is blocked if the OT receptors are blocked peripherally. Since a central PRL action is also essential for the surges to occur, we hypothesized that OT may induce PRL release from lactotrophs, which would be transported into the brain and trigger the rhythm. To test whether a PRL-releasing factor other than OT would induce the PRL rhythm, we compared the effect of peripherally-administered thyrotropin-releasing hormone (TRH) and rat PRL to that of OT. Although all injections increased circulating PRL by 5min, only OT-injected animals expressed the PRL secretory rhythm. As bilateral resection of the pelvic nerve blocks cervical stimulation-induced pseudopregnancy and OT-induced facilitation of lordosis, we injected OT into rats in which the pelvic nerve was previously sectioned. OT failed to induce a PRL secretory rhythm in these pelvic-neurectomized rats. These results suggest that the integrity of the pelvic nerve is necessary for the systemic OT induction of the PRL secretory rhythm in OVX rats. Sources of Research Support: NIH Grants DK43200 and DA19356. Nothing to Disclose: CVH, RC-G, AEG-I, JT, RB, MEF

Pub #	P1-401
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Autocrine/Paracrine Regulation of Somatolactin α Production in Carp Pituitary Cells by Local Release of Somatolactin α and β: Functional Role of Jak2/Stat5, PI3K/Akt and MAPK Signaling Cascades
Author String	Q Jiang, AOL Wong The University of Hong Kong, Hong Kong, China
Body	Pituitary hormones can act locally via autocrine/paracrine mechanisms to modulate pituitary functions, which represent an interesting aspect of pituitary regulation other than the traditional hypothalamic control and feedback signals from peripheral tissues. Somatolactin (SL), a member of the growth hormone/prolactin family, is a pleiotropic hormone with diverse functions in fish models, but its pituitary actions have not been previously examined. Recently, two SL isoforms, SLα and SLβ, have been cloned in grass carp. Based on the sequences obtained, recombinant proteins of grass carp SLα and SLβ with bioactivity in inducing pigment aggregation in carp melanophores were produced. In carp pituitary cells, SLα secretion and cellular content were elevated by static incubation with carp SLα and SLβ, respectively. These stimulatory actions occurred with a parallel raise in SLα mRNA levels with no significant changes in SLβ protein content and gene expression. In parallel experiments, SLα mRNA expression could be reduced by removing endogenous SLα and SLβ using immunoneutralization with the respective SL antisera. At the pituitary cell level, SLα mRNA expression induced by carp SLα and SLβ could be blocked by inhibiting JAK2, STAT5, PI3K, Akt, MEK1/2, and P38 MAPK, respectively. Furthermore, SLα and SLβ treatment could also trigger rapid phosphorylation of STAT5b, Akt, MEK1/2, Erk1/2, MKK3, and P38 MAPK. These results, as a whole, suggest that (i) SLα and SLβ produced locally in the carp pituitary can serve as novel autocrine/paracrine stimulators for SLα release and synthesis, and (ii) SLα production caused by SLα and SLβ stimulation probably are mediated by SLα gene expression via activation of the JAK2/STAT5, PI3K/Akt and MAPK signaling pathways. Sources of Research Support: Grants from Research Grant Council, Hong Kong. Nothing to Disclose: QJ, AOLW

Pub #	P1-402
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Distribution and Characterization of Thyrotroph [Ca2+]i Response to TRH in Pituitary Slices from Adult Male Mice
Author String	DC Del-Rio, LM Rendon, A Hernandez-Cruz, T Fiordelisio Universidad Nacional Autonoma de Mexico, Mexico City, Mexico; Universidad Nacional Autonoma de Mexico, Mexico City, Mexico
Body	The anterior pituitary is a heterogeneous gland with multiple endocrine cell types. The thyrotrophs represent approximately 5% of the population of these cells, synthesize and secrete thyroid-stimulating hormone (TSH) and are positively regulated by TSH-releasing hormone (TRH) from the hypothalamus. TSH binds to its specific receptor on the thyroid gland, regulates the thyroid iodine metabolism, the synthesis of thyroid hormones and thyroid growth. Differences have been reported in anatomical and physiological attributes of some populations of endocrine cell types with respect of their distribution in the pituitary gland. However, the information concerning the thyrotrophs is not plentiful compared with other cell types. Moreover, many studies have been conducted under conditions that compromise the anatomic-functional relationship of the tissue. In this work we identified whether the population of thyrotrophs in the central and lateral region of the gland, when stimulated with TRH, exhibit different responses of [Ca2+]i and characterization of them. To do this, spontaneous activity was recorded and regulated by [Ca2+]i in thyrotrophs in pituitary slices of adult male mice. Image sequences were acquired from the records of the fluctuations of [Ca2+]i by spectrometry and imaging of Ca2±sensitive indicator Fluo-4 AM, in a fluorescence stereomicroscope. TRH was applied in a bath at concentrations of 0.1, 1, 10 and 100nM, with a period of 15min between each dose; at the end, a dose of DA 2[mu]M was applied. Cells responsive to TRH and whose activity was not inhibited by DA were considered as thyrotrophs. The [Ca2+]i response was plotted and analyzed individually. Thus, we can report that compared with the central region, a great number of thyrotrophs of the lateral region presented spontaneous activity and response from low doses of secretagogue. In addition, the average maximum intensity and area under the curve of the response of [Ca2+]i produced at low doses (0.1 and 1nM) of TRH were significantly higher in this same region. The results suggest a physiological difference in the response of [Ca2+]i of thyrotrophs to different concentrations of the agonist with respect to their spatial distribution in the anterior pituitary. Sources of Research Support: CONACYT 81760-Q. Nothing to Disclose: DCD-R, LMR, AH-C, TF

Pub #	P1-403
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Understanding the Cellular Origin of Pituitary Tumors: Isolation and Characterization of Putative Tumorigenic Progenitors/Stem Cells from a Mouse Model of Human Adamantinomatous Craniopharyngioma
Author String	CL Andoniadou, C Gaston-Massuet, P Le Tissier, MT Dattani, JP Martinez-Barbera UCL Institute of Child Health, London, UK; National Institute for Medical Research, London, UK; UCL Institute of Child Health and Great Ormond Street Hospital for Children, London, UK
Body	Somatic stem cells of multiple tissues such as brain, blood, gut epithelium and epidermis, have specific roles in tissue homeostasis and plasticity of cell types. There is evidence that, when mutated, such cells, termed cancer stem cells also underlie tumorigenesis in mice and humans, but their presence in many tumors is elusive. In the murine pituitary gland, somatic stem cells (PSCs) have been previously identified and characterised in vitro and in vivo, but little is known about the signalling pathways involved in their specification and maintenance or their role in tumorigenesis. Combining in vitro stem cell culture and time-lapse microscopy, we demonstrate that the conditional expression in the pituitary of a stable form of beta-catenin and subsequent activation of the Wnt pathway in a mouse model for human adamantinomatous craniopharyngioma (ACP), leads to an enlargement of the PSC compartment and a significant increase in thei proliferation rate, both of which may contribute to tumor formation. These results implicate for the first time this signalling pathway in the control of PSC maintenance. Through flow-sorting isolation of these putative tumorigenic cells we show that they have functional and phenotypic characteristics of PSCs. In addition, microarray analysis has revealed a unique genetic signature in these cells, with significant perturbation of pathways involved in cancer. We will present novel data involving a signalling pathway other than the Wnt pathway, in the pathogenesis of mouse and human ACP and show preliminary results highlighting its relevance as a therapeutic target. Nothing to Disclose: CLA, CG-M, PLT, MTD, JPM-B

Pub #	P1-404
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Analysis of Potential Modifying Loci/Genes in FIPA Indicates a Possible Association of Chromosome 2p16-21 in the Disease Phenotype
Author String	RA Toledo, DM Lourenco, Jr, T Sekiya, SPA Toledo University of São Paulo, School of Medicine, São Paulo, Brazil
Body	Context: The phenotypic diversity and incomplete penetrance observed amongst the patients harboring AIP germline mutations, which predispose to familial isolated pituitary adenoma (FIPA), is compatible with the involvement of modulating genetic factors. Objective: To investigate whether candidate loci/genes may play a role in tumour development of FIPA patients. Patients: Three siblings of a IFS family recently reported to carry an germline AIP mutation, Y268X, but displaying distinctive phenotypes: two of them developed acromegaly at early ages (17 y-old and 21 y-old), while the other was asymptomatic, at 41 y-old. Results: Microsatellite markers spanning the 5q35 (PTTG), 14q32 (MEG3) and 13q14 (Rb) did not segregate with the early onset acromegaly feature in informative patients. Furthermore, several candidate genes (p27Kip1, MEN1, PRKAR1A, GNAS1, GNAI2, GHRH-R, SSTR2, SSTR5 and AHR) were sequenced and no pathological variants were observed. Of note, markers spanning the 2p16-21 oncogenic region co-segregated with development of early, large and functioning pituitary tumors. In addition, we re-analyzed early published data of 2p16 in a family with acromegaly (Gadelha et al., 2000) comparing with the recent data on AIP (Leontiou, 2008) and found a co-segregation pattern similar to the observed in our cases: within the six AIP-mutated adult siblings, five which shared the same 2p16-21 markers developed early and aggressive tumors, while the sixth adult AIP-mutated sibling which presented a different haplotype remained asymptomatic (LOD score 2.5-3.0). The PKCepsilon protooncogene (2p21) was sequenced in our cases. No mutation was observed, only the apparently not pathological variants IVS1+37A>G (exon 1), IVS5-31G>A (exon 6) and IVS14-49G>T (exon 14). Discussion: Altogether, these findings are consistent with a putative role for a gene(s) at 2p16-21 in influencing the penetrance and /or phenotypic variation of AIP-mutated FIPA families. Sources of Research Support: FAPESP Fellowship. Nothing to Disclose: RAT, DML, TS, SPAT

Pub #	P1-405
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Secretagogue Responsiveness and Gene Expression Profiles Differ between Single- and Dual-Hormone-Secreting Acidophils
Author String	FR Boockfor, C Farish, RP Visconti, JL Barth, J Zhang, WS Argraves Medical University of South Carolina, Charleston, SC
Body	Acidophils are the most abundant hormone-secreting cells of the pituitary. This population consists of three cell types; those that secrete GH-only, those that secrete PRL-alone, and those that secrete both GH and PRL (mammosomatotropes; MS). Despite their prominence, very little information is available on the functional characteristics of each cell type, especially properties that would impact their contribution to overall GH or PRL release. The goal of this study was to obtain separate populations of these cells and begin to identify unique functional properties that may influence their release of GH and/or PRL. Because residual hormone remains in the membrane of an endocrine cell following exocytosis, we were able to label monodispersed rat pituitary cells with GH and/or PRL antibody and separate them using Fluorescent Activated Cell Sorting (FACS) into groups of these highly enriched acidophil cell types. Initially, we evaluated the response of PRL-only cells and MS to the well-characterized PRL secretagogue TRH using plaque assays. Average PRL plaque area (indicative of the amount of PRL released) increased approximately 4-5 fold in the presence of TRH with PRL-only cells, but was virtually unchanged when TRH was added to MS. A 3-fold increase in GH plaque size following GRF stimulation suggested that these MS were undamaged and able to respond. High throughput mRNA expression analysis was then conducted to identify transciptomic differences in these populations. Preliminary analysis revealed that among genes detected in all three sample types, 190 were different by > 5 fold or more when comparing single and dual-cells for the same hormone. Unique inductions of several genes were displayed in each cell group suggesting distinctive functional characteristics from one cell type to another. Examination of genes related to secretory granule processing revealed diverse expression profiles between the cell types, as would be expected for cells with systems for packaging and transport of distinct hormone-containing granules. Our results of differences between PRL-only and MS in PRL response to a least one regulatory secretagogue and the divergence of gene expression patterns between cell groups indicate that acidophilic cell types function differently with respect to specific aspects of secretion and sugges that the abundance of each type in the population may play an important role in dictating the release of GH and PRL from the pituitary. Sources of Research Support: R01DK073270 and R01DK073270-03S1 (to FB). Nothing to Disclose: FRB, CF, RPV, JLB, JZ, WSA

Pub #	P1-406
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Somatostatin Receptors and Dopamine 2 Receptor Expression in Various Pathological Types of Clinically Non-Functioning Pituitary Adenomas
Author String	F Gabalec, M Beranek, M Drastikova, T Cesak, D Netuka, V Masopust, J Marek, J Cap Charles University Faculty of Medicine and Teaching Hospital in Hradec Kralove, Hradec Králové, Czech Republic; Charles University Faculty of Medicine and Teaching Hospital in Hradec Kralove, Hradec Králové, Czech Republic; Charles University Faculty of Medicine and Teaching Hospital in Hradec Kralove, Hradec Králové, Czech Republic; 1st Faculty of Medicine, Charles University in Prague and Central Military Hospital Prague, Prague, Czech Republic; 1st Faculty of Medicine, Charles University in Prague, Prague, Czech Republic
Body	Clinically non-functioning pituitary adenomas account for about one-third of pituitary tumors. The majority of them are pathologically classified as gonadotropinomas or null-cell adenomas without hormonal expression. The rest represent silent corticotroph adenomas and plurihormonal tumors. Conservative therapy with dopamine agonists and somatostatine analogues is effective in some cases only depending on the expression of somatostatine (SSTR) and dopamine 2 receptors (D2R). The aim of this study was to quantitatively estimate SSTR and D2R expression in clinically non-functioning pituitary adenomas and correlate the results with adenoma type according to pathological classification. Out of the 87 adenomas investigated, 63 expressed gonadotropins, 7 were silent corticotroph adenomas, 7 were plurihormonal tumors, and only 6 did not express any pituitary hormone on immunohistochemical investigation. With the use of the reverse transcriptase PCR technique, D2R mRNA was expressed in all adenomas with very heterogeneous quantity. The expression was very low in corticotroph adenomas (relative median quantity after normalization to housekeeping gene 0.01) and lower in plurihormonal tumors (median 0.4) than in gonadotroph (median 1.3) and null-cell adenomas (median 1.9). The difference between corticotroph adenomas and plurihormonal tumors in comparison with other pathological types was statistically significant. The expression of D2R did not depend on the presence or absence of gonadotropins. We conclude that D2R expression is very low in corticotroph adenomas and significantly lower in plurihormonal tumors. The positivity of gonadotropins does not predict the D2R quantity. Data for somatostatine receptors will be presented too. Nothing to Disclose: FG, MB, MD, TC, DN, VM, JM, JC

Pub #	P1-407
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Possible Mechanisms of Resistance to Dopamine Agonists in Prolactinoma
Author String	S Shimazu, Y Nagamura, S Yamada, T Usui, A Shimatsu, T Tsukada National Cancer Center, Research Institute, Tokyo, Japan; Toranomon Hospital, Tokyo, Japan; National Hospital Organization, Kyoto Medical Center, Kyoto, Japan
Body	Background: The first line treatment of prolactinoma (PRLoma) is dopamine (DA) agonists, which both normalize plasma prolactin levels and reduce tumor size of DA-sensitive PRLoma. Ten to 15% of cases are resistant to DA agonists from the beginning of the treatment (primary resistance) and are treated by surgery. A few PRLomas initially respond to DA agonists but become resistant after prolonged treatment (secondary resistance). Although the reduction of the dopamine D_2 receptor (D_2R) expression in tumor cells may explain the resistance, the exact mechanism is not fully understood. Methods: We examined 14 cases of surgically resected PRLoma. We divided them into three groups according to responsiveness to DA agonists, the sensitive (n=7), the primary resistant (n=5) and the secondary resistant (n=2) cases. We examined D_2R/ G3PDH mRNA ratio and D_2R short/ D_2R long isoform mRNA ratio. We also investigated DNA methylation patterns in the promoter region of D_2R gene by the DNA bisulphate modification method. Results: The D_2R expression was much lower in the secondary resistant tumors than in others. We also found that the D_2R expression in primary resistant tumors was significantly lower than that in sensitive tumors. The D_2R short/ D_2R long isoform mRNA ratio was not correlated with tumor response to DA agonists. The DNA methylation patterns in the promoter region of D_2R gene were significantly different between sensitive and resistant cases. Conclusion: The resistance of PRLoma to DA agonists is correlated with the reduction of D_2R mRNA levels, suggesting that the silencing of D_2R gene expression is a possible mechanism for DA resistance in PRLoma. Our results also suggest that methylation status of the D_2R gene promoter region is associated with D_2R expression. This is the new clue to identify the mechanisms of drug resistance in pituitary adenoma. Sources of Research Support: Cancer Research Grant; Research Grant from Intractable Disease, MHLW Japan. Nothing to Disclose: SS, YN, SY, TU, AS, TT

Pub #	P1-408
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Somatotroph Adenomas with Low E-Cadherin Expression Respond Differently to Somatostatin Treatment
Author String	T Lekva, S Fougner, T Ueland, JP Berg, J Bollerslev University of Oslo and Oslo University Hospital, Oslo, Norway; University of Oslo and Oslo University Hospital, Oslo, Norway; University of Oslo and Oslo University Hospital, Oslo, Norway; University of Oslo and Oslo University Hospital, Oslo, Norway
Body	Context: The epithelial marker E-cadherin plays a crucial role in epithelial mesenchymal transition (EMT). A decreased expression in somatotroph adenomas has been associated with increased tumor size, invasion, and poor response to somatostatin analog (SA) treatment, but the potential mechanisms of EMT progression in these adenomas is lacking. Objectives: To investigate E-cadherin gene expression in somatotroph adenomas after operation with special focus on the effect of preoperative SA treatment. Patients and Methods: E-cadherin mRNA expression from the adenoma of 49 patients was divided arbitrary into tertiles. Tumor size, gene expression and serum measures of GH and IGF-I, and gene expression of ZEB1, TXNIP and GILZ were compared according to E-cadherin tertiles, with and without preoperative SA treatment. Results: In relation to E-cadherin tertiles, tumor size and IGF-I expression were augmented with lower E-cadherin expression ($p=0.026$ and $p=0.014$), whereas GH expression, GH and IGF-1 serum became lower ($p<0.001$, $p=0.060$ and $p=0.001$). There was no difference in ZEB1, GILZ and TXNIP between the tertiles of E-cadherin expression. In the tertile with the lowest E-cadherin expression, we found no difference in E-cadherin expression with SA treatment compared to, lower expression ($p=0.039$) in the middle group, and higher expression ($p=0.013$) in the tertile with highest E-cadherin expression. A known inhibitor of E-cadherin, ZEB1, was lower ($p=0.025$) in the SA treated group with high E-cadherin, but no difference in the SA treated group with lower E-cadherin expression. GILZ and TXNIP expression were higher ($p=0.029$ and $p=0.019$), whereas SSTR2 expression was lower ($p=0.034$) in the SA treated group with low E-cadherin expression. Conclusions: The data indicates that the adenomas with low E-cadherin expression have dedifferentiated from the normal somatotroph phenotype. SA treated adenomas with low E-cadherin expression have higher TXNIP and GILZ expression, and lower SSTR2 expression, implicating a different response to SA treatment. Nothing to Disclose: TL, SF, TU, JPB, JB

Pub #	P1-409
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Correlation of Expression and Function of Somatostatin Receptors in GH-Secreting Adenoma Cells with Clinical Responsiveness to Somatostatin Analog Treatment
Author String	B Mayr, R Buslei, M Buchfelder, C Schofl Erlangen University Hospital, Erlangen, Germany; Erlangen University Hospital, Erlangen, Germany; Erlangen University Hospital, Erlangen, Germany
Body	**Introduction:** Somatostatin receptor (SstR) agonists (SSA) like octreotide mainly act through SstR subtype 2 in vivo and are clinically effective in about 50 % of acromegaly patients. The molecular reasons for this heterogeneous response to SSA treatment are only partly understood. We studied clinical follow-up data and the expression and function of SstR in primary cultures of GH-secreting adenoma cells in 32 acromegaly patients. **Methods:** Cells isolated from 32 GH-secreting pituitary adenomas were cultured for 3-5 days and the effect o octreotide on calcium- and cAMP-signaling was determined by fura-2 microfluorometry and quantitative RT-PCR for the cAMP target gene ICER. Expression of SstR-2 was determined by immunohistochemistry of paraffin embedded tissues using 2 different antibodies. Clinical follow-up data were obtained to determine the patients' response to SSA treatment. **Results:** 13 of 32 patients received SSA treatment. In 6 of these 13 patients IGF-1 levels were normalized, in 4 patients IGF-1 could be partly lowered (> 25 %), and 3 patients showed no response to SSA treatment. SstR-2 was expressed in all samples. 4 samples, however, showed membrane staining in less than 10 % of cells. Octreotide (100 nM) or SRIF-14 (100 nM) inhibited calcium- and cAMP-signaling in 11 and 10 of the 13 adenoma samples, respectively. All 13 samples exhibited suppression of at least one pathway. Calcium signaling was significantly less suppressed in samples from the 3 non-responsive patients ($p= 0.027$), but SstR-2 staining and inhibition of cAMP-signaling were not different from patients with a full or partial response. **Conclusion:** Most GH-secreting adenoma cells expressed SstR2 and showed functional suppression by octreotide of calcium- and cAMP-signaling. Clinically, however, only 50 % achieved a complete remission in response to SSA treatment. This suggests that factors other than the sensitivity of the adenoma cells to SSA may determine the clinical efficacy of SSA therapy. Sources of Research Support: Grant from Pfizer GmbH, Berlin, Germany. Disclosures: CS: Investigator, Pfizer, Inc. Nothing to Disclose: BM, RB, MB

Pub #	P1-410
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Low Somatostatin Receptor Subtype 2 Expression Predicts the Lack of Biochemical Response of Somatotropinomas to Treatment with Somatostatin Analogs, Independently from the Expression Rate of Dopamine Receptor Subtype 2
Author String	LEA Wildemberg, LV Neto, DF Costa, LM Alves, CM Takiya, MR Gadelha Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
Body	It has been suggested that somatostatin receptors (SSTR) can form homo- and heterodimers with other receptors, such as dopamine receptor subtype 2 (DR2), which may influence their functionality. So, the therapeutic response of somatotropinomas to somatostatin analogues may be dependent on the relative pattern of DR2 and SSTR expression. Objectives To determine SSTR2A and DR2 protein expression in somatotropinomas and to compare it to in vivo response to octreotide LAR treatment. Design and methods SSTR2A and DR2 expression was analyzed by immunohistochemistry in 52 somatotropinomas. Tumors were scored according to percent of immunostained cells: 0 (<25%), 1 (25-50%) and 2 (>50%). Biochemical response to octreotide LAR was assessed by percent IGF-I reduction after three (IGF3) and six (IGF6) months of treatment and patients were divided in responders (IGF-I reduction > 50%) and non-responders (IGF-I reduction < 50%). Biochemical control was also assessed, being considered controlled patients who achieved GH < 1.0 ng/mL and normal IGF-I for age. Results SSTR2A was expressed in 51 (98%, n=52) tumors, while DR2 was present in 45 (94%, n=48) adenomas. SSTR2A was scored as 2 in 33 (64%), 1 in 11 (21%) and 0 in eight (15%) patients. DR2 was scored as 2 in 24 (50%), 1 in 12 (25%) and 0 in 12 (25%) patients. No relation was found between SSTR2A and DR2 expression. Data regarding biochemical response to octreotide LAR was available in 30 patients. Median IGF3 and IGF6 were 47% and 50%, respectively. The IGF3 and IGF6 were significantly different among the three SSTR2A scores ($p = 0.024$ and 0.017, respectively) and were lower in the score 0 than in score 2 ($p = 0.009$ and 0.004, respectively). Seventeen (57%) patients were considered as responders, while 13 (43%) patients reached biochemical control. When it was considered a cut-off point of 25% immunostained cells, there was association between SSTR2A and biochemical response ($p = 0.001$) and control ($p = 0.01$). It was found a sensitivity of 100%, specificity of 54%, negative predictive value of 100% and positive predictive value of 74% for biochemical response, with an accuracy of 80%. No statistically significant relationship was observed between response to octreotide LAR and DR2 protein expression. Conclusion SSTR2A and DR2 were highly expressed in this series of somatotropinomas and there was association between biochemical response and control by octreotide LAR and SSTR2A protein expression, but not with DR2. Nothing to Disclose: LEAW, LVN, DFC, LMA, CMT, MRG

Pub #	P1-411
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	Somatostatin Secretion by Null Cell Type Pituitary Adenomas Down-Regulates Somatostatin Receptors and Suppresses GH Gene Expression
Author String	H Katakami, S Yamada Teikyo University Chiba Medical Center, Ichihara, Japan; Toranomon Hospital, Tokyo, Japan
Body	The null cell type adenoma of the pituitary (**NC**), composing 5-15% of all pituitary adenomas, has been defined the tumor without immunopositivities to any known anterior pituitary hormones, though these **NC** usually have electron-dense secretory granules in their cytoplasm, suggesting secretion of some products. In an attempt to elucidate hypothalamic functions, we simultaneously measured hypothalamic hormones in both cavernous sinus/peritumorous blood (Cn) and peripheral blood (Pe) during transsphenoidal surgery in patients with pituitary tumors, we found by chance a female patient who showed high somatostatin (**SRIF**) levels in Cn, but not in Pe. The IHC of her tumor was immunonegative to any anterior pituitary hormones, but immunopositive to **SRIF**. We have confirmed the production of **SRIF** by most of **NC**, but not by other types of pituitary adenoma, in a large number of pituitary adenomas (N=179), and proposed a new classification for pituitary tumor pathology for **SRIF**-producing **NC** (**NC+**). To further characterize **SRIF** production in **NC+**, we focused on biological aspects of the unique cell type of pituitary adenomas. We carried out a prospective study in a large series of patients with pituitary adenoma of NF (N=69), (N=12, M:F=1:11) and **NC-** (NC without SRIF-production, N=7, M:F=2:5), those of them were verified not only by IHC, but also by quantitative PCR for both hypothalamic and pituitary hormones. We also quantitatively measured the gene expression of **SRIF** Rc (SSTR2, 5) to examine possible down-regulation of the Rc. In 6 out of 9 patients with **NC+**, we found high **SRIF** levels in Cn (19.4-589.4pg/ml), but not in Pe (3.3-7.2pg/ml). All of patients with **NC+** patients with high plasma **SRIF** levels in Cn showed significantly higher tissue **SRIF** contents and gene expression. HPLC analysis of the **SRIF** molecule in tissue extracts of **NC+** showed **SRIF**1-28 was a dominan molecular form. The ratio of the **SRIF**/beta-actin mRNA levels in **NC+** (1.2 ± 0.4) were significantly increased vs. NF (0.002 ± 0.0005), or **NC-** (0.0003 ± 0.0002), respectively. By contrast, the ratio of GH/beta-actin mRNA levels as well as SSTR2/beta-actin or SSTR5/beta-actin mRNAs levels were decreased in **NC+**. These results show that **SRIF** produced by **NC** down-regulate its own receptors and suppress the GH gene expression. Further study will be needed to elucidate the clinical significance of this intriguing type of adenomas. Nothing to Disclose: HK, SY

Pub #	P1-412
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Pituitary Biology & Tumorigenesis (1:30 PM-3:30 PM)
Title	miR-107 Is Overexpressed in Pituitary Adenomas and Inhibits the Expression of Aryl Hydrocarbon Receptor-Interacting Protein (AIP)
Author String	G Trivellin, S Igreja, E Garcia, HS Chahal, H Butz, A Patocs, AB Grossman, M Korbonits Barts and the London School of Medicine, Queen Mary University of London, London, UK; Faculty of Medicine, Semmelweis University, Budapest, Hungary; Hungarian Academy of Sciences, Budapest, Hungary
Body	**Background:** Abnormal microRNAs (miRNAs) expression profiles have been recently associated with sporadic pituitary adenomas, suggesting that miRNAs can contribute to tumor formation. miRNAs are noncoding RNAs which inhibit post-transcriptional expression of target mRNAs by binding to complementary sequences commonly located in the 3' untranslated region (3'UTR). However, the substantial lack of knowledge about miRNAs targets hinders a full understanding of the mechanisms by which they influence tumorigenesis. **Methods:** The expression levels of miR-107, a miRNA involved in the pathogenesis of different types of tumors, were evaluated in 45 human sporadic pituitary adenoma tissues removed at surgery and 14 normal pituitary samples using microRNA screen array and qRT-PCR analyses. Over-expression of a pre-miR-107 precursor and treatment with a miR-107 inhibitor were used to examine the effects of miR-107 expression on cell proliferation and colony formation in human neuroblastoma (SH-SY5Y) and rat pituitary adenoma (GH3) cells. A luciferase reporter assay was used to examine the *in silico* predicted target sites in the 3'UTR of the *aryl hydrocarbon receptor-interacting protein* (*AIP*) gene. **Results:** miR-107 expression was found significantly up-regulated in pituitary adenoma tissues compared to normal pituitaries. Over-expression of miR-107 inhibited cell proliferation of both the rat and the human cell lines tested. Anti-miR-107 increased colony formation by 2.5 fold in human cells. We showed that *AIP*-3'UTR is a functional target of miR-107 as miR-107 inhibits human AIP expression to 53.9±2% of the scrambled miRNA control in a luciferase expression model and it reduces endogenous AIP expression to 53±22% of the scrambled miRNA control in neuroblastoma cells. **Conclusions:** Endogenous miR-107 is over-expressed in pituitary adenomas. *In vitro* mir-107 over-expression reduces, while inhibition increases, cell growth, suggesting that it is a tumor suppressor miR. However, miR-107 inhibits *AIP*, a tumor suppressor gene mutated in some familial isolated pituitary adenoma (FIPA) families, suggesting that while this is a target for miR-107-mediated degradation, the tumor suppressor role of miR-107 is not via AIP. This complex interaction of tumor suppressor genes needs further exploration. Nothing to Disclose: GT, SI, EG, HSC, HB, AP, ABG, MK

Pub #	P1-413
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Acromegaly (1:30 PM-3:30 PM)
Title	SXN101742, a Botulinum Toxin-Derived Targeted Secretion Inhibitor (TSI) Inhibits GH Synthesis and Secretion: A New Concept for the Management of Acromegaly
Author String	E Somm, A Martinez, P Marks, A Toulotte, N Bonnet, S Ferrari, PS Huppi, R Jones, ML Aubert University of Geneva School of Medicine, Geneva, Switzerland; Syntaxin Ltd, Abingdon, UK; University of Geneva School of Medicine, Geneva, Switzerland
Body	**Introduction:** Acromegaly is a chronic condition in which excess growth patterns occur caused by abnormal high levels of GH and IGF-1. The pathology of the disease is most often driven by GH-producing pituitary tumor. In the present study a recombinant targeted secretion inhibitor (TSI) SXN101742, designed to specifically target the pituitary somatotrophs in order to block GH secretion has been investigated in a juvenile rat growth model. The goal of the study was to establish a physiological proof that inhibition of GH secretion by SXN101742 impacts on growth and development in young male rats. **Methods:** A single IV injection of SXN101742 (1mg/kg) was given to male Sprague Dawley rats at 45 days of life to take advantage of the important growth dependence on GH/IGF-1 at this age. We monitored body weight gain daily and circulating IGF-1 at 3-day intervals. On day 55, rats were first measured (nose-anus length) then sacrificed and organs weighed and frozen for further analyses. Gene expression of pituitary hormones, as well as IGF-1 and related transporters in the liver was assessed using real time PCR (ABI). Pituitary GH content was assayed using an ELISA kit (Millipore). Femur morphometry was investigated by scanning with a microCT instrument (Scanco Medical). **Results:** Rats treated with SXN101742 showed arrested weight gain from day 6 after injection. At sacrifice, treated rats weighed less, and their length was significantly shorter. Plasma IGF-1 was decreased 3 days after injection with SXN101742 (43±4%). Moreover, pituitary weight, GH gene expression and GH content were all decreased at sacrifice (by 27±2%, 70±2%, 72±2%, respectively). Interestingly, no change in pituitary gene expression for FSH, LH, TSH, or ACTH was observed while IGF-1, ALS, and IGFBP3 gene expression were reduced in liver by 69±5%, 49±7% and 31±9%, respectively. This inhibition of the GH/IGF-1 axis is biologically relevant since femur length and their cross sectional area were decreased in treated rats by 3.6% and 12.3%, respectively. Furthermore, the most of reported effects were shown to be dose-dependent by studying the lower doses of 0.3 and 0.1 mg/kg. **Conclusion:** A single IV injection of the SXN101742 exerts striking inhibitory action on the somatotropic axis in growing rats with major reductions in key parameters as seen 10 days after this single administration. Therefore this approach is a novel pituitary directed therapy for the treatment of excessive GH/IGF production. Disclosures: AM: Employee, Syntaxin. PM: Employee, Syntaxin. RJ: Employee, Syntaxin. MLA: Consultant, Syntaxin. Nothing to Disclose: ES, AT, NB, SF, PSH

Pub #	P1-414
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Acromegaly (1:30 PM-3:30 PM)
Title	Characterization of Management and Outcomes of Patients with Acromegaly at Vancouver General Hospital over the Last 20 Years
Author String	M Almalki, E Ur, G Wilkins, M Johnson, A Chesover UBC, Vancouver, Canada
Body	Acromegaly is a rare disorder with a prevalence of 60 per million. It is associated with significant morbidity and mortality. The objective of this study was to implement a registry for patient with acromegaly in British Columbia (BC), Canada and to evaluate and describe the demography, epidemiology and effectiveness of treatments used in the condition. The study included 120 patients with a diagnosis of acromegaly since 1980, based on clinical notes. Data was collected retrospectively from patient charts. 'Cure' or control was defined based on GH<2 and IGF-1 within normal limits. Result: 120 patients, (63 female) were included in the analysis with a mean age at diagnosis 35 y (male) and 43y (female). One-third of the patients presented to their general practitioner (37%). Most common presenting features included acral enlargement, coarse facial features, sweating/oily skin, and headache. All cases were due to pituitary adenoma, of which 86.4% were macroadenoma, and of these 33% were invasive. The most common co-morbidities were diabetes (44.4%), arthralgia (43.2%), hypertension (42%), sleep apnea (31.2%), Carpal tunnel syndrome (25.2%), and hormone deficiency (22.8%). The vast majority (83%) of patients was treated surgically and of these 23% went on receive radiotherapy and 73% received medical therapy. When stringent cure criteria are used (based on latest GH and IGF-1 results) the outcomes were 36.7% cured or controlled, 37.5% remained active, 18.3 discordant result and 7.5% were no result reported. Thirty-one percent of patients who underwent surgery and 29% patient who underwent radiotherapy were not cured but tended to be controlled with medical therapy. Majority of the patients (72.5%) were on medical treatment. Somatostatin analogue was the most commonly drug used in 79 % of the time. Conclusions: The preliminary results of this study of acromegaly in BC, showed a prevalence of 27 per million. Cure rate was low following surgery but there was propensity to control disease with adjuvant medical treatment. Nothing to Disclose: MA, EU, GW, MJ, AC

Pub #	P1-415
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Acromegaly (1:30 PM-3:30 PM)
Title	Development of a Novel Mass Spectroscopy-Based Method for Determining Serum IGF-I: Assessment in a Cohort of Newly Diagnosed Subjects with Acromegaly
Author String	AK Annamalai, R Kay, N Kandasamy, G Wark, K Taylor, DJ Halsall, S Pleasance, M Gurnell Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK; , Fordham and Rushden, UK; Royal Surrey County Hospital, Guildford, UK; Addenbrooke's Hospital, Cambridge, UK
Body	**Background:** The recently published 'Consensus on Criteria for Cure of Acromegaly' (Giustina et al, JCEM, 2010) highlighted concerns regarding the quality of currently available insulin-like growth factor-1 (IGF-1) immunoassays which may contribute, at least in part, to the discordance between growth hormone (GH) and IGF-1 that is observed in up to 30% of patients with acromegaly after treatment. The development of mass spectroscopy (MS)-based technology has been proposed as a potential solution to these limitations. **Methods**: Here, we report the development of a stable isotope dilution ultra-performance liquid chromatography tandem MS (UPLC-MS/MS)-based method for the quantitation of serum IGF-1. The method employs Selected Reaction Monitoring (SRM) of two tryptic peptides derived from IGF-1, and relies on solid phase extraction for enrichment of the peptide fraction containing IGF1 rather than immunocapture, so is less susceptible to antibody interference. The UPLC separation of the peptides was performed using a C18 columr prior to MS/MS analysis on a 5500 QTrap MS. The method is not affected by concentrations of IGFBP3 up tc 420nmol/L. **Results:** The method showed good correlation with an IGF-1 immunoassay (Siemens Immulite 2000) over a wide range of serum IGF-1 concentrations (5.4-261 nmol/L by immunoassay) in a cohort of 45 patients that included 25 subjects with acromegaly, assessed both before and after primary medical therapy. The Passing and Bablock regression was: LC-MS/MS(nmol/L) = 1.4*Immunoassay(nmol/L) + 4.4. Analysis of UKNEQAS material with an immunoassay method mean of 22.2 and 45nmol/L returned values of 22.3 and 47.6nmol/L respectively. **Conclusions:** This method relies on entirely different physicochemical principles to the ubiquitous 'sandwich immunoassay' for IGF-1, and thus provides an independent validation of suspicious immunoassay-derived results. A further advantage of the method is that, with the addition of appropriate internal standards, there is potential for extensive multiplexing of serum peptide assays. Nothing to Disclose: AKA, RK, NK, GW, KT, DJH, SP, MG

Pub #	P1-416
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Acromegaly (1:30 PM-3:30 PM)
Title	Cognitive Status in Acromegaly: Possible Recovery after Successful Treatment
Author String	JF Martin-Rodriguez, A Madrazo-Atutxa, A Soto-Moreno, E Venegas-Moreno, E Torres-Vela, P Benito-Lopez, MA Galvez, FJ Tinahones, A Leal-Cerro University Hospital Virgen del Rocío/Consejo Superior de Investigaciones Científicas/University of Seville and Division of Endocrinology, Virgen del Rocío University Hospital, Seville, Spain; San Cecilio Hospital, Granada, Spain; Reina Sofía Hospital, Cordoba, Spain; Virgen de la Victoria Hospital, Malaga, Spain
Body	De novo acromegalic patients show neurocognitive impairment associated to high GH and IGF-I levels1. It has been suggested that this impairment might be a consequence of lasting exposure to GH and IGF-I excess on the central nervous system 1-3. Nevertheless, it remains to be determined whether neurocognitive problem are still present when the disease is cured or controlled with drugs. This research aims to study the neurocognitive state of acromegalic patients after cure and patients with successful pharmacological control o acromegaly. Cognitive functions, emotional status and quality of life (QoL) were assessed in fifteen patients cured of acromegaly (10 women, median age = 50.93) and 15 patients with pharmacologically control of acromegaly (somatostin analogs) (10 women, median age = 48.19). The criteria stablished for cure after pituitary surgery were normal IGF-I levels for age and gender and a GH nadir < 1 ng/mL after an oral glucose tolerance test. The criteria stablished for good control of acromegaly were normal IGF-I levels for age and gender and a basal GH < 2 ng/ml. These patients' data were compared to two age- and gender-matched additional groups, comprised of 15 patients with non-treated active acromegaly and 15 healthy controls. No difference was observed in tests assessing attention, verbal skills and executive functions among the different groups. However, significant differences were found in memory tests (all Ps < 0.01). Concretely, patients with active non-treated acromegaly and patients with good pharmacologically control performed worse in working memory and long-term memory than healthy subjects and cured patients. Further analyses showed a significant correlation between emotional/QoL and severity of memory impairment in pharmacologically controlled patients. In the active non-treated group, no correlation between QoL, depression and severity of memory impairment was observed. Patients with cured acromegaly did not differ from healthy subjects in cognitive functions and emotional/QoL status. Our results confirm memory impairment in active acromegaly that could be due to GH and IGF-I excess. In addition, our findings suggest a good recovery of cognitive impairment after cure of acromegaly while patients with good pharmacological control of the disease show similar memory impairment than that observed in de novo patients. (1) Leon-Carrion J et al., J Clin Endocrinol Metab 2010; 95: 4367 (2) Tanriverdi F et al., Growth Horm IGF Res 2009; 19: 24 (3) Tiemensma J et al., J Clin Endocrinol Metab 2011; 95: E392 Sources of Research Support: Grant from Novartis Oncology. We are indebted to Dr. Monserrat Gilabert for her help in editing the abstract. Nothing to Disclose: JFM-R, AM-A, AS-M, EV-M, ET-V, PB-L, MAG, FJT, AL-C

Pub #	P1-417
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Acromegaly (1:30 PM-3:30 PM)
Title	Memory Complaints in Acromegaly and Their Relationship to Quality of Life and Emotional Status
Author String	A Madrazo-Atutxa, JF Martin-Rodriguez, A Soto-Moreno, E Venegas-Moreno, E Torres-Vela, P Benito-Lopez, MA Galvez, F Tinahones, A Leal-Cerro University Hospital Virgen del Rocío/Consejo Superior de Investigaciones Científicas/University of Seville and Division of Endocrinology, Virgen del Rocío University Hospital, Seville, Spain; San Cecilio Hospital, Granada, Spain; Reina Sofía Hospital, Cordoba, Spain; Virgen de la Victoria Hospital, Malaga, Spain
Body	Recent clinical studies have reported specific cognitive deficits in patients with acromegaly1,2. Among these deficits, memory function seems to be more severely altered, that could be related to specific brain areas malfunction. It has also been suggested that cognitive problems could be linked to patients' low perceived quality of life (QoL). In this current research, we explore how patients with acromegaly perceive their memory problems. Memory complains were assessed in 64 acromegalic patients (37 female; median age = 48.5; 22 de novo non-treated patients, 20 patients with good pharmacological control of acromegaly (16 with somatostatin analogs and 4 with pegvisomant), and 22 patients cured of acromegaly, and 20 healthy controls (median age = 42.5), through a questionnaire measuring five types of memory: working memory, source memory, recognition memory, memory fixation, prospective memory, and procedural memory3. In addition, emotional status 4 and QoL5 were also assessed. Patients with acromegaly reported significantly a higher number of memory complains related to recognition memory ($P = 0.006$) as compared to healthy subjects. De novo patients and patients with good pharmacological control reported significantly higher recognition memory complains compared to healthy subjects, as shown by subgroup analysis ($P = 0.015$ & 0.02, respectively), whereas patients with cure of acromegaly did not differed from healthy controls. The association between the emotional status measure and number of complains in recognition memory displayed a trend towards significance ($P < 0.1$). This statistical trend was also observed for the association between the QoL measure and number of memory complains. The number of memory complains correlate positively with emotional status in the pharmacologically controlled group ($P = 0.04$), but not with QoL. No correlation between QoL, depression and memory complains was observed in the de novo group. In summary, patients with de novo and with good pharmacologically control of acromegaly reported higher memory problems than healthy controls. These memory problems consists of complains about everyday situations in which patients need to correctly remember something that has been encountered before (recognition memory). The number of complains was not associated with depression and QoL in the de novo group, that may suggest that these complains are related to an organic-based memory impairment. (1) Leon-Carrion J et al., J Clin Endocrinol Metab 2010; 95: 4367 (2) Tanriverdi F et al., Growth Horm IGF Res 2009; 19: 24 (3) Leon-Carrion J et al., Revista Espa[ntilde]ola de Neuropsicologia 1999; 2: 37 (4) Beck et al., Manual for the Beck Depression Inventory-II. San Antonio, TX: Psychological Corporation, 1996 (5) Badia X et al.,Health Qual Life Outcomes 2004; 2:13 Sources of Research Support: Grant from Novartis Oncology. We are indebted to Dr. Monserrat Gilabert for her help in editing the manuscript. Nothing to Disclose: AM-A, JFM-R, AS-M, EV-M, ET-V, PB-L, MAG, FT, AL-C

Pub #	P1-418
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Acromegaly (1:30 PM-3:30 PM)
Title	Effects of GH/IGF-I Control on Renal Function, Calcium/Phosphate and Bone Turnover Parameters during Treatment of Acromegalic Patients
Author String	S Grunenwald, C Vaurs, I Tack, D Chauveau, A Bennet, P Caron CHU Larrey, Toulouse, France; CHU Rangueil, Toulouse, France; CHU Rangueil, Toulouse, France
Body	Background: The GH/IGF-1 hypersecretion leads to cardiovascular and metabolic complications. While treatment of acromegaly reverses increased morbidity and mortality, its effects on renal function, calcium/phosphate homeostasis and bone turnover markers are rarely reported. Objective: To compare these parameters in acromegalic patients at diagnosis and after control of the GH/IGF-1 hypersecretion. Methods: We studied glomerular filtration rate (GFR) (assessed by inulin clearance), calcium homeostasis and bone turnover (osteocalcin, crosslaps) parameters in 10 patients (2 men, 8 women, 50 ± 8 years old) (mean ± sd) with micro (n=1) and macro (n=9) GH-secreting adenomas, before and after GH/IGF-1 control with transphenoidal surgery (n=1), pegvisomant (n=1) and/or somatostatin analogues (n=9). Results: Treatment controlled GH (before 20.7±18.8 ng/ml, during 0.6±0.2 ng/ml) ($p < 0.01$) and IGF-1 (before 292±188% of upper limit of normal range [ULNR], during -30±19% of ULNR) ($p<0.01$) levels. Basal GFR was normal and significantly declined from 121 ± 25 to 99 ± 17 ml/min/1.73m2 ($p =0.05$) in controlled patients. After GH/IGF-1 control, plasma total and ionized calcium, phosphate levels, urinary calcium and phosphaturia threshold decreased significantly. Osteocalcin and crosslaps levels were lower after control of GH/IGF-1: at diagnosis after control p plasma total calcium (mmol/l) 2.41 ± 0.06 2.34 ± 0.08 0.02 ionized calcium (mmol/l) 1.26 ± 0.06 1.20 ± 0.05 0.01 phosphate (mmol/l) 1.20 ± 0.16 1.04 ± 0.15 0.03 urinary calcium (mmol/d) 7.34 ± 4.03 3.84 ± 2.27 0.03 phosphaturia threshold (mmol/l) 1.23 ± 0.27 0.92 ± 0.20 0.02 osteocalcin (ng/ml) 52 ± 19 18 ± 8 0.05 crosslaps (pg/ml) 1003 ± 395 443 ± 445 0.01 IGF-1 was positively correlated to plasma ($r =0.4$, $p <0.05$) and urinary ($r =0.56$, $p <0.005$) calcium, phosphaturia threshold ($r =0.41$, $p <0.05$) and bone turnover makers ($r =0.565$ and 0.623, $p <0.005$). GH was positively correlated to urinary phosphaturia threshold ($r =0.596$, $p <0.05$) and bone turnover parameters ($r =0.635$ and 0.595, $p <0.05$). Conclusion: This clinical study demonstrates that control of GH/IGF-1 hypersecretion influences renal function, bone turnover and calcium homeostasis parameters in patients with acromegaly. Nothing to Disclose: SG, CV, IT, DC, AB, PC

Pub #	P1-419
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Acromegaly (1:30 PM-3:30 PM)
Title	Diabetes Mellitus and Acromegaly: Description of a Cohort
Author String	F Costenaro, TC Rodrigues, D Fedrizzi, MD Oliveira, PBd Lima, V Boschi, MA Czepielewski Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil; Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil; Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil
Body	The human growth hormone (GH) is deeply known for its actions related to the metabolism of glucose, lipids and proteins. In situations of excess of GH, particularly in Acromegaly, a diabetes state develops and it can be attributed to reduction of insulin receptor number, or insulin resistance or a post-receptor defect. Diabetes Mellitus (DM) prevalence in acromegalic patients is estimated between 19 and 56%, and its control is mainly associated to the activity of Acromegaly, besides its relation to factors like age, gender, drugs, hypertension and prolonged time as an acromegalic disease carrier. Our main aim was to describe the frequency of DM and the characteristic of an acromegalic patients' cohort followed at an university tertiary hospital. A Cross-sectional study was performed to analyze the metabolic profile of acromegalic patients from the neuroendocrinology outpatient service, using as disease cure the following criteria: IGF1 minor than the upper limit for gender and age; and GH nadir minor than 1ng/dl during oral tolerance test with 75g glucose in the absence of medicines to control Acromegaly. Disease remission was defined when the patient had the normal IGF1 for gender and age using the appropriate medicine of Acromegaly. *IGF-1* was measured by immunoradiometric method and GH by chemiluminescence. There were 59 patients with Acromegaly, 54% women, with a mean age of 55±12 years old. At the moment of this analysis, only 24% of the patients met criteria for cure, 25.4% were in remission and the remaining had active disease. Twenty-two acromegalic patients had diabetes, mean HbA1c of 7.34 ± 2.2%, 10 of them were using oral hypoglycemic drugs. Nineteen patients with DM didn't meet cure criteria. Furthermore, patients with DM were more frequently hypertensive [16/22 (73%) vs. 17/37 (46%), $p = 0.04$] and were on statins [14/22 (64%) vs. 8 / 37 (21%), $p = 0.004$] than patients without diabetes. From cohort's patients, 56% had hypertension and 80% of them have not been cured. After multiple regression analysis, the presence of diabetes was the only factor associated to active Acromegaly [odds ratio: 17.40 (95% CI: 1.08 - 28.0), $p = 0.04$]. This association was independent of age, IGF-1 or GH levels, hypertension and triglycerides level adjustments. Diabetes was frequent among patients with Acromegaly and it was closely related to the control of the underlying disease. Nothing to Disclose: FC, TCR, DF, MDO, PBdL, VB, MAC

Pub #	P1-420
Session Information	POSTER SESSION: TRANSLATIONAL - Pituitary Neoplasia (1:30 PM-3:30 PM)
Title	Evaluation of New Possible Molecular Predictors for Clinical Response to Somatostatin Analog Treatment in Patients with GH-Secreting Adenomas
Author String	F Gatto, RA Feelders, D Ferone, DM Sprij-Mooij, AM Waaijers, PM van Koetsveld, JM Kros, SJ Neggers, SW Lamberts, WW de Herder, LJ Hofland Erasmus Medical Center, Rotterdam, Netherlands; University of Genova, Genova, Italy; University of Genova, Genova, Italy
Body	**Background.** About 60% of patients with GH-secreting pituitary adenomas show a long-term sustained responsiveness to somatostatin analogue (SSA) treatment. No signs of tachyphylaxis to treatment are observed. A number of cellular proteins, such as G-protein coupled receptor (GPCR) kinases (GRK1 and GRK2) and β-arrestins (1 and 2), are involved in desensitization and internalization of the SSTR receptor. Moreover, recent studies have identified other interacting proteins, such as GASP, SNX-1, as well as NSF, that interact with the carboxyl terminus of GPCRs and contribute to receptor sorting decision (recycling or degradation). Overexpression or underrepresentation of these proteins in tumors may affect responsiveness to SSA treatment. **Methods.** We evaluated in 31GH-secreting pituitary adenomas from acromegalic patients the expression of SSTRs (except the subtype 4), the dopamine D_2 receptor, as well as GRK1, GRK2, β-arrestin1, β-arrestin2, GASP, SNX-1 and NSF at mRNA level (quantitative RT-PCR). These data were correlated with the clinical response (circulating IGF-I and GH levels) after short (6 h) and long term treatment with SSA. **Results.** D_2 was expressed at highest level, followed by sst_5 and sst_2. Sst_1 and sst_3 were the least represented. A direct significant correlation (P=0.05) was found between sst_2 expression and the reduction of GH levels after octreotide short term treatment. On the contrary, β-arrestin1 mRNA was significantly and inversely correlated (P=0.01) with the lowering of GH levels. Moreover, we observed a significant correlation (P=0.03) between the octreotide test results and the response of patients after long term treatment (IGF-I levels, expressed as ULN (upper limit of normal)). We observed a significant and inverse correlation between β-arrestin1 (P=0.05) and D_2 (P=0.02) and the reduction of IGF- levels (delta IGF-I) after long term treatment. Moreover, in the group of patients treated for more than 3 months, we observed a direct significant correlatior (P=0.03) between sst_2 mRNA and clinical responsiveness (IGF-I ULN). On the other hand, the expression of GASP was significantly correlated with a worse clinical response. **Conclusion.** These data suggest a possible role of these intracellular proteins interacting with SSTR and D_2 receptor trafficking, such as β-arrestins, in the pathophysiology of GH-secreting adenomas. Measurement of β-arrestin1 and GASP expression may predict long-term response to SSA treatment in acromegaly. Nothing to Disclose: FG, RAF, DF, DMS-M, AMW, PMvK, JMK, SJN, SWL, WWdH, LJH

Pub #	P1-421
Session Information	POSTER SESSION: TRANSLATIONAL - Pituitary Neoplasia (1:30 PM-3:30 PM)
Title	Pituitary Tumor-Transforming Gene (PTTG) as a Novel Marker of Testicular Germ Cell Tumors
Author String	D Milardi, F Pierconti, M Martini, G Grande, T Cenci, A Bianchi, L Tartaglione, G Gulino, G Schinzari, G Mantini, A Pontecorvi, G Rindi, L De Marinis Catholic University, School of Medicine, Rome, Italy; Catholic University, School of Medicine, Rome, Italy; Catholic University, School of Medicine, Rome, Italy; Catholic University, School of Medicine, Rome, Italy; Catholic University, School of Medicine, Rome, Italy; Catholic University, School of Medicine, Rome, Italy
Body	The genomic instability and reduced ability to repair DNA are unique characteristics of germ cell tumours. The pituitary tumour-transforming gene (PTTG) is the major effector of chromosome segregation during mitosis and protects the cell from possible cellular aneuploidy. Overexpression of PTTG has been reported in different endocrine tumors, but has been poorly investigated in germ cell tumors of the testis. We evaluated PTTG immunohistochemical expression in normal testicular tissue, in testicular seminoma and intratubular germ neoplasia unclassified (IGCNU). 28 male patients (18-61 yrs) underwent therapeutic orchiectomy for seminoma (n = 3), seminoma associated with IGCNU (n = 23), IGCNU isolated (n = 2) were studied. Four patients with seminoma had history of cryptorchidism. Two sample of the healthy part of the tumor tissue at a distance of 2 cm and two normal testis by orchiectomy for penis cancer were also used as controls. SPM210 (Santa Cruz Biotechnology, CA, USA) was used as primary antibody monoclonal anti-PTTG, which recognizes the whole protein PTTG. The percentage of PTTG-positive neoplastic cells was evaluated. In normal testicular tissue spermatocytes and round spermatids showed intense staining for PTTG. In IGCNU PTTG showed only focal positivity (20%) of the total neoplastic cellular elements, close to tubular basal membrane. In all seminoma tissues was observed a significant increase in the percentage of immunoreactive elements at the tumor periphery compared to the central compartment, where there was a lower percentage of PTTG-positive elements (29.10±3.2 vs 7.8±0.8%, $p<0.001$). We reported for the first time a PTTG-positive staining in IGCNU, identificating a subclone of IGCNU cells that might be involved in the early stages of testicular tumorigenesis or might represent pluripotent stem cells able to regenerate tumour cells. The finding of a subgroup of PTTG-positive elements in seminoma periphery might identify a population of pluripotent stem cell located on the "front of invasion", which could regenerate the tumor elements and activate the expression of other mediators of neoplastic infiltration. The identification of PTTG in IGCNU might offer a new marker in clinical practise and the specific PTTG-pattern of expression in seminoma support the molecular role in testicular germ cell oncogenesis. Nothing to Disclose: DM, FP, MM, GG, TC, AB, LT, GG, GS, GM, AP, GR, LDM

Pub #	P1-422
Session Information	POSTER SESSION: TRANSLATIONAL - Pituitary Neoplasia (1:30 PM-3:30 PM)
Title	Somatotroph-Specific Deletion of Aryl Hydrocarbon Receptor Interacting Protein Yields Pituitary Tumorigenesis
Author String	CR Ku, MP Gillam, R Kineman, EJ Lee Yonsei University College of Medicine, Seoul, Korea; Northwestern University, Feinberg School of Medicine, Chicago, IL; University of Illinois, Chicago, IL; Northwestern University, Feinberg School of Medicine, Chicago, IL
Body	Germline mutations of aryl hydrocarbon receptor interacting protein (*AIP*) gene have been identified about 30% in a subset of individuals with familial pituitary adenoma. The majority of such mutations result in a truncated and presumably dysfunctional protein, suggesting that *Aip* serves as a tumor suppressor gene. To investigate the *in vivo* role of *Aip* in pituitary tumoriogenesis, we generated somatotroph-specific *Aip* knock out (sAIPKO) mice using Cre-loxp strategy. Mice lacking Aip in pituitary somatotrophs were created by crossing mice with loxp sites flanking exons 5-7 of the *Aip* gene ($Aip^{lox/lox}$) with transgenic mice expressing the Cre-recombinase under control of the rat growth hormone (GH) promoter ($rGHp\text{-}Cre^{tg}$). The targeted deletion of exons 5-7 of the *Aip* gene leads to deletion of the tetratricopeptide repeat (TPR) domains, an area involved in protein-protein interactions, and a region encompassing a hot spot mutation site in the corresponding human genome. Absence of *Aip* expression in somatotrophs of sAIPKO mice was confirmed by immunofluorescence analysis. $Aip^{lox/lox}$ and $rGHp\text{-}Cre^{tg}$ $Aip^{lox/lox}$ (sAIPKO) mice exhibit normal embryonic and postnatal development, and display an unaltered distribution of anterior pituitary cell types. At 48 weeks old age, sAIPKO mice had significantly larger body weight than $Aip^{lox/lox}$ littermate mice (control) ($p<0.001$). Overall, 40% of sAIPKO mice developed pituitary adenomas by 6 months of age; 66% and 33% were of lactotroph and somatotroph origin, respectively. The incidences of pituitary tumor were similar among males and females. sAIPKO mice had no morphologic abnormality in other neuroendocrine organs. On histological examination, pituitary adenomas of sAIPKO mice appeared highly invasive, and demonstrated elevated estrogen receptor (ER) beta, but not ER alpha, expression. Tumorous cells of sAIPKO mice had an increase in the percentage of Ki67 antigen positive population, as compared to $Aip^{lox/lox}$ tissue. There was no evidence of somatotroph or lactotroph hyperplasia adjacent to tumorous tissue. Somatotroph-specific knock out of *Aip* gene induces pituitary tumor in mice, which might involve altered expression of ER beta. Further investigation for the utility of sAIPKO mice in the pathogenesis and treatment of pituitary adenoma will be needed.

Sources of Research Support: NIH K08 DK066044.

Nothing to Disclose: CRK, MPG, RK, EJL

Pub #	P1-423
Session Information	POSTER SESSION: TRANSLATIONAL - Pituitary Neoplasia (1:30 PM-3:30 PM)
Title	Cadherin Changes in Human Pituitary Adenomas Can Be Reproduced in cKO-Men1 and HMGA2 Mouse Models
Author String	N Chauvet, E Galibert, A-C Meunier, V Rigau, G Osterstock, E Baccino, M Fedele, A Fusco, CX Zhang, P L Tissier, A Barlier, P Mollard, N Coutry Institute of Functional Genomics, Montpellier, France; INSERM, Montpellier, France; CNRS, Montpellier, France; University of Montpellier 1 and 2, Montpellier, France; Centre Hospitalier Universitaire Hopital Guy de Chauliac, Montpellier, France; Centre Hospitalier Universitaire Hopital Lapeyronie, Montpellier, France; CNR, Napoli, Italy; Universita degli Studi di Napoli Federico II, Napoli, Italy; CNRS, Lyon, France; University Claude Bernard, Lyon, France; National Institute for Medical Research, London, UK; CNRS, Marseille, France; Centre Hospitalier Universitaire Timone, Marseille, France
Body	The anterior pituitary gland is a highly organised organ with homotypic cellular networks that may optimize communication within the gland (1). The development and fate of these pituitary cell networks may involve cell-cell contact signaling based on a defined pattern of cadherin expression (2). In a wide variety of human malignancies, progression to neoplasia and tumor cell invasion is associated with reduced adhesiveness between cells. The involvement of adhesion molecules in the pathogenesis of pituitary adenomas has been recently highlighted (3). The aims of this study were : 1/ to determine cadherin (Cad) repertoire in human hypophysis samples ; 2/ to assess whether this repertoire could be altered both in human GH or PRL-secreting pituitary tumors, and in animal models developing pituitary adenomas. We performed an exhaustive screening of classical cadherins (21 members) by qPCR on normal human pituitaries. Cadherin repertoire was simplified compared to mouse pituitary since only E-Cad, N-Cad and Cad18 were expressed in human hypophysis (mouse repertoire involved also Cad8 and Cad11). By immunohistochemistry performed on human pituitaries we found N-Cad in corticotrophs, gonadotrophs and thyrotrophs, whilst we detected E-Cad and Cad18 mainly in GH and PRL cells. Interestingly, in mouse pituitary, there is a developmental switch from E-Cad to N-Cad in both GH and PRL cells so that they only express N-Cad and Cad18 in adulthood (2). In human pituitary GH-secreting tumors, the decrease in E-Cad mRNA levels was correlated to tumor invasiveness, while Cad18 mRNA was maintained. In non invasive prolactinomas, E-Cad levels were usually lowered whilst Cad18 did not change. By contrast, in invasive prolactinomas, Cad18 was either up-regulated or unchanged with a concomitant shift between E-Cad and N-Cad. We have used 2 different animal models to assess cadherin repertoire during pituitary tumorigenesis: one in which Men1 gene is inactivated in prolactin cells (developing prolactinomas), and another one in which HMGA2 protein is ubiquitously expressed (GH and PRL-secreting tumors). In both models, N-Cad mRNA levels were strongly reduced, while Cad18 mRNA levels were markedly enhanced. The latter results resembled those observed in both human GH-secreting tumors and non invasive prolactinomas, suggesting that these animal models may be useful to study mechanisms of cell-cell communications, which may be involved in the pathogenesis of pituitary adenomas. (1) Bonnefont X et al. Revealing the large-scale network organization of growth hormone-secreting cells. Pro Natl Acad Sci U S A. 2005 Nov 15;102(46):16880-5. (2) Chauvet N. et al. Characterization of adherens junction protein expression and localization in pituitary cell networks. J Endocrinol. 2009 Sep;202(3):375-87. (3) Ezzat S, Asa SL. The molecular pathogenetic role of cell adhesion in endocrine neoplasia. J Clin Pathol. 2005 Nov;58(11):1121-5. Nothing to Disclose: NC, EG, A-CM, VR, GO, EB, MF, AF, CXZ, PLT, AB, PM, NC

Pub #	P1-424
Session Information	POSTER SESSION: TRANSLATIONAL - Pituitary Neoplasia (1:30 PM-3:30 PM)
Title	A Novel AIP Mutation Related to Familial Isolated Pituitary Adenomas (FIPA)
Author String	I Bilbao Garay, M Alvarez Coca, AF Daly, A Beckers, M Goena Donostia Hospital, San Sebastian, Spain; University of Li[egrave]ge, Li[egrave]ge, Belgium
Body	Introduction: It has been estimated that 15-20% of FIPA families harbor an AIP gene mutation (AIPmut) (1). To our knowledge approximately 50 sequence variants -pathological and otherwise- have been described to date. We report a new FIPA family with an extensive genealogy, in which 4 members have pituitary adenomas in the setting of a novel AIPmut. Patients and Methods: The index patient is a 37 year old man, who presented with childhood onset of somatotropinoma and underwent surgery and radiotherapy at the age of 17. His uncle (now aged 70 years), was diagnosed with a somatotropinoma at the age of 16, and also treated with surgery and radiotherapy. The sister of this latter man (the 72 year old aunt of the index case) presented with secondary amenorrea at age 18, and was later diagnosed with a macroadenoma at the age of 48 after she complained of visual disturbances. She also underwent surgery and radiotherapy. The fourth affected member is the 65 year old cousin of the latter two patients. He is 1,97cm tall, with a long standing hypogonadal phenotype; he had hypopituitarism affecting the gonadal, thyroid and somatotrope axes. His MRI shows a wide sella with an eroded floor, and there is a clinical suggestion of potential apoplexy in the past. A genealogic tree was drawn and due to the FIPA presentation, germline AIP sequencing was performed and showed all four affected subjects to have a novel c.543delT AIPmut, which would predict a truncated AIP protein. Conclusions : This 4-member AIPmut positive FIPA family with a presentation that typifies the range of clinical scenarios encountered in AIPmut carriers with pituitary adenomas (2). As the disease presentation was quite aggressive in these cases and as early intervention has appeared to control disease in some of the individuals, we are currently screening for AIPmut carriers among the family. (1) Daly AF et al J Clin Endocrinol Metab 92: 1891-1896, 2007 (2) Daly AF et al J Clin Endocrinol Metab 95: E373-E383, 2010 Nothing to Disclose: IBG, MAC, AFD, AB, MG

Pub #	P1-425
Session Information	POSTER SESSION: TRANSLATIONAL - Pituitary Neoplasia (1:30 PM-3:30 PM)
Title	Multifunctional Cells in Human Pituitary Are Involved in Pituitary Tumor Genesis -- Pathological Analysis o Microadenomas in the Early Stage Diagnosed by 3T-MRI Fusion MET-PET Scans
Author String	H Ikeda, K Watanabe Research Institute for Pituitary Disease, Southern Tohoku General Hospital, Koriyama, Japan; Research Institute for Neuroscience, Koriyama, Japan

Body

Objective: The value of composite images of MET-PET fusion 3T-MRI in the early diagnosis of both Cushing's disease and acromegaly were reported (Ikeda,2010). To clarify tumor genesis of pituitary microadenoma(MIC), we pathologically analyzed the early stage of MICs, which were diagnosed by methionine (MET)-PET fusion 3T-MRI.

Materials and Methods: Among 300 patients who underwent transsphenoidal surgery in the last 3 years, MRI failed to detect localization of MIC in 28 patients but it was successfully detected by MET-PET fusion images. We investigated 28 patients including 14 cases of Cushing's disease, six cases of GH-secreting adenomas, two cases of TSH-secreting adenomas, and six cases of non-hormone secreting adenomas. Images of 3.0 T MRI, composite images from FDG-PET or MET-PET and 3.0 T MRI were compared with the localization of adenomas verified by surgery. MR imaging and PET images were coregistered using a software workstation. Plasma ACTH, cortisol, GH, IGF-1, PRL, FSH beta, LH beta, TSH beta, free T4 concentrations were measured. CRH and DDAVP loading test, and 1 mg and 8 mg dexamethasone suppression tests were performed. A 75 g-oGTT test and triple combination (CRH, TRH, LHRH) test were also applied in some cases. Surgical specimens were fixed in formalin and stained with HE. Immunohistochemical staining was performed using antibodies raised against ACTH, GH, PRL, TSH, LH-β, FSH-β, Ki67, and keratin.

Results: Twenty-five (86%) out of 29 MIC were multihormone-producing adenomas.
(1) Three out of 14 Cushing's disease showed only ACTH production, but there was production of 1, 2, 3, 4, and 5 other hormones in 2, 4, 2, 3, and 1 cases, respectively. (2) Among 6 cases of GH-producing adenomas, there was no single GH-producing adenoma. There was production of 2, 3, and 4 other hormones in 1, 3, and 1 cases, respectively.
(3)None of TSH-producing adenomas produced only TSH. Two other hormone production were found in 1 case and 5 other hormones were found in 1 case. (4) All cases of non-secreting adenomas showed hormone-production, with 4 types of hormones produced in 4 cases and 5 types of hormones in 2 cases.

Conclusion: MIC have a high cellularity and can produce a large amount of multiple hormones per tumor cell resulting in high amino acid turn over, compared with normal anterior pituitary cells. This is the reason why MET uptake in microadenomas reflects such a high uptake enabling a highly sensitive detection of MIC in MET-PET scans.

(1) Ikeda, H; Abe,T; Watanabe, K. Usefulness of composite methionine-positron emission tomography/3.0-tesla magnetic resonance imaging to detect the localization and extent of early-stage Cushing adenoma. J Neurosurg, 2010 112:750-55.

Nothing to Disclose: HI, KW

Pub #	P1-426
Session Information	POSTER SESSION: TRANSLATIONAL - Pituitary Neoplasia (1:30 PM-3:30 PM)
Title	Molecular Markers as Prognostic Indicators of the Behavior of Pituitary Adenomas
Author String	R Sanchez-Ortiga, L Sanchez-Tejada, O Moreno-Perez, M Niveiro, C Fajardo, A Pico Hospital General Universitario Alicante, Alicante, Spain; Hospital General Universitario Alicante, Alicante, Spain; Hospital General Universitario Alicante, Alicante, Spain; Hospital La Ribera, Alzira, Spain
Body	**Background**: There is an increasing interest in finding specific prognostic markers to predict pituitary adenomas (PA) that will behave aggressively. The most studied marker has been immunohistochemical staining for Ki-67. This study evaluated the prognostic capability of molecular study of growth factors (IGF1R), angiogenic factors (vascular endothelial growth factor [VEGF] and its receptor KDR) and proto-oncogen (PTTG) versus the immunohistochemical expression of Ki-67 in PA. **Design**: In this retrospective descriptive study we analyzed 46 human PA samples: 27 gonadotrophic (GT), 4 corticotrophic (CT), 10 somatotrophic (ST), 3 lactotrophic (LT), 1 thyrotrophic (TT) and 1 null-cell adenomas, based on OMS 2004 classification. We evaluated VEGF, KDR, PTTG and IGFIR mRNA expression by quantitative real-time polymerase chain reaction using Taqman technology and TaqMan[reg] gene expression assays (Applied Biosystems). We use standard curves to estimate the copy number normalized (Cnn) of molecular markers. Immunohistochemical staining was performed in whole sections for Ki-67 (cut-off 3%). In addition we revised the extension in magnetic resonance imaging pre-surgically and recorded sex (men 54.3%, women 45.7%), age (52.8 ± 14.9 years) and pre-surgical treatment (somatostatin analogs (SSa): 5/10 ST; dopamine agonist (DA): 1/3 LT). U Mann-Whitney or Student t and Kruskal-Wallis or ANOVA tests were used for statistical analysis. **Results**: VEGF and IGF1R expression differs significantly between the different histological subtypes (p=0.036 and p=0.003 respectively). There were no statistically significant differences in the expression of Ki 67, KDR and PTTG between different subtypes. Invasive tumors had more VEGF expression than non-invasive (0.57Cnn [P_{25}-P_{75}: 0.29-0.73] vs. 0.20 Cnn [P_{25}-P_{75}: 0.13-0.21], p=0.008). Also invasiveness on MRI showed association with PTTG expression (0.06 Cnn [P_{25}-P_{75}: 0.0-0.09] vs. 0.08 Cnn [P_{25}-P_{75}: 0.07-0.14], p = 0.049). The others markers studied weren't able to identify invasive tumors. None of these markers were modified after treatment with SSa or DA. **Conclusions**: Molecular markers may be useful in the management of pituitary adenomas. However, more studies are needed to predict the specific behaviour of different subtypes of pituitary adenomas. Sources of Research Support: Pfizer. Nothing to Disclose: RS-O, LS-T, OM-P, MN, CF, AP

Pub #	P1-427
Session Information	POSTER SESSION: TRANSLATIONAL - Pituitary Neoplasia (1:30 PM-3:30 PM)
Title	IGF-II Expression Could Be Involved in Tumorigenesis of GSP-Negative Somatotroph Tumors
Author String	AG Diaz, A Barlier, M Kral, AL Germanetti, A Enjalbert, OD Bruno Hospital de Clinicas, Buenos Aires, Argentina; H[ocirc]pital de la Concepcion- Assistance Publique, Marseille, France
Body	Molecular mechanism of tumorigenesis in GH tumours are poorly understood. About forty percent of human somatotroph adenomas contain heterozygous mutations in the GNAS gene that constitutively activate Gs-alpha by substitutions at arginine 201 or glutamine 227. The mutated protein has been named gsp oncogene. In a previous report we have reported on the expression of IGF2 in a selected series of GH tumours. In this work, we tried to find out if there was a correlation between the expression of IGF2 and the presence of gsp oncogene in GH-tumours. We analyzed 29 pathologically confirmed pituitary adenomas of patients with clinical and biochemical acromegaly seen at the Hospital de Clinicas between 2001 and 2008. Tumoral tissues were stored in RNA *later*[reg] at -20[deg]C. Total RNA from tumours was isolated and quantitative real-time reverse transcription-PCR analysis was performed to show the relative expression level of IGF2 in the tumoral tissue by normalization to the expression level of beta glucuronidase (beta Gus). We defined as strong expression of IGF2 when IGF2 copy/beta Gus copy was >1. The mutation of gsp oncogene was searched on cDNA by sequencing after amplifying a fragment encompassing codon 201 and 227 of the Gs-alpha. Ten out of the 29 (34.5%) tumours showed a strong expression of IGF2 (36.1± 14.5 IGF2 copy/ beta Gus copy). Analysis of gsp showed that 11 tumours were gsp (+) (38%) and 18 gsp (-) (62%). Only one of 11 gsp (+) adenomas (9%) expressed high levels of IGF2. In contrast, in gsp (-) adenomas, IGF2 transcript was strongly expressed in 9/18 (50%) (Fisher's Exact Test x^2 p= 0.044). In conclusion, IGF2 overexpression was shown in a substantial proportion of GH- secreting adenomas, being more frequent in gsp (-) tumours. IGF2 might have a role in the initiation or progression of this type of tumours. Nothing to Disclose: AGD, AB, MK, ALG, AE, ODB

Pub #	P1-428
Session Information	POSTER SESSION: TRANSLATIONAL - Pituitary Neoplasia (1:30 PM-3:30 PM)
Title	The FGFR4 Transmembrane Polymorphism Promotes Preferential Signaling To Facilitate Pituitary Growth Hormone Cell Tumorigenesis
Author String	T Tateno, L Zheng, S Asa, S Ezzat Ontario Cancer Institute, Toronto, Canada; Ontario Cancer Institute, Toronto, Canada
Body	**Background**: Fibroblast growth factor receptor 4 (FGFR4) is a member of a family of transmembrane receptors with ligand-induced tyrosine kinase activity which has been implicated in pituitary tumors. A single nucleotide polymorphism (SNP) at codon 388 of FGFR4 substitutes a neutral glycine with a charged arginine residue within the transmembrane domain. This SNP has been associated with increased risk of cancer and progression through unclear mechanisms. Here, we examined the differential properties of the polymorphic variant with wild-type FGFR4 (FGFR4-WT) on cell signaling, hormone production, and pituitary tumor growth. **Methods and Results**: GH4 cells that express growth hormone (GH) and prolactin (PRL) were stably transduced with constructs encoding V5-tagged full length human FGFR4 Gly388 (WT) cDNA or full length human FGFR4 Arg388 (R388) cDNA. The levels of FGFR4, GH and PRL expression, and phosphorylation o FRS2-α, and MAPK were monitored following stimulation with a panel of ligands and through time-courses. Unlike FGFR4-WT cells which supported PRL production, FGFR4-R388 cells potently induced GH expression and colony formation in soft agar. The growth promoting effects of FGFR4-R388 were ascribed to differential Src activation. Using Dastinib as a pharmacologic inhibitor, we show strong preferential growth inhibition of FGFR4-R388, but not FGFR4-WT tumors. We compared GH levels and pituitary tumor size in patients with acromegaly. This examination identified a positive correlation between serum GH levels ($r=0.622$, $p=0.006$) and pituitary tumor size in patients ($n=30$) harboring the FGFR4-R388 genotype. In contrast, a control group of patients ($n=34$) who were FGFR4-WT showed no GH relationship ($r=0.23$; $p=0.468$) with pituitary tumor size. **Conclusion:** These data suggest a potential link between FGFR4 polymorphism, GH hormone production, and pituitary tumorigenesis. Nothing to Disclose: TT, LZ, SA, SE

Pub #	P1-429
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Factors Associated with Delay in Pituitary Adenoma Diagnosis in Patients with Visual Loss
Author String	A Jahangiri, L Blevins, S Kunwar, M Aghi University of California, San Francisco, San Francisco, CA
Body	INTRODUCTION: Duration of visual symptoms associated with nonfunctioning pituitary adenoma (NFA) is a predictive factor for chances of visual improvement. We investigated factors associated with increased duration of visual symptoms in NFA patients METHODS: We retrospectively reviewed NFAs resected at our institution 2004-2010 for duration of visual symptoms, postoperative improvement, and associated factors. RESULTS: 75 patients underwent NFA resection with median visual symptom duration 6.5 months (range 1 week-15 years). Return to baseline vision postoperatively occurred in 44% and 18% of patients with visual symptoms for under and over 6 months, respectively (P=0.03). Univariate nonparametric analyses investigating age of symptom onset, gender, race, insurance, ophthalmologic conditions, income, marital status, ER admission, language, and medical provider found that age was the only variable significantly (P=0.04) prolonging symptom duration, a finding confirmed by multivariate analysis (P=0.03), with race and the presence of ophthalmologic conditions exerting effects that were not quite statistically significant in univariate (P=0.07) and multivariate (P=0.09) analyses. Patients aged 20-39, 40-59, and 60-79 had mean symptom duration of 4, 7, and 12 months, respectively. Seven older patients had symptoms attributed to pre-existing ophthalmologic conditions for a median of 18 months before NFA diagnosis. Amongst age and race subgroups, the largest difference in median symptom duration was between white patients aged 60-79 (5 months) and non-white patients aged 60-79 (24 months) (P=0.04). A logistic regression showed that duration of symptoms, but not age at surgery, predicted postoperative normalization of vision. CONCLUSIONS: We found that older age was associated with delayed NFA diagnosis in visually impaired patients. Contributing factors were the attributing of visual symptoms from NFAs to other ophthalmologic conditions in these patients, and delayed presentation in elderly non-white patients. These findings highlight challenges associated with timely NFA diagnosis in visually impaired patients, a key factor in chances of improvement. Nothing to Disclose: AJ, LB, SK, MA

Pub #	P1-430
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Red Desaturation and Visual Field Deficits in Detecting Chiasmopathy in Patients with Nonfunctioning Pituitary Macroadenoma
Author String	E Shortridge, B Kim, W White, A Little, K Chapple, L Knecht University of Arizona, Phoenix, AZ; Gavin Herbert Eye Institute, University of California, Irvine, CA; Barrow Neurological Institute, Phoenix, AZ; St Joseph's Hospital and Medical Center, Phoenix, AZ
Body	Introduction Nonfunctioning pituitary macroadenomas are slow-growing tumors that can be asymptomatic, or produce mass effects such as headache and visual field loss. Visual deficit is one scenario requiring immediate neurosurgical evaluation. While neuroimaging is necessary for diagnosis, simple and reliable bedside tests are needed to assess a patient's risk for functional compression of the optic chiasm. Confrontation visual fields (CVF) and red desaturation tests are frequently implemented, but data indicating accuracy are limited and inconclusive. We characterized one institute's experience in determining the clinical utility of these tests. Methods In this retrospective case-series, a total of 191 patients with nonfunctioning pituitary macroadenoma underwent transsphenoidal resection between Jan 2005 and Jun 2010 at Barrow Neurological Institute. Macroadenomas were diagnosed by MRI and classified as suprasellar with or without displacement of the chiasm. Patients were assessed for CVF deficit and red desaturation, with non-emergent cases sent for Humphrey visual field (HVF) assessment. Statistical analysis included patients whose pattern of formal visual field loss was consistent with a central chiasmopathy not confounded by other visual pathology. Data were evaluated with Chi-squared analysis and ANOVA. Results Red desaturation detected chiasm displacement with 64.7% sensitivity and 74.5% specificity ($p<.001$), while CVF detected displacement with 23.7% sensitivity and 96% specificity ($p<.01$). HVF deficits were present in 74.5% of patients with chiasm displacement on MRI, with 80% specificity ($p=.01$). Red desaturation occurred in 75.3% of patients with HVF deficit ($p<.001$), whereas CVF deficit occurred in 33.8%. ANOVA comparison of the three visual tests showed that confrontation deficit is less likely to detect chiasm displacement than red desaturation ($p<.001$) or HVF ($p<.001$) tests. ANOVA also showed no significant difference in displacement detection rates between red desaturation and HVF tests. Conclusion Red desaturation testing can be used to assess visual deficit and chiasmopathy with similar sensitivity as with HVF testing in patients presenting with nonfunctioning pituitary macroadenoma. Both tests are superior to CVF testing in detecting chiasmopathy and in predicting formal field deficit. Red desaturation testing can be performed quickly and easily, lending itself to rapid assessment of visual threat and need for surgical evaluation. Nothing to Disclose: ES, BK, WW, AL, KC, LK

Pub #	P1-431
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Correlation between MRI Findings and Histology in Clinically Nonfunctioning Pituitary Adenomas
Author String	H Nishioka, N Inoshita, T Sano, N Fukuhara, S Yamada Toranomon Hospital, Tokyo, Japan; Toranomon Hospital, Tokyo, Japan
Body	Objective Clinically nonfunctioning pituitary adenomas (NFPAs) consist of several histologic subtypes including null cell adenoma (NC), silent gonadotroph cell adenoma (SG), silent corticotroph adenoma (SC), and Pit-1 derived (GH, prolactin, TSH) silent adenoma (SP). The aim of this study is to detect a possible correlation between MRI findings and the histology in various types of NFPAs. Materials We retrospectively studied 390 consecutive patients with NFPA who underwent surgery at Toranomon Hospital between 2008 and 2010. Based on their histologic findings, they were classified into 3 groups: NC&SG 313 cases, SC 39, and SP 36, except 2 diagnosed as plurihormonal adenoma. In addition to age, gender, and MIB-1 index, the following MRI findings were assessed: adenoma size (giant >40mm), configuration of the suprasellar tumor (smooth or lobulated), and invasion to the cavernous sinus (Knosp grade 4; CSI). Results Giant adenoma, lobulated configuration of the tumor extended into suprasellar cistern, and CSI were significantly more common in SC and SP group than NC&SG group. When each adenoma was scored by the presence of these findings from [ldquo]3[rdquo] (all three findings positive) to [ldquo]0[rdquo] (none positive), 76.7% of adenomas in NC&SG group did not show any of these three findings (score [ldquo]0 [rdquo]). Higher score was associated with a decreased frequency of NC&SG group and an increased frequency of SC and SP group (P<0.0001). 87.3% of score [ldquo]0[rdquo] NFPAs were NC&SG group, whereas 52.0% of score [ldquo]3[rdquo] NFPAs were SC and SP group. However, these MRI findings did no exhibit any correlation with MIB-1 index. In addition, there was no significant correlation between MIB-1 index and histology of NFPAs. Regarding with other factors, patients in SP group were significantly younger than patients belonging to other histologic types (P<0.001). Conclusions The three MRI findings (giant in size, lobulated suprasellar tumor, and CSI) are generally considered to indicate biological aggressiveness of the adenoma and our current results suggest that SC and SP behave more aggressively than other types of NFPAs, although MIB-1 index did not show difference between them. Moreover, preoperative MRI can be useful in predicting the histology of NFPAs. Nothing to Disclose: HN, NI, TS, NF, SY

Pub #	P1-432
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Efficacy of External Beam Radiation Therapy in Nonfunctioning Pituitary Adenomas: A Case-Control Study
Author String	G Vargas, B Gonzalez, C Ramirez, M Mercado Centro Medico Nacional, S XXI, Instituto Mexicano del Seguro Social, Mexico City, Mexico
Body	Introduction: The treatment of choice for non-functioning pituitary adenomas (NFPA) is surgery. However, there is no clear consensus as to how to manage patients with residual tumor. Objective: To evaluate the long-term outcome of postoperative external beam radiotherapy (XRT) in patients with NFPA with residual tumor using a case-control approach. Patients and methods: A group of patients with NFPA who underwent transsphenoidal surgery (TSS) once an who received XRT were compared to a control group of subjects who did not receive XRT. The control grou was matched for age, gender and tumor volume. Outcomes analyzed were changes in tumor volume by MRI as well as incidence of pituitary hormone deficiencies at 3, 5 and 10 years of follow up. Results: The XRT group consisted of 51 subjects with a mean age of 56.21 ± 11.7 years, 45% of whom were women; mean follow up was 5.9 years. The control group included 62 patients with a mean age of 53.4 ± 12.3 years and was followed for a mean of 5 years; 46% were women. Among the XRT group, the mean tumor volume at diagnosis was 14130 mm3 and decreased to 1601 mm3 after TSS, right before initiation of XRT. Mean tumor volume decreased further to 1124 mm3 (p=0.002), 816 mm3 (p=0.01) and 188 mm3 (p=0.003) after 3, 5 and 10 of XRT. In the control group, the mean tumor volume after TSS was 1415 mm3; at 5 years o follow up it had not changed significantly (1204 mm3, p=0.93). Radiated patients showed a 50% tumor shrinkage whereas controls had a tumor reduction of only 15%. Among the XRT group, 71% of patients had hypothyroidism, 53% had hypocortisolism, 73% had hypogonadism and 49 % had panhypopituitarism immediately before radiation; these percentages remained unchanged after 3 and 5 years of XRT. In the control group 43% were hypothyroid, 24% were hypocortisolic, 40% were hypogonadal and 17% had panhypopituitarism, after surgery; these proportions did not change at 5 years of follow up. Conclusion: XRT effectively reduces tumor volume in postoperative patients with NFPAs; we recommend implementing it early in the treatment algorithm of these patients. Nothing to Disclose: GV, BG, CR, MM

Pub #	P1-433
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Efficacy of Long-Term Cabergoline Treatment for Nonfunctioning Pituitary Macroadenomas
Author String	J Ah-Lan, J Lesage, H Beauregard, N Aris-Jilwan, R Comtois, C Beauregard, O Serri, S Vallette Centre Hospitalier de l'Université de Montréal, Montréal, Canada; Centre Hospitalier de l'Université de Montréal, Montréal, Canada
Body	Firstline therapy for nonfunctioning pituitary macroadenomas is usually surgery. In patients without signs of optic chiasm compression or with post-surgical residual tumor, dopamine agonists (such as bromocriptine and cabergoline) have been used to control tumor growth, since most of these adenomas express dopamine receptors. There are several published series of tumor response to bromocriptine treatment, but very few with cabergoline, a more potent dopamine agonist. These studies have shown tumor mass stability or decrease in 70-90% of patients[1], compared to watchful waiting where progression was observed in 40-50% of the cases[2]. Objective: To determine the efficacy of cabergoline therapy in nonfunctioning pituitary macroadenomas. Method and Patients: Retrospective study of treatment outcome of 14 patients (7 males) with a nonfunctioning pituitary macroadenoma treated with cabergoline in our center. Cases were selected from medical records review from 1998 to 2011, and have been treated with cabergoline for a minimal follow-up of 1 year. Cabergoline was used in 10 patients who had undergone partial surgical removal of their macroadenoma, in 2 patients after watchful waiting (1-3 years) and in 2 patients as firstline therapy. No patient had received radiotherapy. Results: Age of the patients at the beginning of cabergoline treatment was 60.9 ± 13.4 yrs (range: 38-83). All tumors were macroadenomas, with an average largest diameter of 25 ± 10 mm (range: 17-48). Before addition of cabergoline, tumor progression has been observed in 10/14 (71%) of the cases, 8/10 patients with residual tumor and the 2 patients followed with watchful waiting. All of our patients received cabergoline at an average dose of 1.93 ± 1 mg per week (range: 0.5-3.5) for a follow-up of 32 ± 23.6 months (range:12-78). There was no tumor progression in our series after the addition of cabergoline. Tumor mass remained stable in 10/14 patients (71.4%). Moreover, in 4/14 patients (28.6%), the tumor size decreased by at least 50% (21.5±1.7 mm before treatment vs 9±3.9 mm after treatment) in a mean period of 52 months (range:18-78). Conclusion: Our study confirms the favourable response of non-functioning pituitary macroadenomas to cabergoline. It may prevent tumor growth in the majority of cases and induce tumor regression in some cases. However, randomised placebo-controlled studies in a larger number of patients are needed to further evaluate long-term efficacy and safety of this treatment. (1) Annamaria Colao et al; Medical therapy for clinically non-functioning pituitary adenomas ; Endocrine-Related Cancer (2008) 15 905-915 (2) Yona Greenman et al; Non-functioning pituitary adenomas; Best Practice & Research Clinical Endocrinology & Metabolism; 23 (2009) 625-638 Nothing to Disclose: JA-L, JL, HB, NA-J, RC, CB, OS, SV

Pub #	P1-434
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Patients Previously Treated for Nonfunctioning Pituitary Macroadenomas Have Disturbed Sleep Characteristics, Circadian Movement Rhythm, and Subjective Sleep Quality
Author String	NR Biermasz, SD Joustra, E Donga, AM Pereira Arias, N Van Duinen, M Van Dijk, AA Van der Klaauw, NPM Corssmit, GJ Lammers, K Van Kralingen, G Van Dijk, JA Romijn Leiden University Medical Center, Leiden, Netherlands; Leiden University Medical Center, Leiden, Netherlands; Leiden University Medical Center, Leiden, Netherlands
Body	Context and objective: Fatigue and excessive sleepiness have been reported after treatment of nonfunctioning pituitary macroadenomas (NFMA). Because these complaints may be caused by disturbed nocturnal sleep, we evaluated objective sleep characteristics in patients treated for NFMA. Design: Controlled cross-sectional study Subjects and Methods: We studied 17 patients (8 women, mean age 54 yr), in remission of NFMA during long-term follow-up (8 yr, range 1-18 yr) after surgery (n=17) and additional radiotherapy (n=5) and 17 controls matched for age, gender, and BMI. Inclusion criteria were age 18-65 yr and absence of other co-morbidity except for hypopituitarism and stable hormone replacement therapy. Sleep was assessed by nocturnal polysomnography (PSG), sleep diary, sleep and diurnal movement patterns by actigraphy during 7 days, and quality of life and subjective sleep characteristics by questionnaires. Results: Total sleep duration was not different between patients and controls during PSG night. However, patients spent more time in bed being awake, than controls (P=0.006). Compared to controls, patients had reduced sleep efficiency (P=0.008), less REM-sleep (17.1% vs 25.4%, P<0.001), 10 % more N1 sleep (P=0.001) and more awakenings, in the absence of excessive apnea or periodic limb movements. Actigraphy revealed a longer time in bed, a longer mean sleep duration (7hr7min vs 6hr20min, P=0.007) and profound disturbances in diurnal movement patterns, with more awakenings at night and less activity during the day. Patients scored higher on fatigue scores, especially increased tiredness and sleepdisturbances, and reported impaired quality of life. Conclusion: Patients previously treated for NFMA suffer from decreased subjective sleep quality, disturbed distribution of sleep stages and disturbed circadian movement rhythm. These observations indicate that altered sleep characteristics may be a factor contributing to impaired quality of life and increased fatigue in patients treated for non-functioning macroadenomas. Sources of Research Support: Netherlands Organization for Health Research and Development(Clinical Fellows no:90700195)awarded to NRB. Nothing to Disclose: NRB, SDJ, ED, AMPA, NVD, MVD, AAVdK, NPMC, GJL, KVK, GVD, JAR

Pub #	P1-435
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Tumors Invading the Cavernous Sinus That Cause Internal Carotid Artery Compression Are Rarely Pituitary Adenomas
Author String	ME Molitch, L Cowen, R Stadiem, A Uihlein, M Naidich, E Russell Northwestern University Feinberg School of Medicine, Chicago, IL; Northwestern University Feinberg School of Medicine, Chicago, IL; Massachusetts General Hospital, Boston, MA; Northwestern University Feinberg School of Medicine, Chicago, IL
Body	Introduction: Magnetic Resonance Imaging (MRI) has become the gold standard for imaging of sellar and parasellar masses. Although there are imaging features that help to differentiate different types of tumors, the diagnosis generally cannot be determined definitively prior to tissue examination. Developing criteria for differentiating pituitary adenomas from other types of sellar/parasellar masses is important because it affects the type of therapy that may be needed. Although there are clinical and literature impressions of endocrinologists, neuroradiologists, and neurosurgeons that, of lesions that invade the cavernous sinus, pituitary adenomas do not cause compression of the lumen of the internal carotid artery while other lesions may do so; however, confirmatory studies have not been performed. The goal of this retrospective study was to establish whether the finding of internal carotid lumen compression on MRI is inconsistent with a diagnosis of a pituitary adenoma. Methods: Using the Northwestern Memorial Hospital Radiology search engine, the search terms [ldquo]invasive mass cavernous mri[rdquo] returned over 100,000 results. Careful investigation revealed that 1650 were relevant to our study and of these, 313 MRIs, representing 178 patients showed a mass involving the cavernous sinus. Diagnostic and other information were not available for 32 of these patients, leaving 146 who were evaluable. Of these, 5 showed no actual invasion and 141 showed cavernous sinus invasion. Results: Of the 141 patients whose MRI scans showed cavernous sinus invasion, 83 had encasement of the internal carotid artery of >50% of the circumference of the artery. 76 of these 83 had no carotid compression (55 pituitary adenomas, 21 other lesions) and 7 had carotid compression (1 pituitary adenoma, 2 meningiomas, 1 paraganglioma, 1 Wegener's granuloma, 2 metastatic cancers). Thus, 1/56 (1.8%) of pituitary adenomas and 6/27 (22.2%) [ldquo]other[rdquo] lesions that encased the internal carotid artery caused compression of the artery (p=0.0042, Fisher's Exact Test). Conclusion: A mass lesion that invades the cavernous sinus and encases the internal carotid artery is very unlikely to be a pituitary adenoma if it compresses the lumen of the internal carotid artery. Nothing to Disclose: MEM, LC, RS, AU, MN, ER

Pub #	P1-436
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Enlargement and T1 Hyperintensity of the Anterior Pituitary Gland in Patients with Carotido-Cavernous Fistula Mimicking What Is Observed in Pregnancy: A Common Mechanism?
Author String	JF Bonneville, F Cattin, NC Billon-Grand, A David, A Biondi, F Schillo, A Penfornis University Hospital, Besan[ccedil]on, France; University Hospital, Besan[ccedil]on, France
Body	Anterior pituitary gland hyperintensity on T1-W MR images is encountered in numerous circumstances : presence of hemorrhage (for instance within a preexisting pituitary adenoma), fat (for instance after surgery), proteins (craniopharyngioma, Rathke cleft cyst) or manganese accumulation (for instance after parenteral feeding or hepatic failure) are responsible for the high signal of the anterior pituitary gland on T1. Anterior pituitary gland hyperintensity is sometimes less clearly understood under some circumstances : in neonates or in pregnancy, an increased proteinic synthesis bound to increased hormonal activity has been advocated. Other explanations could be increased quantity of the endoplasmic reticulum, or increase of the secretory granules. At last, for some authors, hyperintensity of the anterior pituitary gland could be the result of altered intracellular water relaxation. In patients with chronic hepatocellular dysfunction, hyperintensity of the anterior pituitary gland is usually considered related to deposition of paramagnetic substance such as manganese. But absence of strict correlation between manganese plasma level and hyperintensity of the anterior pituitary gland is surprising ; some authors have also advocated here the possible role of altered water relaxation in this phenomenon. We present 6 observations of patients presenting with a carotido-cavernous fistula responsible for an impairment of the venous drainage of the pituitary gland : in all cases, the anterior pituitary gland was enlarged and hyperintense on T1-W simulating what is observed during pregnancy. In all cases treated by embolization, the pattern of the anterior pituitary gland was normalized after treatment. These similarities raise the question of an identical mechanism at the origin of the hyperintensity of anterior pituitary gland observed in different circumstances. Nothing to Disclose: JFB, FC, NCB-G, AD, AB, FS, AP

Pub #	P1-437
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Apoplexy of a Silent Pituitary Adenoma as the Only Presentation of a Novel *AIP* Mutation in a Greek Family with Familial Isolated Pituitary Adenoma
Author String	P Xekouki, A Mastroyannis, C Lysikatos, N Patronas, GP Chrousos, CA Stratakis National Institutes of Health, Bethesda, MD; National Institutes of Health, Bethesda, MD; Children's Hospital Aghia Sophia, University of Athens, Athens, Greece
Body	Patients harboring a *Aryl Hydrocarbon receptor-interacting protein (AIP)* gene mutation usually develop aggressive somatotroph macroadenomas early in childhood. We describe a rare family with FIPA due to a novel *AIP* mutation, in which subclinical apoplexy of pituitary adenomas was the first and only presentation i two out of three affected family members. **The proband a:** A $12^{2/12}$-year-old boy was evaluated because of gigantism. Hormonal and imaging investigation revealed increased growth hormone levels (GH):18.6 ng/ml, not suppressed during an oral glucose tolerance test (OGTT) and the presence of a 1.3[times]1.1 cm pituitary macroadenoma with suprasellar invasion. He underwent transsphenoidal resection of the pituitary adenoma and was placed on long-acting somatostatin analogue (SST). Re-evaluation one year post surgery showed normal GH and IGF-1 levels and adequate GH suppression to OGTT. No deficiencies of the other pituitary hormones were detected. Family history revealed a paternal uncle with a history of acromegaly secondary to growth hormone producing pituitary adenoma for which he was operated on. Genetic testing revealed a novel *AIP* exon 1 mutation (c.3-4insC/p.R2fsX43). The same mutation was detected in his clinically unaffected 41-year old father and the $8^{6/12}$-year old brother. Biochemical investigation of the father revealed normal basic GH and IGF-1 levels (0.1 ng/ml and 130 ng/ml , respectively) with normal response of GH to OGTT. The rest of the hormonal evaluation was unremarkable. Basic GH levels of the younger brother were 0.3 ng/ml with normal response to OGTT, whereas IGF-1 levels were low [<25 ng/ml (n.v: 63-279)]. Extensive history revealed no growth abnormalities. Pituitary MRI of the father and younger brother showed the presence of partially empty sella turcica in both a hypoenhancing left sided 4[times]4[times]3 mm pituitary lesion, most probably representing a microadenoma, was also detected in the pituitary MRI of the younger sibling. This is a rare case kindred with FIPA due to a novel *AIP* mutation. MRI findings of the father and younger sibling demonstrate that patients with *AIP* mutations can present with non-secreting somatotroph pituitary adenomas that can lead to silent pituitary apoplexy and/or a partially empty sella turcica. This may explain the lack of disease in several carriers of AIP muattions; it also underscores the need for, pituitary MRI and follow-up for all *A/P* mutation carriers. Nothing to Disclose: PX, AM, CL, NP, GPC, CAS

Pub #	P1-438
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Pituitary Apoplexy during Pregnancy
Author String	SG Rosen, J Kharlip Pennsylvania Hospital, Philadelphia, PA; University of Pennsylvania School of Medicine, Philadelphia, PA
Body	A 32-year-old woman with no significant medical problems was at week 22 of her first pregnancy. After returning from an overseas trip one week prior to admission, she developed a severe headache followed by recurrent nausea and vomiting. Initial hospital admission revealed serum sodium of 127. She was treated with intravenous saline. Her nausea and vomiting abated. She was discharged following normalization of serum sodium levels. Two days later, she was readmitted with recurrent nausea and vomiting. Physical examination revealed a heart rate of 73 beats/min and a supine blood pressure of 102/57 mmHg, but no localizing neurological signs or visual impairment. Laboratory testing revealed serum sodium and potassium of 122 and 3.0 mM. Urine osmolality and sodium levels were 470 mOsm/kg and 175 mM. Her serum levels of free T4 and free T3 were 0.5 ng/dL (normal range 0.9-1.8 ng/dL) and 1.9 ng/dL (normal range 2.3-4.2 pg/mL); TSH concentration was 0.51 mU/L (normal range 0.35-5.50 mU/L). Her serum levels of prolactin, IGF-I, LH and FSH were 8.1 ng/mL (normal pregnancy range 9.7-208.5 ng/mL), 48 ng/mL (normal range 71-352 ng/mL), <0.07 mIU/mL (normal range <0.1-1.5 mIU/mL) and <0.3 mIU/mL (normal range <0.3 mIU/mL). Her 7 AM serum cortisol concentration was 1.6 mcg/dL (normal range 4.3-22.4 mcg/dL) at which time her plasma ACTH concentration was 3 pg/mL (normal range 6-58 pg/mL). Pituitary MRI scan revealed a 1.37 cm intrasellar mass with a hemorrhagic component. There was a 3 mm clearance to the optic chiasm. Given lack of a threatened vision, the hemorrhagic mass was not evacuated surgically. The patient was managed conservatively with corticosteroid and thyroid replacement with resolution of her symptoms. Pituitary hemorrhage resolved by the end of gestation further decreasing her risk of vision loss from pregnancy-related pituitary enlargement. (Images will be included). Induction was performed at week 39; a healthy female infant was delivered vaginally. Pituitary apoplexy had rarely been described in pregnant patients(1). If unrecognized, it is life-threatening to both mother and fetus. A combination of hyponatremia and headache even in the absence of the visual impairment should raise a concern for pituitary apoplexy and prompt an evaluation of the pituitary function. I the absence of visual impairment, pituitary apoplexy during pregnancy may be managed with pituitary hormone replacement therapy leading to a successful pregnancy outcome. (1) de Hiede LJM et al The Netherlands Journal of Medicine 2004; 62:10 Nothing to Disclose: SGR, JK

Pub #	P1-439
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Necrosis of a Posterior Pituitary Adenoma Causing Syndrome of Inappropriate ADH Secretion
Author String	V Budharaju, JR Chintaparthi, C Kohli, J-H Huang, CA Dolinskas, MM Shangold, SG Rosen Pennsylvania Hospital, Philadelphia, PA; Pennsylvania Hospital, Philadelphia, PA; Pennsylvania Hospital, Philadelphia, PA; Pennsylvania Hospital, Philadelphia, PA
Body	There are very few case reports of posterior pituitary tumors causing the syndrome of inappropriate ADH secretion (SIADH). A 55 year-old woman presented with fatigue, nausea, and frontal headaches for three months. Her medications included fluoxetine, estradiol gel and medroxyprogesterone acetate. Physical examination revealed no orthostatic blood pressure changes, normal thyroid gland, clear lungs, no peripheral edema, and normal neurological examination including visual fields. There were no cushingoid or acromegalic features. Laboratory data showed serum levels of sodium and potassium of 129-135 and 4.7mM, respectively. Her blood urea nitrogen was 11 mg/dL and her serum creatinine concentration was 0.6 mg/dL. Her serum osmolality was 276 mOsm/kg (normal range 278-305 mOsm/kg), urine osmolality was 521 mOsm/kg (normal range 50-1200 mOsm/kg), and urine sodium concentration was 36 mM (normal range 28 - 272 mM). In response to cosyntropin 250 mcg, her serum cortisol concentration increased from 12 to 28 mcg/dL at 60 minutes; her baseline 8 AM plasma ACTH concentration was 8 pg/mL (normal range 5 - 27 pg/mL). Her serum TSH concentration was 1.25 mU/L (normal range 0.40- 4.50 mU/L). Chest CT scan showed a 4 mm right lower lobe lung nodule that did not show hypermetabolic activity on PET scanning. Pituitary MRI scan showed a 1.4 cm posterior pituitary mass. Pituitary evaluation revealed serum levels of prolactin, FSH, and IGF-I of 5.9 ng/mL (normal range 2.0 - 20.0 ng/mL), 38 mIU/mL (normal menopausal range 25.8 - 134.8 mIU/mL), and 160 ng/mL (normal range 92-190 ng/mL), respectively. She underwent transsphenoidal pituitary surgery; histopathological examination of the resected tumor revealed necrotic tissue. Although fluid restriction and demeclocycline were required for several weeks after surgery, her serum sodium concentration and serum and urine osmolality normalized at one month and remained stable on ad libitum fluid intake at her six month follow-up visit. Her anterior pituitary function remained normal. It can be postulated that hemorrhage and/or ischemia in a pre-existing posterior pituitary adenoma resulted in necrosis. Neurohypophyseal tumors should be considered in the differential diagnosis of SIADH especially when other common causes are excluded. In patients with a posterior pituitary adenoma and SIADH, surgical resection is the treatment of choice. Nothing to Disclose: VB, JRC, CK, J-HH, CAD, MMS, SGR

Pub #	P1-440
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Gamma-Knife Stereotactic Radiosurgery: A Cause of Ocular Neuromyotonia?
Author String	WC Sze, HI Sabin, P Blackburn, N Ali, WM Drake St Bartholomews Hospital, London, UK; St Bartholomews Hospital, London, UK; University College Londor Hospital, London, UK
Body	Introduction: Ocular neuromyotonia(ONM) is a rare condition, characterised by transient diplopia and strabismus following an evoked muscle activity. It is believed to be due to axonal hyperexcitation causing impaired relaxation of the extraocular muscles. Of the forty cases reported, two thirds resulted from conventional fractionated radiotherapy to the pituitary/peripituitary area(EBRT). Gamma knife stereotactic radiosurgery(GKS) delivers a single dose of focused radiation and is an emerging tool in the management of complex pituitary disease. Three cases of ONM following GKS have been reported. We present two cases of ONM (with illustrative examination videos/pictures) from our cohort of 70 patients whom have received GKS for pituitary tumours. Case 1: 52 year old lady treated with GKS for a right residual cavernous sinus mass in association with severe pigmentation in the context of Nelson's syndrome. She has a background of Cushing's disease treated with EBRT and bilateral adrenalectomy. The Nelson's syndrome have previously been treated with transphenoidal surgery and stereotactic multi-arc radiotherapy. Five months post-GKS, plasma ACTH levels halved but she developed diplopia on right lateral gaze and ONM was diagnosed. Case 2: 32 year old lady with a large invasive somatotroph adenoma and severe clinical acromegaly underwent debulking pituitary surgery and EBRT. She was unresponsive to dopamine-agonist or somatostatin analogue therapy. Biochemical control was achieved with high dose pegvisomant therapy complicated by severe injection site lipohypertrophy. She underwent GKS, resulting in a substantial fall in pegvisomant requirements. Three months later she developed diplopia on left lateral gaze with the eye getting 'stuck'. Treatment with carbamazepine for a diagnosis of ONM led to substantial improvement. Discussion: We present two cases to illustrate that ONM is not a diagnosis exclusive to whole field pituitary irradiation and may occur following GSK. It is interesting that all of the five reported patients with ONM secondary to GSK are female. Concern regarding neuro-ophthalmic injury due to GKS has largely concerned the radiation dose delivered to the optic chiasm. Although rare, we suggest that ONM should be added to the list of potential complications of GKS. Nothing to Disclose: WCCS, HIS, PB, NA, WMD

Pub #	P1-441
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	A Rare Case of Rapid Growth of a Rathke Cleft Cyst in an Adult Patient
Author String	A Seth, N Needleman, A Busta Beth Israel Medical Center, New York, NY; Beth Israel Medical Center, New York, NY
Body	Background: Rathke's cleft cysts (RCC) are embryologic remnants of the craniopharyngeal ducts that arise from the primitive oral cavity. RCC have been reported in all ages, but symptomatic RCC predominantly occur in females with a peak between the fourth and sixth decades (1, 2). RCC present with a wide variety of clinical and radiological features. RCC are usually asymptomatic. Headaches are often the initial presenting symptom and can occur in 81% of symptomatic cases. Visual disturbances and endocrine abnormalities have been reported to occur in 47% and 30% of symptomatic cases respectively (1). RCC are usually slow growing and smaller than 2 cm in diameter. Here we present a case of a rapidly growing RCC in a previously asymptomatic patient. Clinical Case: A 56 year old woman with a history of multiple sclerosis, optic neuritis, and early menopause was referred to endocrinology for evaluation of a pituitary incidentaloma in 2007. An MRI in November 2006 had shown an intra-sellar (0.6 cm x 1.0 cm x 0.8 cm) cyst, confined to the sella turcica, posterior to the pituitary gland, and consistent with a RCC. On initial visit, the patient was asymptomatic with no laboratory abnormalities (including normal pituitary function). MRIs in June 2007 and January 2008 showed no significant interval change. During routine follow up visits in 2008 and 2009, the patient remained asymptomatic with normal blood work. However, a repeat MRI in July 2010 showed significantly increased volume of the cyst (1.6 cm x 1.5 cm x 1.2 cm), elevation and anterior displacement of the optic chiasm, and expansion into the supra-sellar cistern. Laboratory workup was again normal, but the patient complained of occasional headaches. Neuro-ophthalmology examination, including visual field exam, was normal except for optic neuritis attributed to multiple sclerosis. In October 2010, she had successful surgical drainage. Cystic fluid pathology was negative for malignant cells. In subsequent visits, the patient reported resolution of her sporadic headaches. Conclusion: While there have been multiple reports of RCC and presenting symptoms, there are no clear guidelines regarding follow up and specific surgical indications in adults. This case is unique with the sudden and rapid cyst enlargement without clearly associated symptoms, demonstrating the importance of routine monitoring of patients with RCC. (1) J.E. Kim, J.H. Kim, O.L. Kim, S.H. Paek, D.G. Kim, J.G. Chi and H.W. Jung, Surgical treatment of symptomatic Rathke cleft cysts: clinical features and results with special attention to recurrence, J. Neurosurg 100 (2004), pp. 33-40. (2) J.L. Voelker, R.L. Campbell and J. Muller, Clinical, radiographic and pathological features of symptomatic Rathke's cleft cysts, J. Neurosurg. 74 (1991), pp. 535-544. Nothing to Disclose: AS, NN, AB

Pub #	P1-442
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Optimal Surgical Approaches for Rathke Cleft Cyst with Consideration of Endocrine Function
Author String	EY Choe, EJ Lee, SH Kim Yonsei University College of Medicine, Seoul, Republic of Korea; Endocrine Research, Seoul, Republic of Korea; Yonsei Brain Research, Seoul, Republic of Korea; Severance Hospital Integrative Research Institute for Cerebral & Cardiovascular Diseases, Seoul, Republic of Korea; Northwestern University Feinberg School of Medicine, Chicago, IL
Body	BACKGROUND: Surgical indications for Rathke cleft cyst (RCC) are not clear. OBJECTIVE: To evaluate surgical outcomes and to identify the factors associated with aggravated hypopituitarism in transsphenoidal surgery (TSS) for RCC. METHODS: All patients underwent a visaul field test, combined pituitary function test (CPFT), and magnetic resonance imaging (MRI) before and after surgery. Repeated CPFT was performed at 1.5-year intervals. RESULTS: The mean age at the time of surgery was 35 years (range, 9-78 years) and the male/female ratio was 1:1.25 (33/40). The mean follow-up duration after surgery was 59 months (range, 12-166 months). The most common symptoms were headache (84%), visual symptoms (48%), and diabetes insipidus (38%). After TSS, 75% of diabetes insipidus and 96% of visual field defects were resolved and pituitary function improved in 42% of patients. Age at the time of surgery was a significant risk factor for aggravated hypopituitarism. Twelve (16%) patients experienced recurrence, but none required reoperation. Five of the recollected cysts presented different characteristics from those of the initial lesions, and 2 recollected cysts underwent spontaneous regression. CONCLUSION: It is reasonable to conduct minimal incision with radical removal of cyst content to prevent the development of endocrine disturbance and other complications. Individualized risks and benefits must be assessed before reaching a decision regarding surgery and surgical method. Patients with recurrent RCC require careful follow-up with special attention, rather than a hasty operation. Nothing to Disclose: EYC, EJL, SHK

Pub #	P1-443
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Unexpected Pituitary Abscess: Presentation and Spontaneous Resolution of Endocrine Manifestations
Author String	S Sheffer-Babila, M Gangat, M Nicoletta-Gentile, L Lam Children's Hospital at Montefiore, Albert Einstein College of Medicine, Bronx, NY
Body	Introduction: Pituitary abscess is a rare cause of sellar mass and is generally only recognized intraoperatively. The presentation is that of an intracranial mass, with prolonged headache and in some visual complaints. Specific signs of infection such as fever and leukocytosis are present in a minority of cases. It commonly results in pituitary deficiencies which persist despite resolution of the abscess. While there are certain factors which predispose to a pituitary abscess, in the majority of cases there is no preceding history to indicate such increased risk. Pituitary abscess is a potentially fatal entity, particularly when not recognized and managed promptly. Clinical Case: A 19 year-old female presented with months of progressively worsening nocturnal and early morning headache, more recently accompanied by vomiting. Subsequently, she had nonspecific signs and symptoms suggestive of adrenal insufficiency including fatigue, generalized weakness, lightheadedness, and intractable vomiting. Menses had been previously every 4 weeks, but had now been more than 4 weeks since the last. Vital signs showed blood pressure of 92/61. She was ill-appearing. Physical exam was significant for pallor. Neuroophthalmologic exam showed bitemporal hemianopsia. Brain MRI confirmed a 1.9 cm sellar mass, cystic in structure with rim enhancement. Biochemical testing showed hyponatremia (Na 130 mEq/L ,normal 135-145), an undetectable morning cortisol, low TSH, low FSH, low LH, and mildly elevated prolactin. Upon transphenoidal surgical approach, there was unexpected drainage of purulent matter and no mass was identified. Pathology results revealed inflammatory cells and gram-positive cocci. Medical therapy consisted of intravenous antibiotics and hydrocortisone. All pituitary hormones were followed and spontaneously normalized. Post-operatively all symptoms resolved. Menses resumed after a few months. Conclusion: Due to the nonspecific nature of the clinical manifestations, it is important to consider pituitary abscess in the differential diagnosis of a sellar mass. This case depicts the typical presentation of pituitary abscess but is unusual in the recovery of multiple pituitary hormone deficiencies. This case also illustrates several specific and distinct features in biochemical testing and imaging, which may aid in detection of potential cases for rapid and appropriate management. Nothing to Disclose: SS-B, MG, MN-G, LL

Pub #	P1-444
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Spontaneous Reossification of the Sellar Floor in Transsphenoidal Reoperation Associated with Strontium Ranelate Treatment: A Case Report
Author String	MM Pineyro, D Arrestia, M Elhordoy, R Lima, S Wajskopf, MP Serra Hospital de Clinicas, Universidad de la Republica, Montevideo, Uruguay; Hospital de Clinicas, Universidad de la Republica, Montevideo, Uruguay
Body	Spontaneous reossification of the sellar floor after transsphenoidal surgery (TSS) has been rarely reported. Strontium ranelate (SR), a divalent strontium salt, has been shown to increase bone formation. We present a case of Cushing's disease (CD) treated with SR for osteoporosis that presented with significant reossification of the sellar floor at reoperation. A 21-year-old male was referred for evaluation of Cushing syndrome. He had gained weight over the last 5 years. He complained of easy bruising, muscle weakness and intermittent headaches. Past medical history included delayed puberty treated with testosterone for 3 years without further work up, HTN and vertebral fractures after a minor car accident. On physical exam his weight 58 kg (BMI 24), facial plethora, dorsocervical and supraclavicular fat deposition and ecchymosis; yet, he had no abdominal striae. Laboratory results confirmed hypercortisolism: UFC was 578 ug/24 h (28.4-213.7) and serum cortisol after 1 mg DST was 25.8 [micro]g/dl. ACTH levels were high at 83 pg/ml (7.2-63.3). MRI showed a left 5 mm hypointense lesion. An 8 mg DST showed 82.4% serum cortisol suppression (from 33.23 to 5.67 [micro]g/dl). IPSS is not available in the country. The patient underwent TSS, and an adenoma was excised. Postoperative cortisol levels remained high (24,2 ug/dl). DEXA scan showed low bone mineral density on lumbar spine (Z-4.4) and femoral head (Z:-3.8). He was treated with calcium, vitamin D 90.000 U every 3 months and pamidronate 90 mg IV every 3 months (3 times). The patient did not come to follow up visits for more than 1 year; he was prescribed SR 2 mg per day by an outside endocrinologist, which he received for more than 1 year. Almost 2 years after first surgery he was reevaluated and persisted with active CD. UFC was 1413 ug/dl (28.4-213.7). MRI revealed a left 4 mm hypointense mass, with sphenoid sinus occupation by a hyperintense material. CAT scan showed sellar reossification. At repeated TSS sellar bone had a very hard consistency; surgery was complicated with severe hemorrhage and the patient died septic 4 days later. There have been few cases of sellar reossification after reoperation reported in the literature. SR stimulates osteoblast; but no sellar reossificaton has been reported. Yet, this drug has been available for a short period of time. In conclusion, sellar reossification should be taken into account when considering repeat TSS. We speculate i SR may have influenced bone mineralization. Nothing to Disclose: MMP, DA, ME, RL, SW, MPS

Pub #	P1-445
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Atypical Teratoid/Rhabdoid Tumor in Sella Turcica in an Adult
Author String	JIS Mota, W Sobreira, L Pinheiro, FdAA Teixeira Junior, T Ferraz, J Gondim, E Gomes Fortaleza General Hospital, Fortaleza, Brazil; Fortaleza General Hospital, Fortaleza, Brazil; Fortaleza General Hospital, Fortaleza, Brazil
Body	Background: Atypical teratoid/rhabdoid tumors are rare and extremely aggressive malignancies of the central nervous system. This specific neoplasm affects mainly children under 2 years old, occurring predominantly in the posterior fossa. Clinical Case: We report a case of a woman aged 41 that started to present clinical feature of Adrenal Insufficiency associated with frontal headache, diplopia and paralysis of III, IV and VI cranial nerves (Cavernous Sinus Syndrome). Treatment with high doses of hydrocortisone was promptly established with improvement of the symptoms. The serum levels of hormones in the onset of the disease indicated panhipopituitarism: Estradiol <5.0pg/mL, LH <0.1 mUI/mL; FSH 0.452 mIU/ml; Prolactin 0.287 ng/ml (2,5 14,6 ng/ml); Cortisol <1.0 ug/dL (8,7 - 22,4 ug/dL);GH <0.05 ng/mL (<7.0 ng/mL);T4 0.659 mg/dL (0,87 - 1,56);TSH 0.005 mg/ml (0,35 - 5,50mg/mL); IGF-1 62,9 ng/mL (80 a 500 ng/mL). Therefore, it was initiated thyroid and steroid hormones replacement. At the time, the diagnosis of Pituitary Macroadenoma with tumora apoplexy was hypothesized. The Magnetic Resonance Imaging (MRI) revealed a 2,7x2,5x1,5cm infiltrating solid tumor occupying the sellar / supra-selar region. A Transsphenoidal hypophysectomy was performed uneventful. Two weeks after discharge, however, the patient was admitted with eyelid edema progressing to ptosis, hearing loss and severe headache. A new MRI showed an increased and invasive tumor, without possibility of a surgical approach. The patient developed right amaurosis, decline of the general condition and need for endotracheal intubation, being referred to ICU. The histological feature and immunohistochemical profile revealed atypical teratoid/rhabdoid tumor - grade IV (WHO).Microscopically, the cells presented varied morphology. Immunohistochemical stains demonstrated sparse expression of CD45, AE1/AE3 (CKpool) and Chromogranin. It was also demonstrated significative expression, almost universal, of Vimentin - even on cells with eccentric nuclei and acidophilic cytoplasmic inclusions. Palliative radiotherapy was performed at a dose of 3000 cGy in 10 fractions, with no significant improvement. **Conclusion:** Atypical teratoid rhabdoid tumour should be considered in the differential diagnosis of aggressive sella turcica tumours even in middle-aged adults. Nothing to Disclose: JISM, WS, LP, FdAATJ, TF, JG, EG

Pub #	P1-446
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Sella Turcica Localization and Lung Metastases of an Atypical Teratoid/Rhabdoid Tumor in an Adult Female
Author String	F Spasaro, F Giangaspero, L Guccione, P Di Giacinto, L Chioma, M Frajoli, FS Pastore, D Lupoi, E Breda, C Moretti University of Rome Tor Vergata, Rome, Italy; University of Rome La Sapienza, Rome, Italy; University of Rome Tor Vergata, Rome, Italy; Fatebenefratelli Hospital, Rome, Italy; Fatebenefratelli Hospital, Rome, Italy
Body	*Introduction/background*: Atypical teratoid/rhabdoid tumors (AT/RTs) are highly malignant neuroectodermal neoplasms primarily occurring in children less than three years old, originally described within the kidney, higly aggressive when rise in the central nervous system (CNS). These tumors are extremely rare in the adult, and only five cases have been reported in the sellar region. The rarity of these tumors, the preponderance of cases in children and the tumor's polyphenotypical immunoprofile render difficult the diagnosis, frequently mistaken them for other entities (neuroectodermal tumor/medulloblastoma and carcinosarcomas). Frequently in AT/RTs the INI1 gene, located in chromosome band 22q, is inactivated by deletions and/or mutations. Furthermore a polyphenotypic pattern of immunoreactivity is displayed by most AT/RTs including staining for vimentin, epithelial membrane antigen (EMA) and smooth muscle actin (SMA). *Clinical case*: A-60-year-old woman presented with headache, visual impairment and diplopia. RMI showed a 3 cm pituitary mass with suprasellar extension. The patient was treated by transphenoidal neurosurgery with histopathological diagnosis of [ldquo]atypical adenoma[rdquo]. After one month, the patient presented a recurrence and was re-operated and received three months radiation therapy. Eleven months after the first operation, a total body CT scan showed diffuses lung metastasis. Both pituitary and lung tissues were then re-examined and the final histological diagnosis was "AT/RT WHO grade IV with primary location in the sella turcica and secondary location in the lung[rdquo]. Immunoistochemical expression of vimentin, epithelial membrane antigen (EMA), neurofilaments, glial fibrillary acidic protein (GFAP) was demonstrated while a lack of expression of the INI1 protein was found depending by a mutation in the chromosome 22q. The patien undertaken chemiotherapy with adriamycin and vinorelbine, poorly tolerated. *Clinical lesson/conclusion*: The importance of this case report is that it was primarily diagnosed as a pituitary mass, interpreted as macroadenoma and treated by transphenoidal neurosurgery and radiotherapy. Radiotherapy, immediately after surgery, is crucial in the treatment of AT/RT. This approach, performed before the final pathological diagnosis could be available, allowed us to observe a better prognosis in a adult patient affected with AT/RT, which is still alive 16 months after the diagnosis. Nothing to Disclose: FS, FG, LG, PDG, LC, MF, FSP, DL, EB, CM

Pub #	P1-447
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Cancer Metastatic to the Sella: Characteristics Distinguishing It from *De Novo* Sellar Masses
Author String	MM Khan, D Abdelmannan, BM Arafah University Hospitals Case Medical Center, Cleveland, OH; Louis Stokes VA Medical Center, Cleveland, OH
Body	Objective & Methods: Metastatic disease to the sella is an uncommon clinical entity that requires a high level of clinical suspicion for diagnosis. Published data include small series of subjects; thus limiting adequate characterization of this entity. The goals of this study is to characterize the unique features of this entity that can help differentiate it from other sellar masses. We analyzed our own experience over the past 5 years (n=7) and reviewed all pathologically confirmed cases published in the English literature (n=95) for a total of 102 cases. Results: Data analysis reveal equal gender distribution with a median age of 56 yrs. Among the 51 women, th primary cancer was breast in 24, lung in 7, neuroendocrine in 7, thyroid in 6, and other types in the remaining 7. In contrast, the primary cancer in men was lung in 12, renal cell in 12, hepatoma in 6, prostate in 5, colon i 4, and various others in the remaining 12. Metastasis to the pituitary was the first presentation of cancer in 22/51 men and 15/51 women. Symptoms caused by perisellar mass effects included visual changes in 74% and headaches in 46% of patients. At presentation, 49% of patients had other areas of distant metastasis. Among patients who have adequate endocrine evaluation, hypogonadism was demonstrated in 75%, hypothyroidism in 71%, central adrenal insufficiency in 54% and a low plasma IGF-1 in 35%. Serum prolactin level was elevated in 58% of those tested with a mean value of 65 ug/L. Diabetes insipidus (DI) was a prominent symptom in 36% of patients. Imaging studies showed chiasmal compression in 26%, thickened stalk in 19%, erosion of the sella and /or clivus in 17%, and sella enlargement in only 11%. The clinical, biochemical and imaging characteristics were similar in both genders irrespective of the tumor type. Conclusions: Metastatic disease should be highly suspected in patients presenting with visual complaints, DI, a vascular sellar/suprasellar mass with a NL-size sella, mild hyperprolactinemia and partial hypopituitarism, especially if they have a known malignancy. In the absence of surgical intervention, DI is extremely rare in patients with pituitary tumors. Confirmation can only be provided by a biopsy though this is not always practical or necessary. Treatment depends on the primary tumor and available options. Replacement of endocrine deficiencies when appropriate is paramount. Once treated, survival is determined by the aggressiveness of the primary cancer. Nothing to Disclose: MMK, DA, BMA

Pub #	P1-448
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Pituitary Masses in Children and Young People -- To Operate or Not?
Author String	EF Gevers, V Tziaferi, B Ravindranathan, MT Dattani Great Ormond Street Hospital for Children, London, UK; Institute of Child Health, London, UK; National Institute for Medical Research, London, UK
Body	Pituitary size can fluctuate, especially during puberty. Differentiation between physiology and pathology is needed to prevent unnecessary surgical intervention. We present here illustrative cases of pituitary enlargement. The first patient presented at the age of 10 with headaches and hypertension. No cause was identified and antihypertensive treatment was started. Two years later he was progressing rapidly through puberty but had normal endocrine investigations. An MRI revealed a large pituitary, considered to be physiologically enlarged during puberty. A year later, he had an episode of loss of consciousness. A CT revealed no changes. His headaches continued and at the age of 13, an MRI showed a pituitary mass with central non-enhancing signal, abutting the optic chiasm. Visual field testing was normal. Surgery revealed a cyst, which was resected. Histology confirmed a Rathke's cleft cyst. Rathke's cleft cysts are usually asymptomatic but may cause headaches, visual defects, pituitary dysfunction or apoplexy. The second patient presented at the age of 13 with a 2 yr history of weight gain, poor growth, delayed puberty slurred speech and recent headaches. He was severely hypothyroid with a TSH > 375 mU/l. MRI showed a pituitary sellar and suprasellar mass, abutting the optic chiasm, likely due to severe primary hypothyroidism. A year after commencing thyroid hormone treatment the pituitary mass had resolved. The third patient presented at the age of 15 with a 2 yr history of headaches, increasing weight, tiredness and secondary amenorrhoea. She had benign intracranial hypertension. MRI showed a protruding pituitary mass abutting the optic chiasm. Investigation showed insulin insensitivity but no pituitary dysfunction. Seven months later pituitary size had spontaneously normalised. The fourth patient was investigated for complex seizures at the age of 2 and MRI showed a large pituitary. Pituitary function and vision were normal. A year later a normal sized pituitary with a central hyperdense signal was seen, followed by involution a year later, with a consequent reduction in growth rate and IGF1 concentration. No evidence was found for hypophysitis, and no mutations in PROP1 were identified. These cases illustrate the variation in pituitary size over time; Rathke's cleft cyst, hypothyroidism and normal puberty may give rise to pituitary masses resembling adenomas. Multi-disciplinary follow-up and repeat imaging is necessary for appropriate management. Nothing to Disclose: EFG, VT, BR, MTD

Pub #	P1-449
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Endocrinological Outcome after Surgical Resection of Craniopharyngiomas with Large Hypothalamic Involvement: An Adult Population Study
Author String	L Portocarrero-Ortiz, R Lopez-Serna Instituto Nacional de Neurologia y Neurocirugia, Mexico City, Mexico; Instituto Nacional de Neurologia y Neurocirugia, Mexico City, Mexico
Body	Introduction: Craniopharyngiomas show two different histological phenotypes, while the adamantinomatous pattern is more prevalent in children, the squamous papillary form is almost exclusively seen in adults. Considering that most of the published series of cranyopharingioma are based on pediatric populations, studies in adults gain importance based mainly on the reduced number of cases and the possible differences emerging from a histologically different type of tumor. Methods: We reviewed 153 cases of adult cranyopharyngioma surgically treated in our center from 1986 to 2010. Neuroendocrine evaluation was carried-out during the pre and post-operative periods. Hormonal determinations were done by chemiluminescence immunoassay methods. Location and extent of the tumor was evaluated on available films of computed tomography, magnetic resonance imaging and/or radiology reports. Tumor volume was calculated using the formula 4/3 pi.a.b.c, being A,B and C half-diameters in millimeters on axial, coronal and sagittal planes. Statistical analyses were performed using SPSS 11.0.1 (SPSS Inc, Chicago, Illinois). Results with a P value less than 0.05 were accepted as significant. Descriptive data were presented as means with standard deviations or medians with ranges depending on the expected distributions. Results: All of the patients were 16 years or older at the time of diagnosis. Histological phenotype was 85% adamantinomatous and 15% papillary. Distribution by gender was 79 males (51.6%) and 74 females (48.4%). Mean age at diagnosis was 32.4 years (Range 16-77 years). Hormonal alteration as the first sign of disease was found in 12.4% (19 patients). Mean initial volume of tumors was 28.44ml (Range 0.18-100.44 ml). Location of tumors and their vertical extension was considered according to Pertuiset/Samii criteria. The most frequent location was suprasellar and retrochiasmatic in 124 patients (81.04%). Partial or complete hypothalamic involvement (Grades III, IV and V) was found in 90.7%. Among the most frequent post surgical endocrinological complications we found: Hypothyroidism (84%), Hypocortisolism (81.6%), Hypogonadism (65.6%) and Diabetes Insipidus (65.6%). Conclusion: Hypothalamic involvement by craniopharyngiomas with large vertical extension (Grades III-V) in adults is strongly correlated with poor endocrinological outcome after resection, independently of their histological phenotype, age at presentation or gender. Nothing to Disclose: LP-O, RL-S

Pub #	P1-450
Session Information	POSTER SESSION: CLINICAL - Non-Functioning Lesions of the Pituitary-Hypothalamic Area (1:30 PM-3:30 PM)
Title	Analysis of Weight/Height Development and Psychosocial Situation in Long-Term Survivors of Childhood Craniopharyngioma in Relation to Therapeutic Interventions for Weight Regulation
Author String	U Gebhardt, M Wabitsch, A Faldum, G Calaminus, H Mueller Klinikum Oldenburg gGmbH, Oldenburg, Germany; University Ulm, Ulm, Germany; University Muenster, Münster, Germany; University Muenster, Muenster, Germany
Body	Craniopharyngioma are rare embryogenic malformations of the sellar area with low-grade histological malignancy. Approximately 30 new cases per year are diagnosed with childhood craniopharyngioma in Germany. Despite high survival rates, the quality of life (QoL) is frequently impaired in long-term survivors due to sequelae caused by the anatomical proximity to the optic nerve, pituitary gland, and hypothalamus. Obesity and eating disorders are observed in 40% to 50% of patients. In the trial KRANIOPHARYNGEOM 2000 117 patients with newly diagnosed childhood craniopharyngioma were recruited and prospectively observed in regard to risk factors for weight and height development and quality of survival. Severe hypothalamic obesity and endocrine deficits leading to short stature had strong impact on the QoL in survivors. Accordingly, in KRANIOPHARYNGEOM 2007 QoL in patients (age [ge] 5 years at diagnosis; incomplete resection) will be analyzed after randomization of the time point of irradiation after incomplete resection (immediate irradiation versus irradiation at progression of residual tumor). The important question whether severe obesity after childhood craniopharyngioma is a steadily increasing development or reaches a plateau cannot be answered based on the study design of KRANIOPHARYNGEOM 2000/2007. In these trials only patients diagnosed in the year 2001 or later are prospectively observed. Furthermore, the long-term impact of therapeutic interventions on weight development and psychosocial well-being is not part of the study design of KRANIOPHARYNGEOM 2000/2007. The proposed project analyses the long-term weight development of 237 patients diagnosed with childhood craniopharyngioma between 1966 and 2000. The actual degree of obesity will be assessed by body mass index (BMI) and calculated as SDS according to the references of Rolland-Cachera. Data on actual weight, height, psychosocial situation and on the effect of weight stabilizing interventions (medicaments, rehabilitation, bariatric surgery) will be collected by questionnaire. Further anthropometric data on birth weight/height of the patients and parental weight and height will be analyzed. Siblings of the patient will be analyzed for the same parameters and will serve as gender-matched controls. Sources of Research Support: Deutsche Kinderkrebsstiftung, Bonn, Germany. Nothing to Disclose: UG, MW, AF, GC, HM

Pub #	P1-451
Session Information	POSTER SESSION: CLINICAL - Endocrine Nursing Initiatives To Address Patients' Quality of Life (1:30 PM-3:30 PM)
Title	Ease of Administration of Somatostatin Analogs, Octreotide LAR *vs.* Lanreotide
Author String	K Schweinsberg, S Smith, LS Kirschner The Ohio State University Medical Center, Columbus, OH; Tercica a Subsidiary of the Ipsen Group, Brisbane, CA
Body	**Background:** Long acting somatostatin analogs (SSAs) are used in the treatment of endocrine tumors to reduce hormone secretion and potentially control tumor growth. Patients with unresectable growth hormone-secreting tumors (Acromegaly) and patients with carcinoid tumors benefit clinically from SSAs, and are typically on therapy for an extended time. There are 2 long acting SSAs currently available in the United States, Octreotide LAR (OCT LAR) and Lanreotide (LAN). The clinical efficacy of SSAs are considered equivalent. However, there are major differences in the formulations and administration process. These include needle length, volume of injection, intramuscular versus subcutaneous injection, type of solution injected, reconstitution time and injection site side effects. **Study Objective:** The purpose of the study was to investigate the impact of the injection process on clinic and patient time in patients with acromegaly or carcinoid tumors receiving long acting SSAs. **Study Design:** We performed an analysis of injection time, injection issues, and patient response in patients receiving SSAs. Nurses were trained to measure the study parameters and entered real time data immediately following the injection procedure. **Patient Population:** 30 injections of OCT LAR and 21 injections of LAN were evaluated. **Study results:** For patients receiving OCT LAR, the average time to complete the injections including the reconstitution process was 7.21 +/- 0.35 minutes. In comparison, patients receiving LAN completed their injections in 1.38 +/- 0.27 minutes ($p < 0.001$). One injection of OCT LAR was reported to have a needle clog resulting in a second injection to the patient. Injections were generally well tolerated. However, 5 patients in OCT LAR group (16.6%) reported muscle soreness versus the LAN group that reported no injection site issues (0%) ($p < 0.01$). **Conclusion:** In patients receiving long-term SSA therapy, Lanreotide saves both nursing time as well as patient wait time compared to Octreotide LAR. The time savings are mainly due to the added reconstitution step for OCT LAR, which adds over 5 minutes to the total administration time. There is also the potential for needle clogging with OCT LAR which in our experience produced more local site reactions. Although LAN and OCT LAR have similar biochemical efficiency in treating both neuroendocrine tumors and acromegaly, there may be patient and nursing benefits to the use of LAN as first-line SSA therapy. Disclosures: KS: Speaker Bureau Member, Tercica. SS: Employee, Tercica. LSK: Speaker Bureau Member, Tercica.

Pub #	P1-452
Session Information	POSTER SESSION: CLINICAL - Endocrine Nursing Initiatives To Address Patients' Quality of Life (1:30 PM-3:30 PM)
Title	Long-Acting Somatostatin Analog Injection Devices: A Quantitative Time and Perception Study To Explore Nurses' Preferences
Author String	A Burgess, PR Davies, D Adelman Northwestern University, Chicago, IL; The Christie, Manchester, UK; Royal Free Hospital, London, UK
Body	**Introduction**: Somatuline Depot/Autogel (SD) and Sandostatin LAR (LAR) are the two major long-acting somatostatin analogs (SSA) currently available in the US and Europe. The aim of this study was to gain nurses' insights on the use and ease of administration of SSA devices, including Somatuline Autogel new device (SD-ND), in Europe and the US. **Methods/Design**: Qualified nurses treating at least 3 SSA acromegaly and/or neuroendocrine tumor patients/year were interviewed to obtain views and opinions regarding SSA injection use. Nurses were timed while preparing and performing test injections with SD-ND and LAR. Attributes of SD-ND and existing SSA devices were then evaluated via questionnaire and an overall preference score (SSA device attributes weighted by importance to nurses) calculated for each. **Results**: 77 interviews were conducted in France (n=22), Germany (n=19), UK (n=18), and the US (n=18). The most important attributes to nurses were easy/convenient preparation and injection, low risk of clogging, confidence that a full dose has been delivered, and high product efficacy and safety. Also important was prefilled device, ease/speed to teach and prepare, and low risk of needle-stick injuries. During the device try-out, two clogging incidents occurred with LAR; none occurred with SD-ND. Mean time for preparation and administration was significantly ($p<0.01$) lower for SD-ND (66s) compared to LAR (329s). SD-ND scored higher than LAR for nearly all device attributes ($p<0.05$). The five most important attribute improvements for SD-ND vs. LAR were prefilled device, low risk of clogging, and fast/easy administration, low injection volume, and possibility for self-injection. The automatic needle guard was seen as an important device characteristic in avoiding needle-stick injuries. The overall preference score was 63% higher for SD-ND vs. LAR (114 vs. 70; $p<0.01$). **Conclusion**: The new SSA device SD-ND was well accepted among interviewed nurses, and preference over currently available devices was high. Conceivably, the short administration time, confidence that a full dose has been delivered and perceived ease-of-use of the new device compared to existing SSA devices could lead to improvement in clinical practice and provide benefit to patients and caregivers when administering SSAs at home. Sources of Research Support: Cegedim Strategic Data, 90/92 route de la Reine, 92100 Boulogne Billancourt, France; IPSEN Pharma, 65, quai Georges Gorse, 92650 Boulogne Billancourt Cedex, France. Disclosures: AB: Researcher, Ipsen. PRD: Researcher, Ipsen. DA: Researcher, Ipsen.

Pub #	P1-453
Session Information	POSTER SESSION: CLINICAL - Endocrine Nursing Initiatives To Address Patients' Quality of Life (1:30 PM-3:30 PM)
Title	A Survey of Adult Patients in Scotland with Growth Hormone Deficiency (AGHD) Not on Replacement Therapy
Author String	MN Carson, S Philip, AW Patrick, G Leese, JS Bevan, JM Connell Edinburgh Royal Infirmary, Edinburgh, UK; Aberdeen Royal Infirmary, Aberdeen, UK; Ninewells Hospital Medical School, Dundee, UK
Body	Aim: To study the reasons why adults in Scotland with growth hormone deficiency were not on replacement therapy. Methods: We identified adult patients under specialist endocrine review in the four main university hospitals in Scotland with confirmed GH deficiency and not on GH replacement. A cross-sectional case note review was carried out of all patients. Results: 108 patients (50% women; 14% professionals, 98% Caucasian) were included in the audit. Tumours in the sellar region were the main cause of GH deficiency (74%). All patients had baseline pituitary testing and were on other pituitary hormone replacements (thyroxine, 75%; hydrocortisone, 73%; testosterone, 91% men; oestrogens, 72% women; DDAVP, 19%). The main reasons for not starting GH included adequate quality of life (43%), unwilling patient (15%) or a medical contraindication (14%). However only 22 patients of the 45 patients who were identified as having a normal QOL had a formal assessment using a AGHDA QOL questionnaire. Conclusion: The main reason for not starting growth hormone therapy in AGHD patients was perceived quality of life. Sources of Research Support: The Society for Endocrinology. Nothing to Disclose: MNC, SP, AWP, GL, JSB, JMC

Pub #	P1-454
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	High Prevalence of Eating Disorders Not Otherwise Specified in Spain: Population-Based Study
Author String	A Larranaga, MF Docet, RV Garcia-Mayor University Hospital of Vigo, Vigo, Spain; University Hospital of Vigo, Vigo, Spain; University Hospital of Vigo, Vigo, Spain
Body	**Background:** Obesity and the Eating disorders (ED) have become important health problems. Some clinical forms of eating disorders such as Bulimia Nervosa (BN) and Eating Disorders Not Otherwise Specified (EDNOS) are associated with an increased risk for obesity. **Objectives**: To determine the incidence and prevalence of eating disorders taking into account three main diagnostic entities: Anorexia Nervosa (AN), Bulimia Nervosa and Eating Disorders Not Otherwise Specified. **Method**: All new ED cases of both genders, [ge] 15 years old, diagnosed since January 2005 until December 2009 were included in the incidence study. All patients with ED in December of 2009 were included in the prevalence study. This is a prospective, population-based study. Cumulative Incidence rates for population of 15 years old and over per 100,000 inhabitants per year was calculated. Twenty-year Prevalence was calculated as the number of people of 15 years old and over who had ED in a cross-sectional survey performed in December 2009. **Results**: The ED cumulative incidence was 14.1(95%CI:11.4-16.1) cases per 100,000 inhabitans per year. The mean cumulative incidences of AN, BN and EDNOS were not significantly different, being 3.1(95% CI:2.00-4.1), 4.4 (95% CI:3.0-8.00) and 6.5 (95% CI:4.8-7.9), respectively. The prevalence of ED was 82.8 (95% CI:69.4-94.5) per 100,000 inhabitants. The prevalences of AN, BN and EDNOS were 18.6(95% CI: 12.5-24.4), 25.7(95% CI: 18.5-32.5) and 38.3 (95% CI: 29.4-46.5), respectively, being the prevalence of EDNOS significantly higher than the prevalence of AN. **Conclusions:** This is the first population-based study that includes patients of both genders from a wide age-range interval, in which the incidence and the prevalence of ED and its clinical forms were determined in Spain. EDNOS had the highest incidence and prevalence, which is remarkable, since this clinical form is associated with an increased risk for the development of obesity. Nothing to Disclose: AL, MFD, RVG-M

Pub #	P1-455
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Dietary Habits of Women with Turner Syndrome
Author String	W Jez, TJ Irzyniec, A Stachowicz, N Szydlowski-Pesko Medical University of Silesia, Katowice, Poland; Hospital of the Ministry of Interior Affairs and Administration in Katowice, Katowice, Poland; Specialist Hospital No 2, Bytom, Poland
Body	**Introduction:**.Turner Syndrome (TS) affects females and results from a total or partial deletion of one of X chromosomes. Women with TS are substantially shorter than the healthy controls, but they have much higher body mass index and waist-hip ratio. Higher blood pressure (mostly diastolic) as well as elevated LDL and glucose concentrations and decreased HDL levels are characteristic for the TS women too. Obesity is one of the symptoms of TS. Women with TS are claimed to be of an increased risk of the cardiovascular diseases' incidence not only due to the higher prevalence of cardiovascular anomalies. The TS women live shorter and need the medical care from the infancy. **Aim:** This investigation aims at compiling and analyzing the data on dietary habits of women with TS in Poland. **Material and methods**: The survey, which included 25 questions of the basic foodstuff consumption, was taken in the group of the 158 TS women who were split into two subgroups. The first one - 80 subjects were surveyed in 1995-98 and the second one - 78 women in 1999-2004. The control group was comprised of 84 healthy women. The outcomes of all 3 groups were compared to each other. **Results**: Protein products: TS women consume fish, eggs, lean milk and cottage cheese more often than the healthy controls. In turn they strain from whole milk, fat cheese and pork in their diet. Carbohydrates: The percentage of women declaring white bread, grits, potatoes and honey consumption is larger in the TS groups. TS women use sugar to sweeten hot beverages more often than the others too. In turn, they do not consume sweets so often comparing with the healthy controls. Fat food: TS women declared consumption of vegetable oils and margarine more often. The consumption of butter prevailed in the control group. We do not heed any differences in fruits and vegetables consumption between TS and healthy women as well as in consumption of mineral water and fruit tea. The dietary habits of the TS women of two subgroups are more healthful in respectively 15 and 14 out of the 21 investigated sorts of foodstuff, whereas the healthy subjects only in 4 out of 21. **Conclusions:** 1. Dietary habits of TS and healthy women differ form each other. 2. TS women diet is more healthful than in the controls. Nothing to Disclose: WJ, TJI, AS, NS-P

Pub #	P1-456
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	High Prevalence of Symptoms of Inattention in a Clinical Population of Obese Children: Relations with BMI Z-Scores and Parameters of the Metabolic Syndrome
Author String	A Gkourogianni, P Pervanidou, C Kanaka-Gantebein, GP Chrousos Athens University Medical School, Aghia Sophia Children's Hospital, Athens, Greece
Body	Background Recent evidence has shown high rates of comorbidity between obesity and Attention-Deficit/Hyperactivity Disorder (ADHD) in children and adults, suggesting common neurobehavioural pathophysiologic pathways. Indeed, impulsive behaviors related to food intake, as well as deficits in the central dopaminergic reward system, may explain the high rates of comorbidity between these disorders. Objective The aim of this study was to examine the prevalence of ADHD symptoms among children followed at the Childhood Obesity Clinic of our Department. A total of 90 (50 females/40 males) obese children, aged 6-16, were examined for ADHD. Parents were asked to complete a questionnaire based on DSM-IV diagnostic criteria for ADHD. Children were screened for depressive symptoms using the CDI questionnaire. Results Fourteen (10 males and 4 females, 16%) of 90 obese children presented with a high level (over the diagnostic cut-off point) of Attention Deficit Disorder (ADD) symptoms and 5 (5%) with hyperactivity symptoms. Only two children (2%) scored for full ADHD. Four percent of the entire population presented with comorbid symptoms of depression. Children with ADD symptoms were slightly (not significantly) older (12.18±2.6 vs. 10.64 ±2.4) and had a significantly higher BMI z-score (3.8±1.5) than obese children without ADD symptoms (control group) (2.3±1.2, p=0.006). The group with ADD symptoms had also a greater number of positive Metabolic Syndrome manifestations (high triglycerides, low HDL, high blood pressure, high fasting glucose) than the control group (mean number 1.7 vs. 0.8, p=0.02). Conclusions A high prevalence of ADD symptoms, but not symptoms of hyperactivity, is noted in a clinical population of obese children. Children with ADD symptoms had a higher BMI z-score and a greater number of Metabolic Syndrome manifestations than the control group. Although further studies are needed to assess a causal relation between obesity and ADD manifestations, behavioural screening is essential in understanding and treating childhood obesity. (1) Samuele Cortese et al.,Crit Rev Food Sci Nutr. 2008;48(6):524-37. Review. (2) Anderson SE et al., Pediatr. 2006; 6(5):297-301. (3) Blum K. et al., J Psychoactive Drugs. 2000;32 Suppl:i-iv, 1-112. Nothing to Disclose: AG, PP, CK-G, GPC

Pub #	P1-457
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Comparison of Body Composition Study by Dual-Energy X-Ray Absorptiometry (DEXA) in Familial Partial Lipodystrophies and Control Subjects
Author String	CM Valerio, L Zajdenverg, JEP Oliveira, P Mory, R Moises, A Godoy Matos Instituto Estadual de Diabetes e Endocrinologia, Rio de Janeiro, Brazil; Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; Universidade Federal de São Paulo-Escola Paulista de Medicina, São Paulo, Brazil
Body	Familial partial lipodystrophies (FPL) are clinically heterogeneous disorders characterized by selective loss of adipose tissue (1). Affected patients are predisposed to insulin resistance and metabolic complications. Body fat distribution and amount contributes to the metabolic state. Until genetic studies become available for clinical practice, metabolic features and the pattern of adipose tissue loss are the only parameters leading clinicians to consider the diagnosis (2,3). To date few studies have compared regional body fat distribution in FPL and control subjects (4,5,6,7). OBJECTIVE: to evaluate body composition by Dual-Energy X-ray Absorciometry (DEXA) in patients with FPL and control subjects, comparing DEXA measurements, lipid profile and inflammatory markers. METHODS: Fifteen female patients with clinical features of FPL and 16 controls, matched for body mass index, sex and age were studied. Among the 15 patients included, 10 of them have confirmed mutations on LMNA gene so determining diagnosis of Familial Partial Lipodystrophy of Dunnigan (FPLD2). The 5 remaining patients showed no mutations in the LMNA gene. They have not yet been tested for others lipodystrophy familial syndromes. RESULTS: DEXA revealed a marked decrease in trunkal fat and a 3 folds decrease in limbs fat percentage in affected patients (*p=0,0001*). Comparative analysis showed that Fat Mass Ratio (FMR) between trunk and lower limbs [ge] 1.80 improved accuracy for diagnosing FPLD2 with a cut-off point of 1.84. Furthermore, affected women showed hypoleptinaemia (median of 3,6 vs. 17,2 ng/ml in controls), insulin resistance (6 out 10 had diabetes type 2 and two had impaired fasting glucose) and an aggressive lipid profile with lower levels of HDL-cholesterol (median of 41 vs. 59 mg/dl in controls) and hypertriglyceridemia (*p=0,0002*). Eighty percent of LMNA-mutated women had clinical phenotype of Polycystic Ovarian Syndrome and seven exhibited hypertension. After adjustment for anthropometric data, gynoid fat was the only parameter independently associated to leptin levels (*p=0,002, R2=0,52*). CONCLUSION: In this study, assessment of body fat distribution by DEXA permitted phenotypic diagnosis of FLPD2, once 10 out 15 exhibited LMNA mutation. A consistent pattern of marked reduction in subcutaneous fat of lower extremities was observed in affected patients. To our knowledge this is the first time that cut-off values of objective variables were proposed for evaluation of FPLD2. (1) Garg A. Acquired and Inherited Lipodystrophies. N Engl J Med 2004; 350:1220-1234. (2) Hegele RA, Joy TR, Al-Attar S, Rutt BK. Lipodystrophies: windows on adipose biology and metabolism. Journal of Lipid Research 2007, 48:1433-1444. (3)Garg A, Agarwal AK. Lipodystrophies: disorders of adipose tissue biology. Bioch Biophys Acta 2009, doi 10.1016/j.bbalip.2008.12.014 (4) Al-Atar s, Pollex RL, Robinson JF. Quantitative and qualitative differences in subcutaneous adipose tissue stores across lipodystrophy types shown by magnetic resonance imaging. BMC Medical Imaging 2007; 7:3. (5) Garg A, Peshock RM, Fleckenstein JL. Adipose tissue distribution pattern in patients with familial partial lipodystrophy (Dunnigan Variety). J Clin Endocrinol and Metab 1999; 84:70-174. (6)Valerio CM, Godoy-Matos A, Moreira RO. Dual-Energy X-ray Absorptiometry Study of Body Composition in Patients with Lipodystrophy. Diabetes Care 2007, 30(7):1857-1859. (7) Pandey SN, Pungavkar SA, Vaidya RA. An imaging study of body composition including lipodeposition pattern in a patient of familial partial lipodystrophy (Dunnigan type). J Assoc Physicians India 2005; 53:897-900. Nothing to Disclose: CMV, LZ, JEPO, PM, RM, AGM

Pub #	P1-458
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Assessment of the Usefulness of Bioelectrical Impedance in the Prediction of Insulin Resistance
Author String	V Perea, A Jimenez, GB Aranda, M Mora, J Viaplana, MJ Coves, J Ferrer, J Vidal Hospital Clínic of Barcelona, Barcelona, Spain
Body	**Background**: Total body fat, and especially abdominal fat mass have been associated with insulin resistance. Despite body mass index (BMI) and waist circumference (WC) being respectively useful proxies of total and central body fat, more especific and convenient fat distribution mesaurements are needed. **Objectives**: To investigate in morbidly obese (MO) subjects the association between insulin resistance and fa mass estimates derived from bioelectrical impedance (BIA) measurements and validated with DXA specifically in this population. **Subjects and Methods**: Cross-sectional study in 640 MO non-diabetic subjects (74.6% women, age 42.0 ± 10.8 years, BMI 47.1 ± 6.4 kg/m^2). Insulin resistance was estimated as the HOMA-R index. BIA-derived (Tanita BC418) total fat mass (TFM) and android fat mass (AFM) estimates were calculated using specific formula previously developed and validated with DXA in our group. Pearson's partial correlation and multiple linear regression models (both adjusted for gender and age) were used to test the association between anthropometric variables (BMI, WC, BIA-TFM and BIA-AFM) and HOMA-R index. Receiver Operator Curve (ROC) analysis was used to determine which variable was the best parameter to detect insulin resistance (defined as HOMA-R>2.95, 80^{th} percentile of our reference healthy non-obese population). **Results:** In a partial correlation analysis, all the anthropometric variables were significantly but weakly correlated with HOMA-R (BMI: r=0.218, p<0.001; WC: r= 0.227,p<0.001; TFM: r=0.228, p<0.001; AFM: r =0.240, p <0.001). A predictive model with age, gender, and BMI as independent variables yielded a significant estimate of for HOMA-R (R^2=0.116). Substituting BMI by BIA-TFM did not increase the predictive value (R^2=0.091). Likewise, age, gender, and WC significantly predicted HOMA-R (R^2=0.119), but BIA-AFM did not improve prediction (R^2=0.115). The best predictive model was the one including gender, age, WC and BMI (R^2=0.127). ROC analysis showed that all fat mass estimates were weak to detect insulin resistance (AUC<0.80), with WC being the best among the 4 evaluated parameters (BMI:AUC=0.67, p<0.00, WC:AUC= 0.72, p<0.001; TFM:AUC=0.67, p<0.001; AFM:AUC=0.70, p<0.0011). **Conclusions:** In a population of morbidly obese non-diabetic subjects, BIA-derived estimates of total fat mas or android fat mass do not improve the prediction of insulin resistance compared with classic anthropometric measurements. Nothing to Disclose: VP, AJ, GBA, MM, JV, MJC, JF, JV

Pub #	P1-459
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Conviction and Confidence for Dietary Changes Predict Early Weight Loss in a Lifestyle Modification Intervention
Author String	M-E Domingue, J-P Baillargeon, C Brown, V Lebrun, M-F Langlois University of Sherbrooke, Sherbrooke, Canada
Body	Introduction: Interdisciplinary lifestyle intervention, the most effective preventive strategy for diabetes, encounters high drop-out and non-responder rates. We designed a questionnaire based on patient expectations readiness to change and conviction/confidence levels for lifestyle modification, the weight-loss readiness tool (WLRT), to assess initial and early predictors of response to lifestyle intervention. Methods: Prospective study including 70 subjects with impaired glucose tolerance and BMI 27-40 kg/m2. The WLRT and anthropometric measurements are assessed at baseline and 6, 12 and 52 weeks. Patients are followed every 6 weeks for 1 year by our hospital-based lifestyle modification interdisciplinary team and have access to group education. Recruitment took place between November 2008 and May 2009. Study will end in May 2010. Results: Mean age at baseline is 58.2±11.3 and mean BMI 32.5±3.0. Mean weight variation at 6 and 12 weeks is 1.45Kg (CI: -0.91 to -1.99; $p<0.001$; n=65), -2.55Kg (CI: -1.70 to -3.40; $p<0.001$; n=59). At 12 weeks, weight variation is significantly correlated to 6 questions of the WLRT, relating to conviction and confidence of subjects for weight loss, particularly for dietary changes (r: -0.25 to -0.32; $p<0.05$; Spearman correlation). Moreover, WLRT results at 12 weeks showed that patients progress in their readiness to change during the first weeks of intervention. Conclusion: Our intervention results in a significant weight loss at 12 weeks that is correlated to conviction and confidence level of patients as assessed by the WLRT. Analyses at one year will allow identifying response predictors to our intervention and thus guide resources allocation. Sources of Research Support: The Lawson Foundation. Nothing to Disclose: M-ED, J-PB, CB, VL, M-FL

Pub #	P1-460
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Impact of Weight Loss on Obesity-Specific Health-Related Quality of Life in an Employed Population
Author String	AE Rothberg, LN McEwen, AM Miller, CF Burant, WH Herman University of Michigan, Ann Arbor, MI
Body	Obesity impacts many aspects of health-related quality of life (HRQOL) including physical function, self-esteem, sexual life, public distress, and work function. Weight loss may improve HRQOL. The purpose of thi study was to examine the impact of a very low calorie diet (VLCD) during the first 12 weeks of a 2-year medical weight loss program on HRQOL as assessed by the Impact of Weight on Quality of Life (IWQOL) -Lite in patients from a single employer group enrolled in a single managed care health plan. Domains of the IWQOL-Lite were examined by sex before and after weight loss and by percentage change in body mass index (BMI) from baseline. We studied 52 salaried employees before and after 3 months of treatment with a VLCD. Mean age (±SD) was 49±7 years and mean baseline BMI was 40±5 kg/m^2. Mean baseline BMI did not differ by sex. There were 24 men and 28 women. Hypertension (50%), dyslipidemia (38%), diabetes (33%), obstructive sleep apnea (23%) and depression (23%) were prevalent at baseline. Mean weight loss at 3 months was 57±20 lbs in men and 42±17 lbs in women. Mean change in BMI from baseline was -20±6% in men and -17±6% in women. At baseline, women had poorer HRQOL than men in the domains of physical function (59 vs 67) and self-esteem (43 vs 64), and had lower total HRQOL than men (61 vs 71). At follow-up, women had greater improvements in the domains of physical function (+26 vs +19), self-esteem (+29 vs +17), and work function (+13 vs +7) than men. At follow-up, women and men exhibited comparable total HRQOL (84 vs 85). Greater change in BMI was most strongly associated with improvements in the domains of public distress (100% improvement in 3^{rd} tertile compared to 1^{st} tertile of % change in BMI), physical function (1.6 fold improvement), and sexual life (1.5 fold improvement). Obesity had a greater impact on HRQOL in women than men, largely due to its impact on physical function and self-esteem. Following 3 months of a VLCD and substantial weight loss, and despite lesser weight loss and lesser % improvement in BMI in women than in men, women had greater improvement in HRQOL so that at follow-up, women and men exhibited comparable total HRQOL. In women and men, the magnitude of change in BMI had the greatest impact on public distress, physical function, and sexual life. In this employed, white collar population, weight loss was especially beneficial for women. Sources of Research Support: National Institutes of Health, P30DK089503-01, Michigan Nutrition Obesity Research Center. Nothing to Disclose: AER, LNM, AMM, CFB, WHH

Pub #	P1-461
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Effect of a Lifestyle Intervention on Adiposity and Fitness in High-Risk Subgroups of Preschoolers (Ballabeina): A Cluster-Randomized Trial
Author String	I Niederer, F Burgi, V Ebenegger, C Schindler, P Marques-Vidal, S Kriemler, JJ Puder University of Basel, Basel, Switzerland; University of Lausanne, Lausanne, Switzerland; University of Basel, Basel, Switzerland; University of Lausanne, Lausanne, Switzerland

Body

Background

Overweight (OW) and low fit (LF) children have a clustered risk for later cardiovascular disease. We therefore investigated, whether a preschool-based lifestyle intervention was effective in OW and LF children.

Methods

Forty preschool classes were randomly selected and 1:1 randomized into an intervention and a control arm after stratification for language region (French vs. German part of Switzerland). The intervention included a physical activity (PA) program, lessons on eating habits, media use and sleep, and adaptation of the built environment. Primary outcomes were changes in BMI and aerobic fitness; secondary outcomes changes in percent body fat, sum of 4 skinfolds (SF), waist circumference and motor agility. Potential interactions of intervention with high baseline BMI (OW [ge]90th national percentile) and low fitness (lowest sex- and age-adjusted quartile of aerobic fitness) were tested and stratified analyses performed.

Results

652 preschool children (mean age 5.2 ± 0.6 yrs, 20% OW, 25% LF) participated. In the total population, the intervention had beneficial effects on body fat and both fitness measures. Compared to their counterparts, OW children experienced more beneficial effects for most and LF children for all adiposity measures (p for interactions <0.1), while there were no interactions for fitness measures. OW children showed a significant intervention effect (adjusted changes (95% CI)) in sum of 4 SF (-3.6 mm (-6.5 to -0.8 mm), p=0.011), waist circumference (-2.2 cm (-3.2 to -1.2 cm), p<0.001) and agility (-1.0 s (-1.9 to -0.1 s), p=0.023). LF children showed a significant intervention effect in BMI (-0.3 kg/m^2 (-0.5 to -0.03 kg/m^2), p=0.014), percent body fat (-2.1% (3.2 to -1.0%), p<0.001), sum of 4 SF (-5.0 mm (-7.4 to -2.7 mm), p<0.001) and waist circumference (-1.62 cm (-2.7 to -0.5 cm), p=0.004) but not in fitness measures.

Discussion

This intervention was also effective in high risk preschoolers and represents a promising option for OW and LF children.

Sources of Research Support: Swiss National Science Foundation.

Nothing to Disclose: IN, FB, VE, CS, PM-V, SK, JJP

Pub #	P1-462
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Effect of a Resistance Weight Training Intervention on Body Composition and Left Ventricular Structure and Function in Obese Adolescents
Author String	R Dahiya, S Shultz, S Clark, K Kostner, M O'Riordan, N Byrne, A Hills, GM Leong Mater Health Services, South Brisbane, Australia; Queensland University of Technology, Kelvin Grove, Australia; Mater Health Services, South Brisbane, Australia; Rainbow Babies and Children's Hospital, Cleveland, OH; The University of Queensland, St Lucia, Australia

Body

Introduction: Obesity and insulin resistance are associated with left ventricular (LV) diastolic dysfunction and atherosclerosis leading to heart failure or coronary heart disease in adults (1). As these changes may occu as early as during puberty (2)(3), we examined the effects of a 16-week resistance weight training interventio on body composition and cardiac structure and function in obese adolescents.
Methods: 14 obese adolescents (16.1±1.6 y; M:F 6:8; BMI SDS: 1.97±0.37) were recruited for a 16-week resistance weight training intervention. Participants completed 10 repetitions of 15 exercises during 3 testing sessions each week. Intensity and volume were progressively increased. Height, weight, waist and hip circumference were used to calculate body mass index (BMI) and waist-to-hip ratio (WHR), respectively. Body composition was assessed using dual energy x-ray absorptiometry (DXA). Bilateral carotid ultrasound was used to assess carotid intimal media thickness (cIMT) and 2D echocardiography was used to evaluate LV systolic and diastolic function. LV filling pressures were assessed by pulse wave tissue Doppler using the peak of early LV septal diastolic tissue velocity (E').
Results: Although the BMI SDS did not alter significantly, a decrease in WHR (P=<0.001), percentage arm fat (P=<0.001), percentage truncal fat (P=0.04), and an increase in total lean mass (P=0.007) and lean mass in the arms (P=<0.001) was observed. Of the 7 subjects who had a reduction in truncal fat by at least one standard deviation, 5 subjects had an increase in tissue velocities (E') by at least one standard deviation. No significant change was found in left atrial volume, cIMT, left systolic function and LV mass index.
Conclusion: The 16-week resistance weight training intervention was effective in improving body composition by reducing the WHR, and adipose tissue in the trunk and arms. Subjects who had a reduction in truncal fat, an indicator of visceral fat, were more likely to show an improvement in diastolic LV relaxation. Larger studies are needed to confirm these findings and further evaluate whether resistance training or aerobic exercise is beneficial in improving insulin resistance and LV diastolic function.

(1) Kosmala et al., JCEM; 2008 Oct;93(10):3748-54.
(2) Shah AS et al., Diabetologia 2010; 1974-1977
(3) Urbina EM et al., Circulation. 2009;119:2913-2919

Sources of Research Support: Golden Casket Research Grant, Mater Health Services.

Nothing to Disclose: RD, SS, SC, KK, MO, NB, AH, GML

Pub #	P1-463
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Endothelial Function and Insulin Sensitivity Improved by Exercise during Weight Maintenance after Weight Reduction, but Not by Diet, in Type 2 Diabetes
Author String	KA Han, KW Min, JH Lee, KS Pak, HK Lee Eulji University School of Medicine, Seoul, Korea
Body	**Background and aims**: The aim of the study was to compare the effects of diet or exercise induced weight loss programs for 3 months and following weight maintenance for 3 months on the endothelin dependent and independent vasodilation in type 2 diabetes. **Materials and methods**: Total 39 women with type 2 diabetes were randomly assigned to control (C, N=14), diet (D, N=11), exercise (E, N=14) and completed the 3 month weight loss program, and then maintained their body weight(BW) for following 3 months. The restriction of calorie intake (< 1400 kcal/day) was done for D, walking for 60 minutes at moderate intensity (3.6 to 5.2 METs) five times a week for E. Diet was monitored with 3 day diet record, and physical activity with accelerometer. We assessed anthropometric parameters, homeostasis model assessment of insulin resistance (HOMA- IR), flow mediated vasodilation(FMD) and endothelin independent vasodilation(EID) at baseline, 3 months, and 6 months. **Results**: At baseline, the participants' age was 54.9±7.4 years and BMI was 27.2±3.4kg/m^2 (BW: 66.8±8.7kg) without the differences across 3 groups. Body weight (BW) decreased from baseline by 4.9±1.5kg in D, and by 1.4 ±1.6 kg in E during weight loss program (p=0.001, p=0.008), and didn't change significantly during following maintenance in both intervention groups period. *Percent change of FMD from Vaseline were not different among 3 groups at 3 months and improved only in E* at 6 months (*p*=0.032). EID didn't change in both intervention groups at 3 and 6 months. Decrease in HOMA-IR were not significant in D at 3 months (*p*=0.06), and 6 months. HOMA-IR decreased gradually and made significant difference only at 6 months in E (*P*=0.04). **Conclusion**: These results suggest that exercise resulted favorable effects on insulin resistance and endothelia function during weight maintenance, which were not prominent immediate after weight reduction in type 2 diabetes. Diet didn't change insulin resistance and endothelial function despite of significant weight reduction. Nothing to Disclose: KAH, KWM, JHL, KSP, HKL

Pub #	P1-464
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Association between IGFBP-1 Levels, Fatty Liver Disease and Metabolic Syndrome in Obese Children before and after Weight Loss
Author String	T Reinehr, J Woefle, CL Roth Vestische Kinderklinik, Datteln, Germany; University Bonn, Bonn, Germany; Seattle Children's Hospital Research Institute, Seattle, WA

Body

Background: Insulin-like growth factor binding protein 1 (IGFBP-1) is as marker of insulin resistance. We hypothesized that IGFBP-1 is associated to non-alcoholic fatty liver disease (NAFLD) and metabolic syndrome (MetS), disorders which are related to insulin resistance.
Methods: We examined 51 obese children (mean age 12.1±2.3, 55% male, mean BMI 31.8±4.8kg/m[sup2]). Hepatic ultrasound, fasting IGFBP-1, transaminases, waist circumference, glucose, insulin, blood pressure, triglycerides, and HDL-cholesterol concentrations were determined at onset and end of the one-year lifestyle intervention [ldquo]Obeldicks[rdquo]. Briefly, this outpatient intervention is based on physical exercise, nutrition education, and behavior therapy including the individual psychological care of the obese child and his/her family.
Results: IGFBP-1 correlated significantly ($p<0.01$) to most parameters of the MetS (waist circumference:r=-0.45; systolic blood pressure:r=-0.38, diastolic blood pressure:r=-0.30; triglycerides:r=-0.29 HOMA:r=-0.30). In multiple logistic regression analysis adjusted for age, gender, pubertal stage, and BMI, the risk for NAFLD was inversely related with IGFBP-1 (odds ratio 0.35 per additional IGFBP-1 unit; 95%CI 0.16-0.80;p=0.012). The 11 obese children with NAFLD had significantly ($p<0.01$) lower IGFBP-1 levels (1.5±1.3ng/ml) than the 40 obese children without NAFLD (4.2±4.1ng/ml). The 9 obese children with MetS had significantly ($p<0.01$) lower IGFBP-1 levels (1.6±1.3ngm/l) than the 42 obese children without MetS (4.0±3.8ng/ml).
In the course of 1y, changes of IGFBP-1 correlated significantly to changes of HOMA (r=-0.62), and triglycerides (r=-0.22). Separating the children according to their weight loss demonstrated that the 25 children with the greatest reduction of their weight (BMI-SDS -0.74±0.28) reduced their HOMA (-1.1±2.1, p=0.023), triglycerides (-21 ±32mg/dl, p=0.003), ALT (-7 ±14U/l, p=0.025), and AST levels (-4±8U/l, p=0.013), and increased their HDL-cholesterol (+4±9mg/dl, p=0.041). A trend towards an increase of IGFBP-1 was observed (+1.4±1.2 ng/ml, p=0.012).
In the 26 children with the lowest degree of weight loss (BMI-SDS +0.07±0.14), transaminases and IGFBP-1 did not change significantly and no parameter of the metabolic syndrome improved significantly.
Conclusion: The strong relationships between IGFBP-1, insulin resistance, and the associated comorbidities MetS and NAFLD suggest that IGFBP-1 might be a promising marker for these entities in obesity.

Sources of Research Support: TR: received grant support (2008-2010) from of the German Ministry of Education and Research (Bundesministerium für Bildung und Forschung Obesity network: LARGE, grant number 01 GI0839, and National Genome Research Network, NGFNplus (01GS0820)) and Ipsen; CR: received grant support (2003-2004) from Bonfor Research Foundation, University of Bonn, Germany; JW: received grant support from Ipsen.

Nothing to Disclose: TR, JW, CLR

Pub #	P1-465
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Adipose Tissue IL-8 Expression Associates with Abdominal Fat Accumulation and Decreases after Weight Loss
Author String	M Alvehus, K Simonyte, T Andersson, J Buren, I Soderstrom, E Rask, C Mattsson, T Olsson Ume[aring] University Hospital, Ume[aring], Sweden; [Ouml]rebro University Hospital, [Ouml]rebro, Sweden
Body	The menopausal transition is characterized by increased central body fat accumulation, associated with an increased risk of type 2 diabetes and cardiovascular disease (CVD). A putative mediator may be development of a low-grade inflammatory state, in similarity with obesity. Notably, interleukin (IL)-8 has been suggested to be an important link between inflammation and CVD in obesity. We collected anthropometric data, serum blood samples and adipose tissue from subcutaneous adipose tissue biopsies in cohorts of normal weight pre- and postmenopausal women and obese (mainly premenopausal) women before and two years after gastric bypass (GBP) surgery. Serum levels of inflammation-related proteins were assessed and the inflammatory profile of adipose tissue was investigated by real-time PCR. Percentage body fat and waist hip ratios were 30% and 6% higher ($p<0.001$ and $p<0.01$, respectively) in post- compared to premenopausal women, while body mass index (BMI) and waist circumference did not differ. Subcutaneous adipose tissue expression and serum levels of IL-8 were 64% and 41% higher in post- versus premenopausal women ($p<0.05$ and $p<0.01$, respectively). IL-8 expression associated with waist circumference, independent of menopausal status ($\beta=0.51$, $p<0.001$). Adipose tissue IL-6 expression and serum monocyte chemoattractant protein (MCP)-1 levels were 51% and 108% higher in post- versus premenopausal women ($p< 0.01$ and $p<0.001$, respectively). BMI, waist circumference and percentage body fat decreased significantly by 33%, 26%, and 23% after GBP surgery, with a concomitantly significant 267% increase in insulin sensitivity, as estimated by HOMA-IR. Adipose tissue expression of IL-8, MCP-1 and tumor necrosis factor (TNF)-α decreased after GBP surgery-induced weight loss (90%, $p<0.001$; 72%, $p<0.01$; and 63%, $p<0.001$, respectively). Insulin levels associated significantly ($r_s=0.71$, $p<0.01$ for IL-8; $r_s=0.61$, $p<0.05$ for MCP-1; $r_s=0.59$, $p<0.05$ for IL-6; and $r_s=0.59$, $p<0.05$ for TNF-α) with adipose inflammatory gene expression before, but not after, GBP surgery-induced weight loss. Abdominal fat accumulation is linked to an increased low-grade inflammation in adipose tissue and serum, especially of IL-8, in normal weight postmenopausal women. Significant weight loss in obese women is followed by reduced adipose tissue inflammation and improved metabolic outcome. Our data also suggest a link between adipose tissue inflammation and insulin resistance. Nothing to Disclose: MA, KS, TA, JB, IS, ER, CM, TO

Pub #	P1-466
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Limiting Excess Weight Gain in High-Risk Pregnancies: A Randomized Controlled Trial
Author String	CL Harrison, CB Lombard, M Gibson-Helm, A Deeks, HJ Teede Monash University, Melbourne, Australia
Body	Background: Dramatically escalating weight globally has increased the proportion of women entering pregnancy overweight or obese. During pregnancy excessive gestational weight gain (GWG), especially if superimposed on pre-existing excess weight, increases complications including caesarean delivery, maternal hypertension and gestational diabetes mellitus (GDM), as well as increasing long-term obesity risk. With prevention of obesity a WHO priority, pregnancy presents an ideal opportunity to intervene. We have previously shown that a low intensity lifestyle intervention can prevent weight gain in healthy young women. OBJECTIVE: To evaluate the efficacy of an adapted low-intensity lifestyle behavioural intervention aimed at reducing excess weight gain in pregnancy, targeting overweight women at risk of developing GDM. METHODS: In a randomised controlled clinical trial intervention, women who were overweight (Body mass index [ge]25.0kg/m^2 or [ge]23kg/m^2 if high risk ethnicity), <15 weeks gestation and at high risk for developing GDM based on known risk factors (age, weight, ethnicity, family history of diabetes, previous GDM) were recruited from a hospital-based clinic and randomised to intervention (n=106) or control (n=99) group. The intervention group received a 4 session behavioural education intervention (healthy lifestyle, behavioural change and weight gain monitoring). Controls received generic information only and both groups received standard antenatal care. Women were followed from early pregnancy to 6 weeks post-delivery. Here we present the data from 28 weeks gestation, with final data pending. The main outcome variable was weight gain. RESULTS: Mean (±SEM) age and BMI was 32.0±0.3yrs and 30.5±0.42kg/m^2 respectively, with no significant difference between groups. The intervention significantly impacted on excess weight gain at 28 weeks gestation with a mean gain of 5.9±0.3kg (2.3±0.1kg/m^2) versus controls with a mean gain of 6.8±0.3kg (2.7±0.1kg/m^2); p<0.05 [95% CI: -1.7 to -0.04kg]. CONCLUSIONS: A non-intensive behavioural intervention in early to mid-pregnancy significantly reduced excess weight gain compared to education during pregnancy, in overweight women at high risk for GDM in pregnancy. This work has significant public health implications. Sources of Research Support: BRIDGES Grant from the Global Diabetes Foundation. BRIDGES, an International Diabetes Foundation project is supported by an educational grant from Eli Lilly and Company. Nothing to Disclose: CLH, CBL, MG-H, AD, HJT

Pub #	P1-467
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Improving Physical Activity in High-Risk Pregnancies: A Randomized Controlled Trial
Author String	HJ Teede, CL Harrison, M Gibson-Helm, CB Lombard The Jean Hailes Foundation, School of Public Health and Preventive Medicine, Monash University, Melbourne, Australia; Southern Health, Melbourne, Australia
Body	Background: Dramatically escalating weight globally has increased excess weight in pregnancy. Reduced physical activity contributes to excess preconception weight and pregnancy related weight gain, increasing complications (caesareans, gestational diabetes mellitus (GDM)). We have previously developed a validated screening tool to identify high risk GDM women, demonstrated accuracy of pedometers versus gold standard accelerometers in pregnancy and shown that a low intensity behavioural lifestyle intervention improves physical activity in healthy non pregnant women. OBJECTIVE: To evaluate an adapted low-intensity lifestyle behavioural intervention in optimising physical activity in pregnancy in overweight women at risk of GDM. DESIGN and METHODS: In this randomised controlled trial overweight women (body mass index [ge] 25.0kg/m^2) <15 weeks gestation, at high GDM risk, were recruited from hospital-based clinics and randomised to intervention (n=106) or control (n=99). At baseline (hospital booking:12-15 weeks gestation) a 26-28 weeks and at 6 weeks postpartum women had anthropometric and physical activity assessments (Yamax pedometer: steps blinded from participants). Between 15-28 weeks the intervention group received a 4 session behavioural program (healthy lifestyle and behavioural change with physical activity prioritised), compared to controls (a single education session). Both groups received standard antenatal care. Main outcomes were weight gain (reported elsewhere) and physical activity. RESULTS: 28 weeks gestation data is presented with postpartum results pending. Mean (±SEM) age was 32.0±4.6yrs and BMI was 30.5±6.1kg/m^2 with no significant differences between groups (p=0.3). Pedometer data was complete for n=79 interventions and n=69 controls. Baseline steps were similar in both groups (6090±348 vs 5574±349steps/day, p=0.3). By 28 weeks both groups had reduced step counts, yet the intervention group had a 23% higher step count than controls (5225±381 vs 4251±288 steps/day, p<0.05). CONCLUSIONS: A non-intensive behavioural intervention in early to mid-pregnancy reduces pregnancy related decline in physical activity, compared to education alone, in overweight women at high GDM risk, with significant public health implications. Sources of Research Support: International Diabetes Foundation project is supported by an educational grant from Eli Lilly and Company. Nothing to Disclose: HJT, CLH, MG-H, CBL

Pub #	P1-468
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Hypogonadism among a Population of Obese Men: Prevalence, Risk Factors, Mechanisms and Reversibility after Weight Loss Induced by Gastric Bypass Surgery
Author String	V Ippersiel, A Lepot, D Gruson, J Jamart, D Maiter, J-P Thissen Mont-Godinne Academic Hospital, University of Louvain, Yvoir, Belgium; Saint-Luc Academic Hospital, University of Louvain, Brussels, Belgium; Mont-Godinne Academic Hospital, University of Louvain, Yvoir, Belgium
Body	**Context**: Obesity in men is frequently associated with low levels of testosterone, loss of libido and/or erectile dysfunction. Several mechanisms have been proposed to explain this hypogonadism. **Objective**: To estimate the prevalence of hypotestosteronemia among a population of obese men, to determin the risk factors and the mechanisms for this condition, and to study its reversibility after significant weight loss obtained by gastric bypass surgery. **Design**: A prospective study including 75 consecutive patients between 2007 and 2010. **Setting**: Obesity clinic in a teaching academic hospital. **Patients**: 75 men aged 43±11 yr with a body mass index (BMI) 41.3±7.3 kg/m[sup2] were studied at baseline Fasting levels of total (TT) and free (FT) testosterone, sex hormone binding globulin (SHBG), LH and FSH, estrone (E1), estradiol (E2), prolactin, IGF-1, lipid profile and HbA1c were measured in the morning. 75-g oral glucose tolerance and HOMA tests were also performed. Body composition was assessed by bioelectrical impedance and ADAM (Androgen Deficiency in Aging Males) questionnaire was recorded. Among these patients, 17 underwent bariatric surgery and were re-evaluated after 3 and 12 months. **Results**: At baseline, 39% of obese men had hypotestosteronemia, while the ADAM questionnaire was positive in 93%. Mean levels of TT and FT were 10.9±2.4 and 0.195±0.052 nmol/L, respectively. TT and FT were inversely related to BMI ($p<0.05$), while TT also correlated negatively with waist circumference ($p=0.012$) and body fat mass ($p=0.022$). No significant correlation was found between testosterone and glucose, HbA1c or HOMA-S. SHBG and LH correlated positively with BMI ($p<0.05$), but not E1 and E2 levels. After bypass surgery, weight loss (-40.6±16.3 kg) was associated with an increase in TT (+3.97±2.24 nmol/L, $p=0.001$) but not FT. Erectile function was also improved. A significant decrease was also observed for E2 ($p<0.05$) and the ratio E2/TT, reflecting reduction in aromatase activity.There were no changes in SHBG, LH and E1 levels. **Conclusion**: Low testosterone levels are frequently observed among obese men and correlated with the degre of abdominal adiposity, but not strongly associated with sexual dysfunction. Weight loss induced by gastric bypass surgery leads to normalized TT and to decreased E2 and E2/TT ratio, suggesting a role of excessive aromatization in the hypotestosteronemia associated with obesity. Nothing to Disclose: VI, AL, DG, JJ, DM, J-PT

Pub #	P1-469
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Hormonal Response to Natural Dietary Antioxidants in Obese Patients with Insulin Resistance: Multivariate Analysis Approach
Author String	A Mancini, M Magini, R Festa, S Raimondo, E Cavallaro, MC Mele, GAD Miggiano, A Pontecorvi, GE Martorana Catholic University of the Sacred Heart, Rome, Italy; Catholic University of the Sacred Heart, Rome, Italy; University of Marche, Ancona, Italy; INRCA, Ancona, Italy; Catholic University of the Sacred Heart, Rome, Italy
Body	In previous works, we have observed that natural dietary antioxidants ameliorate insulin-sensitivity in insulin-resistant (IR) obese subjects, lowering HOMA-IR and enhancing the effect of insulin-sensitizing drugs at a significant extent. In order to delve the molecular mechanisms linking oxidative stress (OS), IR and metabolic syndrome (MS)-related manifestations, we have extended the study investigating the effects of dietary antioxidants on biochemical and hormonal parameters (basal glycemia, oral glucose tolerance test, total- LDL and HDL-cholesterol, triglycerides, uric acid, albumin, transaminases, C reactive protein, insulin, IGF-1 and thyroid hormones) in a larger group of obese (n= 55, 19 males and 36 females, 18-66 ys, mean±SD BMI 36.3±5.5 Kg/m^2), with IR defined as HOMA index >2.5. Patients were divided in four groups according to different treatments: hypocaloric diet without (group A) or with metformin 1000 mg/day (group B); hypocaloric diet rich in natural antioxidants without (group C) or with metformin (group D). A personalized program, with mean caloric intake of 1500 Kcal, 25% proteins, low glycemic index CHO and a calculated antioxidant intake of 800-1000 mg/daily, from fruits and vegetables, was administered to group C and D. Analysis of variance for repeated measures was performed to evaluate the effect of the treatments in the four groups considered as a single group (F1) and the differences among the four treatment groups (F2). F1 showed a significant decrease ($p<0.05$) in BMI, total- and LDL-cholesterol, triglycerides, sGOT, CRP, free-T3, free-T3/free T4 ratio and a significant increase in IGF-1, without significant differences among groups. F2, instead, showed a significant decrease in insulin peak, insulin area under the curve in OGTT and HOMA index, due to the particular response of group D with respect to the other groups : -[Delta] (CI-95%) = 97 (153-42), 7869 (13504-2233), and 2.5 (5-0), respectively. These data suggest that some variations are related to body weight decrease, irrespective of the different treatments, while insulin response is specifically affected by the diet enriched with natural antioxidants, suggesting an independent mechanism, which could be related to OS influence on IR. Nothing to Disclose: AM, MM, RF, SR, EC, MCM, GADM, AP, GEM

Pub #	P1-470
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Weekly Administration of Sustained-Release Growth Hormone Reduces Abdominal Visceral Fat and Waist Circumference in Adults with Abdominal Obesity
Author String	JW Hong, WK Lee, YD Song, EJ Lee Yonsei University College of Medicine, Seoul, Republic of Korea; National Health Insurance Cooperation Ilsan Hospital, Ilsan, Republic of Korea
Body	**Objectives:** Administration of recombinant human growth hormone (rhGH) in obesity has been known to lead to a decrease in visceral adiposity and an increase in lean body mass. Most studies have used supraphysiologic doses of rhGH which were administered daily or every other day. We aimed to evaluate whether weekly administered low dose of sustained-release rhGH (SR-rhGH) could play a therapeutic role in the treatment of abdominal obesity. **Methods:** Prospective, single-arm, open-label, multicenter pilot study was carried out. Participants were 26 adults, 40-65 years old with abdominal obesity (male: waist circumference >90cm, female: waist circumference>85cm). The subjects were given 3 mg of SR-rhGH, administered subcutaneously, weekly for 26 weeks. The main outcome was measured using fat distribution, body composition, and waist circumference. **Results:** After 26 weeks, SR-rhGH treatment reduced abdominal visceral adipose tissue (VAT) (140.35 ± 75.97 to 128.43 ± 73.85 cm^2, p= 0.0038) and subcutaneous adipose tissue (SAT) (198.32 ± 55.57 to 185.77 ± 56.25 cm^2, p=0.05). In the subgroup analysis for abdominal VAT according to sex and age, VAT significantly decreased after GH treatment in women only (p=0.0146), and not men (p=0.1681). When classified by age, only subjects in their 60s showed significant change in VAT after GH treatment (p= 0.0013). Average waist circumference decreased from 96.25 ± 6.41 to 91.93 ± 6.13 cm (p<0.0001) after treatment. However, body weight or lean body mass did not show any significant change. There were no severe adverse events or drop-outs during the study. **Conclusions:** SR-rhGH treatment for 26 weeks reduced abdominal visceral/subcutaneous fat and waist circumference. Further studies may be considered on the role of weekly administered SR-rhGH as a treatment for abdominal obesity, especially in older women subjects. Nothing to Disclose: JWH, WKL, YDS, EJL

Pub #	P1-471
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Efficacy and Safety of Topiramate in Weight Loss: A Meta-Analysis of Randomized Controlled Trials
Author String	CK Kramer, CB Leitao, LC Pinto, LH Canani, MJ Azevedo, JL Gross Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil
Body	Topiramate was associated with weight loss in clinical trials. Conversely topiramate is not an approved drug for the treatment of obesity. Data from individual trials might not be sufficient to support clinical decision, and a robust evidence of its safety is lacking. Therefore, the aim of the present study was to assess the benefits and harms of topiramate in the treatment of obesity by a systematic review and meta-analysis of randomized controlled trials. The databases Medline, Embase, and Cochrane were searched (beginning in 1950 to April 2010). Randomized controlled studies with at least 16 weeks of duration that report the effect of topiramate on weight loss and adverse events were eligible for inclusion. Ten studies were included (3320 individuals). Patients treated with topiramate lost an average of 5.34kg (95%CI -6.12 to -4.56) of additional weight as compared to placebo. According to meta-regression analysis, treatment duration and dosage were associated with the efficacy of topiramate treatment. Evaluating trials using topiramate 96-200 mg/day the weight loss was higher in trials with >28 weeks of duration [-6.58kg (95%CI -7.48 to -5.68)] than in trials with less than 28 weeks [-4.11kg (95%CI -4.92 to -3.30)]. In an analysis considering weight loss greater than 5%, the chance of significant weight loss was higher in the topiramate treatment group than the placebo: pooled OR 6.02 (95%CI 4.81 to 7.53) (I^2 31.1%, P = 0.11), and the number needed to treat (NNT) was 2.63. The same was true for weight loss greater than 10%: OR 7.16 (95%CI 5.48 to 9.36) (I^2 17.8%, P = 0.25), and NNT = 3.7. Data of 6620 individuals were available for adverse events evaluation and those more frequently observed were paresthesia [OR 8.70 (95%CI 6.90 to 11.0)], taste impairment [OR 8.61 (95%CI 5.35 to 13.87)], and psychomotor disturbances [OR 7.82 (95%CI 3.71 to 16.46)]. The OR for adverse events leading to topiramate withdrawal was 1.94(95%CI 1.64 to 2.29) compared to the control group, corresponding to a number needed to harm (NNH) of 13.7. In conclusion, our findings suggest that topiramate[acute]s prescription as an anti-obesity agent is associated with substantial weight loss. However unpleasant side effects were a limitation of its use and proper warnings about side effects are needed for topiramate prescription. These findings suggest that topiramate might be a useful adjunctive therapeutic tool in the treatment of obesity. Nothing to Disclose: CKK, CBL, LCP, LHC, MJA, JLG

Pub #	P1-472
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Formoterol, a Highly β2-Selective Adrenergic Agonist, Increases Energy Expenditure, Fat Utilization and Protein Anabolism in Men
Author String	P Lee, RO Day, V Birzniece, S Sutanto, JR Greenfield, KKY Ho Garvan Institute of Medical Research, Sydney, Australia; St Vincent's Hospital, Sydney, Australia; St Vincent's Hospital, Sydney, Australia

Body

The sympathetic nervous system regulates energy expenditure (EE) and substrate metabolism. Sympathetic stimulation of β2-adrenoceptors in adipose tissue and skeletal muscle enhances fat utilisation and protein anabolism (1). However, therapeutic exploitation of β2-adrenegic agonism for these metabolic benefits had been hindered by limited specificity of available β2-adrenergic agonists, which cross-stimulate cardiac β1-adrenoceptors and induce tachycardia. Formoterol is a new generation, highly β2-selective adrenergic agonist The metabolic effects of formoterol in humans have not been studied.

The aim is to investigate the effects of formoterol on energy and protein metabolism. We undertook a) a dose-finding study in 4 subjects, administered 80, 160 and 320 [mu]g daily of oral formoterol for 1 week each and b) a detailed metabolic evaluation in 8 men before and at the end of 1 week treatment. EE and fat oxidation (Fox) were quantified by indirect calorimetry with diet-induced thermogenesis measured over 120 minutes after a standardised meal. Changes in whole body protein metabolism were assessed using a 3-h primed constant infusion of 1-[13C]leucine, from which rates of leucine appearance (LRa) and leucine oxidation (Lox) were estimated. Statistical analysis was performed after log-transformation where appropriate.

In the dose finding study, 160 [mu]g achieved a maximal increase in resting EE and Fox without inducing tachycardia. In the metabolic study, this dose increased resting EE by 13±2% ($p<0.001$), Fox by 23±4% ($p<0.01$) but not heart rate ($p=0.2$). Basal plasma non-esterified free fatty acid concentration rose by 16±2% ($p<0.05$). Post-prandial EE was enhanced by 10±3% ($p=0.03$). Formoterol significantly reduced LRa ($p<0.001$) and Lox ($p<0.01$) by 9±2% and 14±3%, respectively. Lox as a proportion LRa was significantly lower after formoterol treatment by 6±1% ($p=0.03$).

In summary, formoterol 160 [mu]g/day increases resting energy expenditure, fat utilisation and protein anabolism, without inducing tachycardia. From this first metabolic evaluation of formoterol in humans, we conclude that formoterol imparts beneficial metabolic changes and may be a potential therapy for obesity and sarcopaenia.

(1) Lynch GS et al., Physiol Rev. 2008; 88: 729-67

Sources of Research Support: National Health Medical Research Council Australia.

Nothing to Disclose: PL, ROD, VB, SS, JRG, KKYH

Pub #	P1-473
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Letrozole Normalizes Serum Testosterone but Has No Clinical Effects in Men with Obesity-Related Hypogonadotropic Hypogonadism
Author String	S Loves, J de Jong, A van Sorge, D Telting, A Hermus, H de Boer Rijnstate Hospital, Arnhem, Netherlands; Rijnstate Hospital, Arnhem, Netherlands; Rijnstate Hospital, Arnhem, Netherlands; Rijnstate Hospital, Arnhem, Netherlands; Radboud University Medical Centre, Nijmegen, Netherlands
Body	Introduction: Hypogonadotropic hypogonadism is frequently observed in morbidly obese men, due to aromatase-dependent conversion of androgens to estrogens in adipocytes. The clinical impact of this sex hormone imbalance is not known. Aim: To evaluate the clinical effects of aromatase inhibition in obesity-related hypogonadotropic hypogonadism. Methods: Double-blind, placebo-controlled, 6-month trial in severely obese men (BMI > 35 kg/m^2) with obesity-related hypogonadism (serum total testosterone < 10 nmol/l). Predefined drug regimen (letrozole or placebo): Starting dose 1 tablet/week, subsequent dose escalation every month up to a maximum of 7 tablets/week or until a serum total testosterone of 20 nmol/L. The dose was reduced if serum estradiol decreased below 40 pmol/L. Results: 42 patients were included and 39 completed the study according to protocol: 18 on Letrozole and 21 receiving placebo. Mean age 44.6 ± 1.1 years (mean ± SE), BMI 41.1 ± 0.8 kg/m[sup2]. At baseline, both groups were well matched for all study parameters. Placebo treatment did not affect serum hormone levels, whereas Letrozole decreased serum estradiol from 119.1 ± 10.1 to 59.2 ± 6.1 pmol/L (P = 0.0001, normal range (NR) 40 - 160 pmol/L), increased serum LH from 3.3 ± 0.3 to 8.8 ± 0.9 U/L (P < 0.0001, NR: 2.0 - 9.0 U/L) and free testosterone from 244 ± 19 to 691 ± 39 pmol/L (P < 0.0001, NR: 225 - 625 pmol/L). Both groups demonstrated a comparable decrease in body weight of about 5 kg, and a decrease in abdominal circumference of about 4 cm. Changes in fat free mass, fat mass and bone density also did not differ between groups. Glucose metabolism, lipid profiles, physical exercise capacity and psychological characteristics did not change during treatment. Conclusion: Despite a marked rise in serum free testosterone, low dose aromatase inhibition had no somatic o psychological effects in men with obesity-related hypogonadotropic hypogonadism. We hypothesize that, witl respect to non-sexual somatic and psychological parameters, males primarily thrive on oestrogens, not testosterone. Sources of Research Support: Novartis. Disclosures: SL: Independant clinician/investigator, Novartis Pharmaceuticals. HdB: Independant clinician/chief investigator, Novartis Pharmaceuticals. Nothing to Disclose: JdJ, AvS, DT, AH

Pub #	P1-474
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Synergistic Effect of Spironolactone and Fluexetin for Weight Reduction Is Superior to Sibutramine
Author String	S Sakkal, M Sakkal, M Mounla-Sakkal Metablic Care Center, Aleppo, Syrian Arab Republic; Kalamoon University, Deir Atieh, Syrian Arab Republic
Body	**Objective:** Weight reduction is a challenge in modern practice. Lifestyle change is the cornerstone but medications have an important supporting role. Medications used separately fails since the many pathways effecting satiety,energy, and weight need combination therapy. We tested synergy of 2 medications compared to Sibutramine. **Methods:** 250 patients, who reached maximum weight reduction from exercise, diet were given one of five: 1) Sibutramine. 2) Spironolactone. 3) Fluextine.4) Spironolactone and Fluexetine.5) all three (50 patients in each group). Measurements were made for weight, height, waist, hip,W/H, W/Ht,metabolic outcomes before and after intervention. 25 patients did not complete the study. Data for mean averages available for 225 participants, age 35 (16-58). **Results:** *At baseline* : Mean Weight was 90.6 Kg , Height 160.2 Cm, BMI 35 , Waist 105.3 cm ,hip 115.5 , W/H 0.93 , W/Ht 0.65 , TG 99 , Cholest. 190 , LDL/HDL 2.9 , TG/HDL 1.9 ,FBS 90. *After therapy* :Mean weight became 79 Kg, BMI 30 , waist 98 cm ,hip 111, W/H0.88 , W/Ht 0.61 , TG ,Cholest ,LDL/HDL ,FBS were all normal. Percentage difference between baseline and after intervention were: Weight %11.4 , BMI %15 ,waist %7, hip%4.5,W/H %5 ,W/Ht %6.4. Statistical analysis showed significance for all. After 3 months of therapy difference was more profound in between groups as seen in the following:Wt Before Sibutramine89.4*, Wt After79*, hange.Kg10.4,%change11.7.Wt Before Spirono91.2**, Wt After85**, Change.Kg6.2,change6.7 Wt Before Fluexet 90.4**,Wt After86**, Change.Kg4.4,%change5.Wt Before Spir/Flu. 92*, Wt After78* ,Change.Kg14,%change15.Wt Before All three93*, Wt After74*, Change.Kg19, %change20.P value: *0.001 **0.05. **Discussion**: With pro active weight reduction program, of exercise prescription and healthy diet, medications gives a good supporting role. Some are better used in synergy, to minimize side effects and increase benefits. Spironolactone alone or Fluexetine alone have some benefit (%5-7) but the synergy between the two may exceed the effect of Sibutramine (%15 Vs %12).When all three are used the benefit is enhanced without side effects increase. The drop out is acceptable. The benefits are decrease in central obesity, BMI, waist, waist/hip ratio and possibly metabolic syndrome incidence. **Conclusion: Synergy** between Spironolactone and Fluexetin is very promising, safe combination for weight reduction. It may exceed sibutramine effect, decrease central obesity, BMI, and W/H ratio. Nothing to Disclose: SS, MLS, MM-S

Pub #	P1-475
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Does [ldquo]Endermologie[rdquo] Affect BMI or Central Obesity or Prevent the Metabolic Syndrome? A Metabolic Perspective
Author String	ML Sakkal, S Sakkal, MM Sakkal Metabolic Care Center, Aleppo, Syrian Arab Republic; Kalamoon University, Deir Atieh, Syrian Arab Republic

Body

Objective:

Endermologie is used to alter fat in the subcutaneous adipose tissue.Few studies done on effect for body contouring,and none on metabolic effects.. We tested its metabolic effects on weight,BMI, central obesity and waist to height ratio(W/Ht).

Methods:

33 healthy women (age17-48), who reached maximum benefit of weight reduction program. Measurements made for wt, ht, waist, hip, thigh, upper arm, W/H, W/Ht, TG ,Chol. ,LDL/HDL,TG/HDL, FBS before and after Endermologie. Each session, after hydration, included negative lymph nodes suctioning,kneading message, followed by peripheral compression for 20 minutes each. Patients were their own control, satisfaction questionnaire, P Value used for comparison.

Results:

Baseline : Mean Wt was 82.4 Kg , Ht 161.3 Cm, BMI 31.9 , Waist 105.3 cm ,hip 115.5 ,thigh 67.8 ,upper arm 36 , W/H 0.93 , W/Ht 0.66 , TG 97 , Cholesterol 193 , LDL/HDL 2.8 , TG/HDL 1.8 ,FBS 93.

After Endermologie: Mean Wt and(percentage) became 78.5 Kg(%9.2), BMI 29.7(%7.8) , waist 97.5 cm(% 7.3) ,hip109.6(%4.8) , thigh 64.3(%7.6) , upper arm 33.1(%8.8) W/H 0.89(%3) , W/Ht 0.60(%6). TG , Ch , FBS were same.Patients satisfaction was %90.

Statistical analysis showed significance for all contour parameters, P value of 0.001(Weight, BMI, waist, thigh, upper arm, W/Ht). P value of 0.005 (hip, W/H).

Patients completing 20 sessions had more benefits : lessWt%10.6,BMI%10.5 , waist %9.1, hip%6.6 ,thigh% 11.4 ,upper arm %11.4,W/H%2.7,W/Ht %6.7.

Discussion:

Study shows significant benefit in overweight and obese individuals who reached maximum benefit of robust weight reduction program. The changes in Wt,BMI, waist, W/Ht, has many known benefits for postponing the metabolic syndrome in addition to its significant psychological cosmetic and circulatory benefits.

Participants has no evidence of metabolic abnormality despite their abnormal Waist and may represent an early stage when prevention of Met Synd. might be ideal. Longitudinal studies are needed to answer the question of Endermologie impact on incidence of the Metabolic Syndrome.

In the largest published study in the literature; 14 sessions of treatments showed a reduction in of 1.83 cm. (1)

Conclusion:

When done correctly, Endermologie is very effective in reducing Weight ,BMI , Waist approximately %8-10 and waist to Height ratio by %5 which has a very satisfactory effects to participants from cosmetic and psychological perspective and, may have a significant impact on incidence of Metabolic Syndrome.

(1)Chang P, Wiseman J, Jacoby T, Salisbury AV, Ersek RA Aesthetic Plast Surg. 1998 Mar-Apr; 22(2):145-53).

Nothing to Disclose: MLS, SS, MMS

Pub #	P1-476
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Laparoscopic Adjustable Gastric Banding (LAGB) Failure: Experience of a Single Center
Author String	I Malik, R Marcus, W Andrew, B Gul St George's Healthcare NHS Trust, Tooting, London, UK
Body	**BACKGROUND:**The percentage of excess weight loss (%EWL) reported in literature for LAGB varies between 30% & 63% at 1 year. Inadequate weight loss is an important cause of LAGB failure, removal of gastric band and major re-operation. It is therefore vital that LAGB should not be considered the procedure of choice for all forms of obesity and strict selection criteria should be employed before this procedure is chosen over other bariatric surgeries. **OBJECTIVE:**The main objective of this study was to analyse the causes of gastric band failure, and complications associated with the procedure in a single centre. **METHODS:** A retrospective notes review was carried out on patients who had undergone LAGB but the procedure was considered to be a failure during the period between 1997 and 2004. Failure of LAGB was defined as an excess weight loss (EWL) of < 30% at one year; or the development of major band-related complication leading to band removal and/or conversion to another bariatric procedure. **RESULTS:**Between 1997 and 2004, 24 patients were identified with LAGB failure. The most common reason for LAGB failure was inadequate (<30% EWL) weight loss in 37.5% (9/24) of patients due to poor compliance with dietary restrictions. Five of these nine patients, after pre-operative assessment, were advised against LAGB and were advised to have another bariatric procedure. This was based on a perceived lack of motivation to follow restricted diet following gastric banding. A negative correlation (r=-3) was found between pre-op BMI and %EWL at 1 year. Other causes of LAGB failure were band erosion in 25% of cases and this required removal of the band; symptoms due to oesophageal dilatation and band slippage which occurred in 16.6% and 4.2% of patients respectively. Overall gastric band had to be removed in 58.3% (14/24) of patients, the causes being band erosion (43%); symptomatic oesophageal dilatation (28.5%); inadequate weight loss (14.3%); band slippage (7.1%) and pouch dilatation (7.1%). Six out of 14 patients, who had gastric band removed, underwent second bariatric operations. **CONCLUSIONS:**In this study inadequate weight loss is the main cause of gastric band failure. There is also a high rate of other complications associated with LABG. Pre-operative BMI and psychological assessment o eating behaviour could predict post-operative weight loss and could guide better choice of bariatric procedure Nothing to Disclose: IM, RM, WA, BG

Pub #	P1-477
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Bariatric Surgery Alters the Cortisol/Cortisone Ratio in Adipose Tissues to Levels Lower Than Those in Leaner Controls
Author String	P Methlie, TS Myhra, SN Dankel, J Gjerde, DJS Fadnes, BJ Christensen, V Vage, G Mellgren University of Bergen, Bergen, Norway; Haukeland University Hospital, Bergen, Norway; F[oslash]rde Central Hospital, F[oslash]rde, Norway; F[oslash]rde Central Hospital, F[oslash]rde, Norway; Haukeland University Hospital, Bergen, Norway; University of Bergen, Bergen, Norway

Body

Background And Aims

Accumulating evidence suggests that glucocorticoid dysregulation plays an important role in obesity. The intracellular enzyme 11β-hydroxysteroid dehydrogenase type I (11β-HSD1) reactivates cortisol from cortisone. Hence, tissue-specific modulation of cortisol by 11β-HSD1 could be important in the pathogenesis of obesity. Bariatric surgery is an effective therapeutic option, but only few studies have examined the impact of surgery on glucocorticoid levels in adipose tissues. Most clinical studies on the tissue metabolism of glucocorticoids are based on *11HSD1* gene expression analysis, which may not be sufficient because other factors could influence hormone levels. Liquid chromatography mass spectrometry offers highly sensitive and specific measurements of glucocorticoids in adipose tissue. The ratio cortisol/cortisone (F/E-ratio) provides a possibly more accurate assessment of 11β-HSD1 activity *in vivo*. We aimed to compare subcutaneous adipose tissue (SAT) collected from patients one year after bariatric surgery to SAT from leaner controls, utilizing gene expression analyses (qPCR) and direct hormone measurements.

Results

SAT was collected from 15 obese subjects (9 [female]) one year after bariatric surgery and from 22 leaner controls (8 [female]) undergoing elective hernia repair. Their median (range) BMI were 35 (26-39) and 24 (20-32) kg/m^2, respectively. The F/E-ratio after bariatric surgery was lower compared to controls (median 2.08 vs. 3.28, $p<0.01$), but *11HSD1* expression did not differ (0.76 vs. 0.98, $p=0.21$). F/E-ratio and *11HSD1* expression correlated strongly in the bariatric surgery group (Spearman's rho 0.82, $p<0.001$), and moderately in the control group (rho 0.61, $p<0.01$). BMI after bariatric surgery correlated highly with *11HSD1* expression (rho 0.86, $p<0.00001$), while the association was weaker in the leaner subjects (rho 0.36, $p=0.10$). BMI correlated with F/E-ratio after bariatric surgery (rho 0.77, $p<0.01$) and in controls (rho 0.53, $p<0.05$).

Conclusions

We show that subjects having undergone bariatric surgery have lower F/E-ratio in SAT than the leaner controls. These data suggest that bariatric surgery alters the glucocorticoid metabolism in SAT in direction of lower cortisol regeneration, despite the higher BMI post-surgery. Even so, these subjects tended to have stronger associations between the glucocorticoid status and BMI, supporting the hypothesis that altered metabolism of cortisol plays a causative role in obesity.

Sources of Research Support: Samarbeidsorganet Helse Vest RHF, Meltzerfondet and Programstyret for ern [aelig]ring at the University of Bergen.

Nothing to Disclose: PM, TSM, SND, JG, DJSF, BJC, VV, GM

Pub #	P1-478
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Progressive Resistance Exercise Training in Class III Obese Surgical Candidates
Author String	RL Miciek, TW Storer, A Elmi, A Thompsen, A Nahigyan, A Bourland, C Apovian Boston Medical Center, Boston, MA; Boston University School of Medicine, Boston, MA
Body	**Background**: Class III obese (BMI [ge] 40) patients are the fastest growing segment of the obese population. Current medical therapies remain inadequate. Weight loss surgery is effective but associated with complications, increased costs and may adversely affect quality of life. Exercise may be one way to improve health and fitness in obese patients with resistance training particularly beneficial in modifying body composition and improving physical function. To date there has been no investigation into the role of intense progressive resistance exercise (PRT) to improve physical performance and mobility in Class III obese subjects. We hypothesized that these patients would adhere to a 10 wk PRT program and would demonstrate significant improvement in muscle performance and physical function. **Methods**: A total of 14 Class III obese patients (BMI=46.3±4.5) were recruited. Baseline and end of study assessments were conducted for muscle strength, muscle endurance, 6-min walk distance (6-MWD) and short physical performance battery (SPPB). Training consisted of twice weekly sessions with nine major muscle group exercises. Weeks 1-2 consisted of two sets of 12 repetitions (reps) with loads perceived as somewhat hard using Borg's 6-20 RPE scale. Thereafter, loads were increased as tolerated with the aim of completing at least 8 reps but not more than 12 with RPE 16-20. Adherence was defined as number of sessions attended divided by 20 possible sessions. **Results**: Eight (2 males) of the 14 subjects completed the study averaging 82% adherence. The remaining six subjects attended 36% of sessions for an overall adherence rate of 61%. For the completers, BMI decreased by 2.7 kg/m^2. The change in total weight lifted for the nine exercises from wks 1 to 10 was 116% (±63%), P=0.029. Leg press and chest press 1-RM improved by 21% (±19%) and 13% (±10%), P=0.025 and P=0.013, respectively; leg press endurance improved 110% (±91%), P=0.003. Chest press endurance and SPPB scores did not change. **Conclusions**: We have demonstrated that morbidly obese individuals are able to adhere to 10 weeks of PRT and more than double their training stimulus. Moreover, substantial improvements in muscle strength and endurance were observed. We conclude that PRT is a feasible intervention for Class III obese patients. Future studies should examine additional outcomes such as modulation of the inflammatory process as well as postoperative complication rates in those undergoing bariatric surgery. Nothing to Disclose: RLM, TWS, AE, AT, AN, AB, CA

Pub #	P1-479
Session Information	POSTER SESSION: CLINICAL - Obesity: Assessment & Treatment (1:30 PM-3:30 PM)
Title	Correlation between Acute Reduced Circulating Acylation Stimulating Protein and Early Insulin Resistance Improvement after BPD-DS Gastric Surgery in Morbidly Obese Diabetes Subjects
Author String	MN Munkonda, J Martin, A Carrington, P Poirier, K Cianflone Institut Universitaire de Cardiologie et de Pneumologie, Québec, Canada; University of the West Indies, Cave Hill, Barbados
Body	***Background*:** Physiological mechanisms involved in early resolution of insulin resistance and type 2 diabetes mellitus after biliopancreatic diversion with duodenal switch surgery (BPD-DS) are still unknown. Our objective was to evaluate the early effects of BPD-DS on level of Acylation Stimulating Protein (ASP), an adipokine involved in lipid and glucose metabolism. We hypothesized an association between acute ASP response and insulin resistance and type 2 diabetes improvement. ***Methods*:** Fifteen men and fifty-four women, 18 years of age or older, with an indication for bariatric surgery (BMI[ge] 40 kg/m2 or BMI [ge] 35 kg/m2 with risk factor) were invited to participate in this study. Patients were examined before and 1 day, 5 days, 6 and 12 months after surgery. ***Results*:** The mean level of ASP, C3, insulin, glucose, HOMA-IR, cholesterol, HDL-cholesterol, LDL-cholesterol were decreased significantly during the first 5 days and over 12 months after BPD-DS in all groups. Acute change of ASP and C3 levels correlated significantly with early change in glucose, fructosamine, HOMA β-cell, HOMA-IR, HOMA-IS and lipoprotein ratio, all markers of insulin resistance and diabetes improvement. ***Conclusions*:** We established evidence that acute down regulation of ASP and C3 level is associated with early improvement of insulin resistance and type 2 diabetes after BPD-DS surgery through normalization of both glycemia and insulin secretion. Sources of Research Support: Canadian Institutes of Health Research. Nothing to Disclose: MNM, JM, AC, PP, KC

Pub #	P1-480
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Insulin Action & Gut Hormones (1:30 PM-3:30 PM)
Title	GLP1 Receptor Agonists Reduce Food Intake While Differentially Activating Neurons in the Hypothalamus and Hindbrain
Author String	K Coughlan, J Jones, D Kubasiak, T Gareski, TJ Unger, S Will, A Qadri, X Li, M Perreault Pfizer, Cambridge, MA
Body	GLP1 and oxyntomodulin (Oxm) are structurally related gut peptides that are released by L-cells in response to food intake. Administration of these peptides and their analogs has been shown to decrease food intake in rodents and humans. In order to elucidate the central action of various GLP1R agonists, we examined neuronal c-fos expression in the hypothalamus and hindbrain in mice 1 and 3 hours following peripheral administration (IP dose, 80ug/kg) of Oxm, Exendin 4 (Ex-4), Liraglutide, and a stable oxyntomodulin analog peptide (OAP). Each of these peptides has an EC50 ranging from 0.2 to 7 nM against the mouse GLP1 receptor and all analogs, except Oxm, significantly reduced food intake in mice following peripheral administration. Ex-4 was the most potent peptide tested and produced significant c-fos activation 1hr post dose in the Nucleus of the Solitary Tract (NTS), Dorsal Motor Nucleus of the Vagus (DMNV), and Area Postrema (AP) of the hindbrain but this activation in the hindbrain largely subsided by 3hrs. Interestingly, Ex-4-induced neuronal activation was seen in the Arcuate nucleus (ARC) of the hypothalamus 3hrs, but not 1hr post-dose, consistent with the known GLP1-ergic pathway in which enteroreceptive GLP-1 expressing neurons in the NTS project to GLP-1R expressing areas of the hypothalamus, including the ARC. OAP and Oxm both showed trends of c-fos activation in the NTS at 1 hr, but OAP only showed activation in the NTS plus the rostral DMNV at 3hr. Liraglutide did not significantly increase neuronal c-fos activation in any brain region examined at the 1hr timepoint, but c-fos activation was significantly increased in the AP and rostral DMNV, as well as the medial ARC at 3hr. These results demonstrate that while these peptides all act through GLP1 receptors in the brain to reduce food intake in rodents, there are differences in the timing of neuronal activation in specific regions of the hypothalamus and hindbrain, likely due to differences in structure, stability and/or binding properties. Nothing to Disclose: KC, JJ, DK, TG, TJU, SW, AQ, XL, MP

Pub #	P1-481
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Insulin Action & Gut Hormones (1:30 PM-3:30 PM)
Title	The Insulin Receptor Is Closely Associated with Caveolae on the Surface of the Endothelial Cell
Author String	CT Upchurch, Z Liu, EJ Barrett University of Virginia, Charlottesville, VA

Body

Insulin's movement from the capillary plasma compartment to the interstitium of skeletal muscle is rate limiting for insulin mediated glucose disposal, and yet the mechanism for this step has not been firmly established. Previous studies have shown that insulin moves across the tight endothelial barrier of the muscle capillary not by paracellular leakage but rather by a transendothelial mechanism which is dependent on the insulin receptor (IR) of the endothelial cell's (EC) plasma membrane. Previous studies have also suggested that the IR is closely associated with the plasma membrane structures known as caveolae. Since caveolae are known to be involved with endocytosis, it is possible that caveolae serve as the site of insulin uptake by the endothelial cell. Total Internal Reflection Fluorescence (TIRF) microscopy is an optical microscopy technique that provides highly resolved images of structures at or near the plasma membrane (PM). For this study, we utilized TIRF microscopy to study the association between the IR and the caveolae protein CAV1 on the surface of the EC.

Bovine aortic endothelial cells (bAECs) were grown in complete cell media, fixed, permeabilized, and then double stained with primary antibodies to IR and CAV1 and species specific secondary antibodies. Using TIRF microscopy, we observed a very high degree of co-localization between the IR and CAV1 within 70nm of the cell surface. Quantifying this association demonstrated a $97 \pm 6\%$ overlap of the IR with CAV1.

We then examined the effect of serum starvation on the cell surface content and co-localization of IR and CAV1. Serum starvation for 16 hrs increased the fluorescence intensity of the IR and CAV1 at the cell surface by 200% and 198%, respectively ($p=.002$, for each). Adding insulin (50 nM) to the serum free media did not affect the intensity of IR but decreased that of CAV1 ($p=\leq.07$) at the cell surface. The overlap of the IR with CAV1 did not differ between the serum fed and either starved vs. starved plus insulin ($p\geq.05$).

In summary, IR and CAV1 are in close association on the surface of the EC. Serum starvation leads to simultaneous migration of both IR and CAV1 to the PM while maintaining co-localization. The addition of insulin selectively decreased CAV1 at the cell surface.

Sources of Research Support: NIDDK RO1 057878 awarded to EJB.

Nothing to Disclose: CTU, ZL, EJB

Pub #	P1-482
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Insulin Action & Gut Hormones (1:30 PM-3:30 PM)
Title	Dipeptidyl Peptidase 4[en dash]Deficient Rats Are Protected from Lipotoxicity in a High-Fat Diet[en dash] Induced Insulin Resistance Model
Author String	S Ben-shlomo, I Zvibel, Z Halpern, R Oren, S Fishman Tel Aviv Sourasky Medical Center and the Sackler Faculty of Medicine Tel Aviv University, Tel Aviv, Israel

Body

Background/Aims: Glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are secreted in a nutrient-dependent manner, stimulate insulin secretion and are rapidly degraded by dipeptidyl peptidase 4 (DPP4). We showed that elevated GLP-1 in normoglycemic DPP4-deficient rats (DPP4-) suppressed lipogenesis (1). The present study investigated the effect of chronically elevated GLP-1 and GIP on glucose and lipid metabolism in a high fat diet (HFD) model, in order to determine whether DPP4 inhibitors can serve as treatment for non-alcoholic fatty liver disease.
Methods: Body and serum parameters and hepatic triglyceride (TG) content were assessed in wild type (WT) and DPP4- rats fed HFD for 2 months. Expression of genes involved in lipogenesis, lipid secretion or uptake were determined in liver and visceral fat (VF). Insulin sensitivity was determined by glucose tolerance test.
Results: DPP4- had significantly reduced serum TG and cholesterol, but similar hepatic TG levels and expanded VF mass compared to WT. In addition, DPP4- displayed increased hepatic expression of lipogenesis-associated genes, and attenuated expression of CYP7A1, the rate-limiting enzyme in the conversion of cholesterol to bile acids. Since bile acids were shown to stimulate GLP-1 secretion via TGR5 receptor, we hypothesize that reduced lipogenesis results from reduced GLP-1 and bile acids concentrations. Indeed, compared to regular chow, DPP4- fed HFD had lower serum GLP-1 levels. However, hepatic TG content was the same in DPP4- and WT, due to increased TG secretion, as indicated by enhanced ApoB mRNA and by the VLDL secretion assay. VF of DPP4- displayed enhanced mRNA for lipoprotein lipase, the enzyme responsible for TG uptake, and for lipogenesis genes. Furthermore, GIP directly increased lipogenesis in adipose tissue explants. mRNA expression of insulin sensitizer adiponectin was increased and insulin signaling antagonists TNF-a and IL-1b were reduced, in VF of DPP4-. Consequently, insulin sensitivity was enhanced in DPP4- rats
Conclusions: The inhibitory effect of GLP-1 on hepatic lipogenesis is abolished in HFD-induced insulin resistance, due to reduced bile acids concentrations and serum GLP-1. However, DPP4- rats maintain low serum TG and cholesterol and increased insulin sensitivity, owing to increased TG secretion and peripheral uptake up-regulated by GIP and changes of VF cytokine profile.

(1) Ben shlomo s et al, j hep 2010 in press

Nothing to Disclose: SB-S, IZ, ZH, RO, SF

Pub #	P1-483
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Insulin Action & Gut Hormones (1:30 PM-3:30 PM)
Title	High-Fat Diet in C57Bl/6 Mice Induces a Rapid Decrease in Insulin Sensitivity and an Acute Phase Response
Author String	FM Campbell, JE Drew, A Tups, N Hoggard, PF Nicol, AJ Farquharson, C Koch, C Grant, AC Morris, LM Williams Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, UK; Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, UK; Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, UK; Philipps University, Marburg, Germany
Body	High-fat diet-induced insulin insensitivity is seen within 3 days in C57Bl/6J mice. To further investigate the mechanisms underlying this induction we fed 12 week old male mice a low- or high-fat diet: 10% or 60% (Kcal) (D12450B and D12492 respectively, Research Diets, USA) for 3 days and 1 week. Mice were also pair-fed the high-fat diet restricted to the caloric intake of the low-fat diet for 3 days. Insulin sensitivity was assessed by intraperitioneal glucose tolerance tests (IPGTT) and plasma proteomics was used to determine changes in circulating proteins. Body composition was measured using MRI, liver lipid content measured by oil red O staining and ileum and liver gene expression were measured using real-time RT-PCR. Mice on the high-fat diet showed increased adiposity, liver lipid content and insulin insensitivity with a 48% increase in area under the curve (AUC) compared to control animals after 3 days. Insulin sensitivity improved after 1 week on the high-fat diet increasing to a 26% difference in AUC, while liver lipid and adiposity continued to increase. Plasma proteomics revealed profound changes in circulating proteins after 3 days on the high-fat die which when identified by mass spectroscopy and confirmed by immunoblotting were found to be mainly acut phase proteins. Gene expression in the ileum and liver confirmed rapid changes in expression of genes encoding acute phase proteins. Both the decrease in insulin sensitivity and the induction of acute phase proteins were seen in the pair-fed mice after 3 days on the high-fat diet indicating that the fat content of the diet and not the increased caloric intake caused the acute phase response and decrease in insulin sensitivity. The level of acute phase proteins in the plasma appears diminished after 1 week on the high-fat diet. Thus, the acute phase response and initial reduction in insulin sensitivity in C57Bl/6 mice on a high-fat fed is transient and the result of increased fat in the diet. Sources of Research Support: Scottish Government Rural and Environment Research and Analysis Directorate (RERAD). Nothing to Disclose: FMC, JED, AT, NH, PFN, AJF, CK, CG, ACM, LMW

Pub #	P1-484
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Insulin Action & Gut Hormones (1:30 PM-3:30 PM)
Title	Effects of Insulin and Insulin Resistance on Ghrelin Secretion in a Primary Culture of Rat Stomach Cells
Author String	J Gagnon, Y Anini Dalhousie University, Halifax, Canada
Body	Ghrelin is an orexigenic peptide hormone primarily produced in the endocrine cells of the stomach. The circulating levels of ghrelin are highest in the fasted state and decrease postprandially. To investigate the mechanism(s) regulating ghrelin secretion, we developed a primary culture of new born (PD 8) rat stomach cells. Whole rat stomachs were enzymatically digested and the isolated cells were placed into culture. We firs demonstrated that ghrelin positive cells in culture co-express prohormone convertase 1, ghrelin O-acyl transferase and obestatin. After 24 hours in culture, cells were incubated with different treatments for 4 hours. Total and acylated ghrelin levels were measured in the culture media and cell extracts. The basal level of ghrelin secretion was 315±21pg/ml/4hrs for total ghrelin and 7.63±3.22pg/ml/4hrs for acylated ghrelin. Forskolin (10uM) significantly increased ghrelin secretion by 43% over the control ($P<0.01$). We then used this cellular model to investigate the mechanism by which insulin regulate ghrelin secretion. We first demonstrated that ghrelin cells express the insulin receptor by double immunoflourescence. Insulin (10 nM) significantly reduced total and acylated ghrelin secretion by 40% ($P<0.01$) and 50% ($P<0.01$) respectively. The insulin mediated inhibition of ghrelin secretion was completely blocked by the PI3K inhibitor LY294002 (10uM, $P<0.05$). Furthermore, insulin (10 nM) significantly increased the phosphorylation of Akt by 73% ($P<0.05$), and reduced cAMP levels by 35% ($P<0.05$). To determine if insulin resistance (IR) affects ghrelin secretion, IR was induced by 18 h pretreatment with 100 nM insulin. Insulin mediated inhibition of ghrelin secretion was significantly attenuated ($P<0.05$) as was AKT phosphorylation ($P<0.05$) in IR cells. These findings indicate that insulin acts directly on ghrelin cells to inhibit ghrelin secretion and that insulin resistance in vitro is associated with dysregulated ghrelin secretion. Sources of Research Support: Canadian Insitutes of Health Research operating grant; Natural Sciences and Engineering Research Council Doctoral Scholarship. Nothing to Disclose: JG, YA

Pub #	P1-485
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Insulin Action & Gut Hormones (1:30 PM-3:30 PM)
Title	GLP-1 Receptor Activation by Exenatide Induces Expansion of Pancreatic Duct Glands
Author String	B Gier, AV Matveyenko, DW Dawson, SM Dry, PC Butler Larry Hillblom Islet Research Center, Los Angeles, CA; Department of Pathology and Laboratory Medicine, Los Angeles, CA; Jonsson Comprehensive Cancer Center, University of California Los Angeles, Los Angeles CA
Body	Background & Aims: Pancreatic duct glands (PDGs) expand in response to acute inflammation but when chronically expanded may induce chronic pancreatitis (CP) and with genetic predisposition pancreatic cancer. Type 2 diabetes (T2DM) and obesity are associated with an increased risk of CP and pancreatic cancer. Recent reports suggest that glucagon like peptide-1 (GLP-1) mimetic treatment may induce pancreatitis. In contrast, metformin is protective against both CP and pancreatic cancer in humans. We investigated the effect of the GLP-1 analog exenatide (Ex) on proliferation patways in human duct cells and the PDG compartment. Results: Rats treated with Ex (10 [mu]g/kg BW/day; n=10) for 12 weeks as expected had decreased bodyweight and reduced blood glucose levels *vs* control (ctrl) animals. The number of PDGs/[mu]m main duct and total PDG area were increased ($p<0.05$ and $p<0.001$) in the Ex treated group.Moreover, PDG replication rate (measured by Ki-67) was increased ($p<0.05$) in Ex treated animals. This marked epithelial proliferation was seen histologically by multilayering and villiform features. GLP-1 receptor (GLP-1R) was present in PDGs (humans and rodents). To investigate the mechanism of GLP-1 inducted cell proliferation, human ductal cells (HPDE) were treated with Ex (10 nM). Ex induced a time-dependant phosphorylation of the mitogen-activated kinases ERK1/2 ($p<0.001$). GLP-1R activation also caused activation of the cAMP/PKA pathway: phosphorylation of CREB in HPDE cells was significantly increased after 10 min and reached a plateau at ~3(min ($p<0.05$).This effect was potentiated in the presence of the Kras mutation, and counteracted by metformir ($p<0.05$). In addition, cyclin D1 protein was maximally induced by Ex at ~6h ($p<0.05$). Conclusions: Treatment of rats with GLP-1 analog exenatide induced expansion of the pancreatic duct gland compartment by increasing cell proliferation. Exenatide activated cAMP/PKA/pCREB and MAPK induced proliferation pathways in human duct cells. Metformin abrogated these proliferative effects. The long-term effects if widely prescribed GLP-1 analogs on human pancreatic duct epithelium remain incompletely described. Our data indicates GLP-1 therapy increases ductal cell and PDG proliferation. If unopposed and persistent, such long-term pancreatic ductal proliferation may amplify the pre-existing risk of CP and pancreatic cancer in patients with obesity and T2DM. Nothing to Disclose: BG, AVM, DWD, SMD, PCB

Pub #	P1-486
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Insulin Action & Gut Hormones (1:30 PM-3:30 PM)
Title	Repression of GKAP (cGMP-Dependent Kinase Anchoring Protein) Causes Insulin Resistance in 3T3-L1 Adipocytes
Author String	Y Ando, Y Shinozawa, Y Iijima, Y Ooi, Y Watanaka, K Chida, F Hakuno, S-I Takahashi Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo, Japan
Body	Tumor necrosis factor (TNF)-α and other cytokines have been shown to cause insulin resistance in adipocytes *in vivo* and in cell cultures. We have shown that long-term treatment of 3T3-L1 adipocytes with TNF-α for 24 h decreases insulin-dependent tyrosine phosphorylation of insulin receptor substrate (IRS)-1, followed by inhibition of the PI 3-kinase pathway including Akt activation, leading to impairment of glucose uptake induced by insulin. In the course of studying this mechanism, we found that IRSs form high-molecular-weigh complexes and components of these complexes modulate the availability of IRS-1 to insulin receptor tyrosine kinase. This study was undertaken to identify IRS-associated proteins which change insulin-dependent IRS tyrosine phosphorylation in 3T3-L1 adipocytes treated with TNF-α. We performed yeast two-hybrid screenin using IRS-1 as bait and 3T3-L1 adipocytes cDNA library as prey and identified GKAP as an IRS-associated protein. GKAP is a 42-kDa protein and is reported to function as an anchoring protein for cGMP-dependent kinase-Iα to regulate its intracellular localization and access to substrates. Interaction of GKAP with IRS-1 was confirmed by co-immunoprecipitation in HEK293T cells expressing both of these proteins. The N-terminal region (amino acid residues 1-95) of GKAP was responsible for its interaction with IRS-1. To investigate the roles of GKAP on insulin signaling, we repressed endogenous GKAP levels in 3T3-L1 adipocytes using siRNA and assessed insulin-dependent activation of insulin signaling and glucose uptake. We found that GKAP knockdown did not affect insulin receptor autophosphorylation induced by insulin, but did decrease insulin-dependent tyrosine phosphorylation of IRS-1, binding of p85 regulatory subunit of PI 3-kinase to IRS-1 and phosphorylation of Akt. Moreover, insulin-dependent glucose uptake was inhibited by GKAP knockdown. Our results demonstrated GKAP increases availability of IRS-1 to insulin receptor tyrosine kinase, resulting in maintenance of insulin signaling and insulin-dependent glucose uptake in 3T3-L1 adipocytes. Taken together with the results showing that the GKAP protein level was suppressed by long-term TNF-α treatment, we concluded that TNF-α-induced insulin resistance was caused by repression of GKAP at least in a part. Nothing to Disclose: YA, YS, YI, YO, YW, KC, FH, S-IT

Pub #	P1-487
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Insulin Action & Gut Hormones (1:30 PM-3:30 PM)
Title	Increased Risk of Intrauterine Malformations Associated with Gestational Hyperglycemia in Alx3-Deficient Mice
Author String	P Garcia-Sanz, M Mirasierra, M Vallejo CSIC, Madrid, Spain; Instituto de Salud Carlos III, Madrid, Spain

Body

The risk of alterations in fetal development leading to congenital malformations is increased significantly by hyperglycemia in diabetic pregnancies. During development, expression of the homeodomain transcription factor Alx3 in the craniofacial mesenchyme is important for correct neural tube closure, and is dependent on the presence of folic acid (1). In adult animals, Alx3 is expressed in pancreatic islets and is important for the maintenance of glucose homeostasis (2). Alx3 deficiency results in increased apoptosis both in the developing mesenchyme and in adult pancreatic islets, leading to the generation of neural tube closure defects and altered glucose homeostasis, respectively.

In this study, we investigated whether Alx3-deficiency increases the risk of congenital malformations during gestational hyperglycemia. In addition, we investigated whether the presence of Alx3 in developing embryos is required for the expression of genes that respond to high glucose concentrations of maternal origin.

Alx3 knockout, heterozygote and appropriate control mice were used. The occurrence of intrauterine malformations was examined in embryos 10.5 days post coitum. Severe gestational diabetes was induced in female mice treated with streptozotozin. The resulting hyperglycemia was counterbalanced by implantation of insulin pellets and the mice were allowed to mate. Hyperglycemia reappeared after 4-5 days post coitum, resulting in gestational diabetes. Embryos were examined for malformations, or used for histological and molecular analyses.

Gross embryonic malformations and relatively frequent uterine reabsorptions were observed in Alx3-null mice, but not in wild type embryos. These defects were not altered by folic acid administration to pregnant mice. In heterozygote embryos, the incidence of malformations was lower than in Alx3-deficient embryos, and decreased further when dams were treated with intraperitoneal injections of folic acid during pregnancy. Quantitative RT-PCR revealed that expression of genes encoding proteins involved in the defense mechanisms against oxidative stress was induced by maternal hyperglycemia in wild type but not in Alx3-deficient embryos. In addition, genes encoding transcriptional and developmental regulators were selectively affected.

These data indicate that expression of Alx3 constitutes an important component of some of the protective mechanisms against the occurrence of developmental malformations during gestational hyperglycemia.

(1) Lakhwani S et al., Dev Biol 2010; 344:869
(2) Mirasierra M et al., Diabetologia 2011; 54:403

Sources of Research Support: Spanish Ministry of Science and Innovation BFU2008-01283; CIBERDEM is an initiative of the Instituto de Salud Carlos III.

Nothing to Disclose: PG-S, MM, MV

Pub #	P1-488
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Insulin Action & Gut Hormones (1:30 PM-3:30 PM)
Title	Metabolic Synchronization of the Liver Circadian Clock
Author String	D Landgraf, DJ Drucker, H Oster Max Planck Institute of Biophysical Chemistry, G[ouml]ttingen, Germany; Samuel Lunenfeld Research Institute, Toronto, Canada
Body	In mammals, the master circadian clock of the suprachiasmatic nuclei (SCN) and peripheral clocks found throughout the body coordinate 24 h rhythms of behavior and physiology. At the molecular level these clocks are based on interlocked transcriptional translational feedback loops (TTLs) including positive (Bmal1/Clock) and negative (Per1/2, Cry1/2) components. Under ad libitum feeding conditions hepatocyte clocks are stably entrained to the light/dark (LD) cycle via the central circadian pacemaker of the SCN. When food access is restricted to certain periods of the day (restricted feeding) the liver clock is reset to synchronize with the metabolic signal of food uptake. This process is independent of the SCN clock which remains locked to the LD cycle. This phenomenon suggests an SCN-independent direct entrainment pathway of peripheral organs by metabolic cues. The aim of this study was to characterize the molecular pathways of food synchronization of the liver clock. Gastrointestinal peptide libraries were screened to identify factors capapble of resetting circadian rhythms of lucifearse activity in organotypic liver slice cultures of Per2::Luc knock in mice. This screen identified severa candidates including ghrelin which previously had been implicated in food-mediated synchronization of circadian activity rhythms. One of the candidates was used for further studies. Hormone treatment caused an acute induction of the clock genes Per1 and Per2 in vitro and in vivo. Preliminary data suggest that this effect is mediated via the cAMP/PKA pathway and activation of CREB. When knocking out the corresponding receptor in mice, mutant animals showed disturbed circadian adaptation to daytime feeding schedules. In summary our findings reveal a novel, SCN-independent pathway for liver clock resetting in response to a metabolic zeitgeber. Sources of Research Support: German Research Foundation (DFG). Nothing to Disclose: DL, DJD, HO

Pub #	P1-489
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Insulin Action & Gut Hormones (1:30 PM-3:30 PM)
Title	Unacylated Ghrelin Improves Insulin Sensitivity in Early-Phase Obesity
Author String	P Delhanty, M Huisman, I van den Berge, T Abribat, A Themmen, A-J van der Lely Erasmus MC, Rotterdam, Netherlands; Alize Pharma, Lyon, France
Body	In the US 24 million children and adults, and in Europe more than 50 million adults are diabetic. Of these, 90-95% have type 2 diabetes (T2D). It is also recognized that an even greater population is insulin resistant due to overweight and it is thought that treatment at this early phase of the metabolic syndrome may prevent the occurrence of overt diabetes and associated pathologies. We are in the process of developing unacylated ghrelin (UAG) analogues as therapeutics for insulin resistance and T2D. Towards this goal, we have used a mouse protocol in which we pretreat mice with the analogues, then initiate a short-term high fat diet (HFD) to induce insulin resistance but not diabetes. Twelve week old C57BL/6 mice (n=10) were fed either normal chow (12% kcal from fat), or a HFD consisting of 41% kcal from fat, for two weeks. We assessed animal weight, food intake, fat mass, fed and fasting plasma glucose concentrations, and glucose tolerance (glucose tolerance tests) during the study, then insulin sensitivity (insulin tolerance tests) at the end of the study. Body weight was significantly increased by the HFD during the study period, and fat mass was markedly increased by approximately 2.5-fold compared with animals on control diets. This occurred despite a decrease in food intake in this group. The animals on the HFD became glucose intolerant and insulin resistant. Infusion of UAG and analogues had no consistent effects on fasting or fed glycemia or glucose tolerance in this short-term model. Importantly, though, insulin sensitivity was significantly improved, as assessed by ITT, in agreement with our preliminary data in obese diabetic ob/ob mice. UAG agonists may be of use as therapeutics in the treatment of insulin resistance in metabolic syndrome. Nothing to Disclose: PD, MH, IvdB, TA, AT, A-JvdL

Pub #	P1-490
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Insulin Action & Gut Hormones (1:30 PM-3:30 PM)
Title	A Stabilized Apelin Analog Impairs Glucose Homeostasis in Diet-Induced Obese Mice
Author String	D Kubasiak, T Gareski, T Unger, S-P Yeh, S Will, X Li, A Qadri, N Huang, M Perreault, R Gimeno, C Baber S Ranganath Pfizer, Cambridge, MA; Pfizer, Cambridge, MA
Body	Apelin is a peptide expressed by a number of metabolically important tissues, including adipocytes, hypothalamic neurons, and endothelial cells. Apelin expression in adipose tissue is regulated by insulin signaling, and circulating levels are increased in patients with obesity and insulin resistance. Apelin is synthesized as a 77 amino acid precursor which is proteolyzed by a yet uncharacterized pathway to yield multiple bioactive forms, including apelin-36, apelin-17, and apelin-13. All three apelin isoforms bind and activate APJ, a G-protein coupled receptor, and injection of apelin-13 in mice has recently been shown to improve glucose tolerance and insulin sensitivity by activating AMPK in skeletal muscle; furthermore, apelin deficient mice display impaired insulin sensitivity. Apelin-13 has an EC50 of 1 nM in an *in vitro* APJ activation assay, but is highly unstable in mouse serum in vitro and rapidly disappears from the circulation after intraperitoneal injection in vivo (t1/2 <1 min), thus limiting the use of apelin-13 in *in vivo* experiments. To further investigate possible pharmacological effects of apelin-13, we developed a stabilized analog of apelin-13, SR-265, which activated APJ with an EC50 of 268 nM, had serum stability of 3 hours, and an in vivo half life of 1.5 hours. SR-265 was characterized further in diet-induced obese (DIO) mice and its effect was compared to native apelin-13. We found that intraperitoneal injection of apelin-13 had no significant effect on glucose homeostasis as assessed in an oral GTT. In contrast, the administration of analog SR-265 significantly impaired glucose tolerance in DIO mice, without concomitantly altering glucose-stimulated insulin secretion. These data suggest the possibility that the sustained, elevated levels of modified apelin-13 may have negative effects on glucose homeostasis and that elevated apelin levels possibly contribute to the pathophysiology observed in obese and diabetic patients. Nothing to Disclose: DK, TG, TJU, S-PY, SW, XL, AQ, NH, MP, RG, CB, SR

Pub #	P1-491
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Insulin Action & Gut Hormones (1:30 PM-3:30 PM)
Title	Short-Term Exposure to Angiotensin II Enhances Insulin Sensitivity in L6 Cells
Author String	S-J Oh, W-C Ha, J-I Lee, J-H Kim, J-M Lee, S-A Chang, O-K Hong, H-S Son Uijeongbu St Mary's Hospital, Uijeongbu-si, Republic of Korea; St Paul's Hospital, Seoul, Republic of Korea; Seoul St Mary's Hospital, Seoul, Republic of Korea
Body	Introduction: Angiotensin II (ATII) is an important substance that increases insulin resistance in individuals with hypertension, obesity, or type 2 DM and many studies have shown that ACE inhibitor or ARB improves insulin resistance. However, it is reported that acute infusion of ATII can increase insulin sensitivity in healthy individuals. So the biological actions of ATII on insulin sensitivity remain controversial. In this study we investigated the effect of ATII on the insulin action in L6 cells. Method: After treating with ATII (10^{-7}M) and/or insulin (10^{-7}M) (for 10 min) and/or ARB (angiotensin receptor blocker, eprosartan 0.2, 2, 20, and 200 uM was pretreated for 30 min), we observed the phosphorylation (Ser^{473}) of Akt and the insulin binding capacity in L6 cells. Result 1) In both ATII alone and ATII + insulin groups, the phosphorylation of Akt was increased. 2) After treating with ATII, insulin binding capacity was increased up to 60 % 3) When pretreating with eprosartan, ATII-induced Akt phosphorylation was reduced but insulin-stimulated Akt phosphorylation was not affected. 4) When pretreating with eprosartan in ATII groups, the insulin binding capacity was decreased, dose dependently. Conclusion: When treating with ATII for short time, ATII increases insulin sensitivity by improving the insulin binding capacity and insulin signaling in L6 cell. But when L6 cell is exposed chronically to ATII or added with some substances or hormones that can increase insulin resistance, the results may be changed. Nothing to Disclose: S-JO, W-CH, J-IL, J-HK, J-ML, S-AC, O-KH, H-SS

Pub #	P1-492
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Insulin Action & Gut Hormones (1:30 PM-3:30 PM)
Title	Effect of Peripheral Serotonin on Plasma Metabolite Concentrations and Glucose Uptake in Several Tissues
Author String	H Watanabe, K Saito, R Saito, T Nakano, S Ohwada, K Watanabe, T Yamaguchi, H Aso Tohoku University, Sendai, Japan
Body	[Objective] Serotonin is a monoaminergic neurotransmitter with activities that modulate central and peripheral functions. Serotonin affects food intake, sleep, anxiety, sexual behavior and mood in the central nervous system. On the other hand, the functions of serotonin in peripheral tissue have not yet been fully elucidated. Further, serotonin is thought not to be able to pass the blood-brain barrier. Thus, there are two independent serotonin systems: one in the central nervous system and the other in the periphery. Recently, serotonin has been shown to be involved in glucose metabolism. These data suggest peripheral serotonin has functions in metabolic homeostasis. In this study, we have investigated the physiological effect of peripheral serotonin on the plasma levels of metabolites and the glucose uptake in several tissues. [Results] Mice (C57B/6, male, 7 weeks old) were fasted for 12 h before the experiment. After the intraperitoneal injection of 1 mg serotonin, the plasma glucose and insulin levels were significantly elevated between 60 and 270 min after the injection. The concentration of bile acids in plasma was increased between 30 and 90 min after injection. In contrast, plasma triglyceride, cholesterol and NEFA concentrations were decreased. In order to determine what kind of serotonin receptors were related to these serotonin functions, mice were pretreated with three kinds of serotonin receptor antagonists: ketanserin ($5HTR_{2A}$), SB-269970 ($5HTR_{7}$) and methysergide ($5HTR_{1,2,7}$), at 30 min before serotonin injection. Serotonin-induced elevation of plasma glucose levels was attenuated by each of these. The serotonin-induced increase of plasma insulin concentrations was only antagonized by methysergide. Additionally, the decrease in the plasma TG, NEFA and cholesterol levels were each caused by serotonin acting through different 5HTRs. To determine the mechanism by which serotonin elevates plasma glucose concentrations, we investigated the effect of serotonin on the uptake of 2-deoxy-D-glucose (2DG). The plasma levels of 2DG in serotonin-injected mice were 4-fold greater than that in PBS-injected mice. Serotonin increased the 2DG uptake in the white and brown adipose tissues, but didn't affect that in the liver and muscle. These results suggest that the hyperglycemia induced by serotonin is likely caused by inhibiting glucose uptake from blood to the tissues, and that peripheral serotonin accelerates the metabolism of lipid through the diverse 5HTRs. Nothing to Disclose: HW, KS, RS, TN, SO, KW, TY, HA

Pub #	P1-493
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Insulin Action & Gut Hormones (1:30 PM-3:30 PM)
Title	An Acute Increase in Plasma Corticosterone-Induced Tissue-Specific Changes in Corticosterone and Glucose Metabolism in Select Target Tissue of the Laying Hen (*Gallus domesticus*)
Author String	CR Ralph, AJ Tilbrook, PH Hemsworth, BJ Leury University of Melbourne, Melbourne, Australia; Monash University, Clayton, Australia
Body	The effect of corticosterone (CS) on glucose metabolism is variable, possibly influenced by the nutritional sate of the animal. This study tested the hypothesis that an acute increase in plasma CS would induce tissue specific changes to intracellular CS and glucose metabolism in the laying hen. Forty-eight hens were evaluated over 180min. ACTH (0.2 [micro]g/kg) was injected and simultaneous tissue and plasma samples were obtained 15min before and 15, 30, 60, 90, 120, 150 and 180min after the injection. At each time 6 hens were decapitated and the trunk blood collected whilst the liver and a skeletal muscle sample were dissected and immediately frozen. Tissue and plasma samples were assayed for CS and glucose concentration. Insulin was assayed in plasma. Glucose-6-phosphate dehydrogenase (G6PDH) Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) and Pyruvate Carboxylase (PCx) activity were assayed in both tissues. There was no change in glucose concentration in the tissue or plasma. There was an acute increase in plasma CS with significant ($P<0.01$) increases at 15, 30 and 60min. There was a parallel acute increase in skeletal muscle CS at 15, 30 and 60min ($p<.001$). The change in hepatic CS did not parallel the plasma. Hepatic CS increased at 15 min ($p<.05$) remained elevated at 30min ($p<.001$), returned to baseline at 60 min and then increased again at 180 min ($p<.001$). An increase in the rate of G6PDH activity in hepatic cells indicated that the acute increase in plasma CS stimulated hepatic glycogenesis. A decrease in the rate of G3PDH activity indicated that glycogenolysis in hepatic cells was not stimulated and a decrease in the rate of PCx activity indicated that gluconeogenesis was not stimulated in hepatic cells. An increase in the rate of G3PDH activity in the cells of the skeletal muscle indicated the glycogenolysis had been stimulated. These results indicate that an acute increase in plasma CS can produce tissue specific changes in intracellular CS and glucose metabolism. The effect of CS on glucose metabolism may differ based on energetic needs and these changes may assist the animal to cope with the stressor. These data also suggest that when sufficient hepatic glucose and insulin are present an increase in CS may antagonize the effects of insulin. Nothing to Disclose: CRR, AJT, PHH, BJL

Pub #	P1-494
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Type I Diabetes & Diabetic Complications (1:30 PM-3:3(PM)
Title	Ectopic Islet Neogenesis in Combination with an Inhibition of Local T-Cell Activation Reverses Autoimmune Diabetes in NOD Mice
Author String	R Li, JK Lee, M-s Kim, V Liu, E Buras, K Ozer, L Chan, V Yechoor Baylor College of Medicine, Houston, TX
Body	Type 1 diabetes is caused by T cell-mediated autoimmune destruction of insulin-producing cells in the pancreas. Induced islet neogenesis has the potential to be a cure for type 1 diabetes. In our previous study, using helper-dependent adenoviral vectors (HDAd) to deliver Ngn3,an islet development factor, in combination with betacelllin (Btc), an islet growth factor, into STZ-induced diabetic mice, we showed that induction of ectopic islets with insulin positive cells in the liver led to a complete reversal of diabetes (Developmental Cell ,2009). To extend this further, we used NOD mice, a model of autoimmune diabetes. However, therapy with HDAd-Ngn3 and HDAd-Btc, that was effective in a non-autoimmune model of STZ-diabetes, failed to restore euglycemia in diabetic NOD mice. This may be secondary to a resurgence of T cell-mediated destruction of new insulin producing cells in the liver. We hypothesized that co-expressing CD274 , an important component of the co-inhibitory signaling pathway that inhibits T-cell activation, driven by rat insulin promoter only in the new b-cells, with Ngn3 and Btc, may protect the newly induced ectopic insulin producing cells in the liver from T-cell mediated destruction. To test this, NOD mice were treated with a single tail vein injection of HDAd-CD274 (1x10^11 viral particles [vp]), HDAd-Ngn3 (5x10^11vp) and HDAd-Btc (1x10^11vp) at the time of onset diabetes. 14 of 19 diabetic mice (73%) treated with Ngn3, Btc and CD274, responded with a return of euglycemia within 2-4 weeks and regained their body weight after therapy. In contrast, untreated diabetic NOD mice remained hyperglycemic and rapidly lost weight eventually succumbing within 6-8 weeks. An intra peritoneal glucose tolerance testing performed after 4 weeks, 8 weeks of treatment with Ngn3+Btc+CD274 revealed a normal glucose tolerance accompanied by normal in vivo glucose-stimulated insulin secretion in the responder mice. This treatment induced periportal islet-like cell clusters in the liver which displayed β-cell-specific transcripts and produced all four major islet hormones. W also show that the newly formed islet-like clusters were protected by CD274 via suppressing local T-cell proliferation and activation. Our results demonstrate that inducing ectopic islet neogenesis with Ngn3 and Btc gene delivery and protecting them from destruction by CD274-induced prevention of local T-cell activation, has the potential to be a curative therapy for type 1 diabetes. Yechoor V et al.,Dev Cell. 2009 ;16(3):358 Nothing to Disclose: RL, JKL, M-SK, VL, EB, KO, LC, VY

Pub #	P1-495
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Type I Diabetes & Diabetic Complications (1:30 PM-3:3(PM)
Title	Prevention of Autoimmune Diabetes in the Rat with an Allele-Specific Antibody That Recognizes the Vβ13 T Cell Receptor Beta Chain
Author String	JP Mordes, Z Liu, L Cort, R Eberwine, J Leif, EP Blankenhorn University of Massachusetts Medical School, Worcester, MA; Drexel University College of Medicine, Philadelphia, PA
Body	To identify new potential intervention strategies for autoimmune type 1 diabetes (T1D), we have investigated several rat models of the disorder. T1D is relatively common among inbred rat strains with a high risk class II major histocompatitibity comples (MHC) haplotype. We first dissected the powerful *Iddm14* diabetes susceptibility locus in eight T1D susceptible vs. resistant rat strains by single nucleotide polymorphism (SNP) haplotyping. We identified an allele of a T cell receptor (TCR) beta chain gene, *Tcrb-V13S1A1* (encoding Vβ13a) as a likely candidate gene. We report here that, in three separate trials, treating LEW.1WR1 rats, which are susceptible to T1D, with a depleting anti-Vβ13 monoclonal antibody reduces diabetes frequency from ~75% (N=50) to ~17% (N-30, $p<0.001$). We also analyzed the phenotype of infiltrating T cells recovered from the cultured islets of LEW.1WR1 rats exposed to a diabetogenic trigger. Within 5 days, up to 22% of CD4+ T cells recovered from islets were Vβ13+, most of these CD25+FoxP3-, suggesting that they are not regulatory T cells. We also recovered Vβ13 transcripts from pre-diabetic islets and observed a limited number of Jβ variant transcripts, indicating an oligoclonal TCR response to pancreatic beta cells. These data indicate that, in susceptile rats, Vβ13+ usage by diabetogenic T cells is required to recognize a critical T1D autoantigen.The data also suggest that it may be possible to prevent T1D with a very narrowly targeted therapy. Sources of Research Support: ADA 7-08-RA-106 from the American Diabetes Association. Nothing to Disclose: JPM, ZL, LC, RE, JL, EPB

Pub #	P1-496
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Type I Diabetes & Diabetic Complications (1:30 PM-3:3(PM)
Title	Insulin-Coated Microneedle Arrays for Bolus Delivery to Diabetic Rats
Author String	TC Daley, M Prausnitz, E Felner Emory University, Atlanta, GA; Georgia Tech University, Atlanta, GA

Body

BACKGROUND: Microneedles have been developed to create transport pathways for small drugs, macromolecules, and fluid flow in a painless manner. They range in length from 150 - 900 mm. This microscopic length permits appropriate delivery of fluid to the capillaries but limits the extension into the abundant nerve-ending region of the dermis, thereby minimizing pain. The advantages for using microneedles to deliver insulin over conventional methods, include a more rapid insulin action and a less painful delivery route. Hollow glass microneedles have been fabricated for delivery of insulin to diabetic animals and humans. For effective drug delivery through solid microneedles, however, microneedles must be coated with the drug and then inserted into the skin. There are no reports of the evaluation of insulin delivery comparing an insulin coated microneedle to the hypodermic route in diabetic animals or humans.
METHODS: We studied 3 rats with streptozotocin-induced diabetes. One rat served as the control and did not receive insulin. The other 2 received 0.5 units of Lispro (Humalog, Eli Lilly, Indianapolis, IN, USA) insulin. One received the insulin via the conventional subcutaneous route and the other received the insulin from an array of five solid microneedles that were coated with Lispro insulin mixed with carboxymethylcellulose (CMC). Lateral tail vein samples for glucose were obtained every 30 minutes for 6 hours.
RESULTS: Prior to administering insulin, all 3 rats had blood glucose levels > 200 mg/dL. As expected, the diabetic rat that did not receive insulin had no reduction in blood glucose over the 6-hour period. It had an area-under-the-glucose-curve (AUGC) of 2167 mg/dl-hr. The rat receiving the conventional delivery had a reduction in blood glucose of 274 mg/dL, had a glucose nadir (34 mg/dL) 120 minutes after insulin delivery, and had an AUGC of 637 mg/dL-hr. The rat receiving microneedle delivery had a reduction in blood glucose of 196 mg/dL, had a glucose nadir (100 mg/dL) 60 minutes after insulin delivery, and had an AUGC of 1044 mg/dL-hr.
CONCLUSION: We were able to demonstrate that solid coated microneedles provide another means for effective insulin delivery. We observed an appropriate decline in blood glucose levels after the administration of insulin with solid coated microneedles. Insulin-coated microneedles may provide an acceptable alternative for insulin delivery.

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Nothing to Disclose: TCD, MP, EF

Pub #	P1-497
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Type I Diabetes & Diabetic Complications (1:30 PM-3:3(PM)
Title	Islet Transplantation on Biodegradable Polymer Scaffolds for Type 1 Diabetes in Syngeneic and Xenogeneic Murine Models
Author String	RF Gibly, X Zhang, DB Kaufman, WL Lowe, LD Shea Northwestern University, Evanston, IL; Northwestern University, Chicago, IL; Northwestern University, Chicago, IL

Body

Introduction:
Islet transplantation as a cell therapy for Type-1 diabetes offers the potential of a real cure. The current process infuses islets into the hepatic vasculature; a site that may negatively impact the islets. Here we present an extrahepatic approach for controlling the islet microenvironment to maximize survival and host reintegration. Islets were delivered on a porous, biodegradable polymer scaffold, in contrast with the isolation strategies of most biomaterial approaches. We demonstrate functional islet engraftment and survival in syngeneic and xenogeneic murine models using a minimal mass of islets.

Methods:
Poly(lactide-co-glycolide, PLG) microspheres were used to fabricate biocompatible, porous and degradable scaffolds for islet transplantation (1). Scaffolds architecturally define the transplant environment, support islets, and can present ECM molecules (2) or locally deliver trophic factors. Diabetes reversal, weight gain, and histology of explants were used to assay the effectiveness of scaffolds for syngeneic islet transplants into C57BL/6 mice and human islet transplants into NOD-*scid* gamma mice.

Results:
Scaffolds with varying architecture were studied for their impact on transplanted syngeneic islet function in mice at a peritoneal fat pad site. Scaffolds formed using 6% PLG microspheres reversed diabetes within one day when using 125 islets and within an average of 11.9 days with only 75 islets (approximately 1/3 of the normal pancreatic mass), while using 2% PLG microspheres has required 125 islets and an avg. of 20 days (1). Histology demonstrates complete host cell infiltration, islet revascularization, and biocompatibility based on the lack of an inflammatory capsule. Human islets transplanted on scaffolds in the peritoneal fat of diabetic NOD-*scid* gamma mice demonstrated similarly successful engraftment and reversal of diabetes.

Conclusions:
Utilizing PLG scaffolds and an extrahepatic site, islets can be delivered as a cell-based therapy for Type-1 diabetes. In the epididymal fat pad, scaffolds support successful transplant of what is considered a minimal mass of islets at traditional liver or kidney capsule sites. This platform enables localized control of the islet distribution and microenvironment by presenting ECM and locally delivering trophic factors to enhance survival, engraftment and revascularization in a robust, modular package.

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Sources of Research Support: NIH/NIDDK: RO1 EB003806, R21 EB009502, RO1 EB009910.

Nothing to Disclose: RFG, XZ, DBK, WLL, LDS

Pub #	P1-498
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Type I Diabetes & Diabetic Complications (1:30 PM-3:3(PM)
Title	Optimizing Reduction of Basal Hyperglucagonemia and Improvement in Pulsatile Glucagon Counterregulation (GCR) in the Insulin-Deficient Pancreas
Author String	LS Farhy, AL McCall University of Virginia Health System, Charlottesville, VA
Body	**Background.** GCR is the first line of defense against hypoglycemia compromised in diabetes. Our recent studies suggest that the decrease in GCR caused by progression of insulin deficiency is inversely proportional to the increase in basal glucagon secretion (1-3). We have also suggested that partial glucagon inhibition may improve GCR (4). Here, we continue the *in silico* analysis of the use of α-cell inhibitors (ACI) to repair defective GCR with regard to their ability to suppress separate components that constitute the secretion of glucagon. **Methods.** We use our mathematical model of the pancreatic network which assumes that α-cells are suppressed by glucose, β-cells, and auto-feedback and which has been shown to precisely reconstruct the GCI control mechanism and its impairment in diabetes (1-4). We use this model to predict the impact on GCR caused by ACI depending on the level at which they inhibit the feedback-independent (basal) and feedback-regulated (pulsatile) glucagon (FIG and FRG, respectively). This was achieved via multiple simulations in which hypoglycemia-stimulated GCR was monitored at different levels of FIG inhibition (FIGI) and FRG inhibition (FRGI) by ACI. **Results.** ACI can restore normal GCR levels only if they suppress FIG. If an ACI suppresses also FRG, restoration of GCR levels requires at least 40% FIGI and more FRGI demand higher FIGI. However, if an ACI suppresses FRG > 55% normal GCR levels cannot be restored. Normalizing glucagon levels during both eu- and hypoglycemia is also possible, but requires additional FIGI. Restoring the fold increase in glucagon ir response to hypoglycemia to levels similar to the normal pancreas by ACI requires at least 40% FIGI if FRGI=0 to 20%, 60% FIGI if FRGI=40 to 60%, and 80% FIGI if FRGI=80%, **Conclusion.** Our system-level analysis predicts that in insulin deficiency ACI can repair defective GCR only if they suppress basal glucagon. If an ACI suppresses also the pulsatile glucagon release, the suppression of basal glucagon should dominate over the inhibition of pulsatile glucagon. Determining the relationship between inhibition of basal and pulsatile release by a particular ACI is possible via deconvolution analysis of glucagon concentration time series which can distinguish the level of suppression of both secretory components simultaneously. Our results therefore suggest an experimental strategy to determine whether a given ACI is a potential candidate for GCR repair in insulin deficiency. (1) Farhy LS, Du Z, Zeng, Veldhuis PP, Johnson ML, Brayman KL, McCall AL. Amplification of pulsatile glucagon secretion by switch-off of -cell suppressing signals in Streptozotocin (STZ)-treated rats. Am J Physiol Endocrinol Metab, 2008; 295: E575 - E585. (2) Farhy LS, McCall AL. System-Level Control to Optimize Glucagon Counterregulation by Switch-Off of -Cell Suppressing Signals in -Cell Deficiency. Journal of Diabetes Science and Technology, 2009;3(1):21-33 (3) Farhy LS, McCall AL. Pancreatic network control of glucagon secretion and counterregulation. Methods in Enzymology, Dec, 2009, Vol. 467:547-81. (4) Farhy LS, McCall AL. Models of Glucagon Secretion, Their Application to the Analysis of the Defects in Glucagon Counterregulation and Potential Extension to Approximate Glucagon Action. Journal of Diabetes Science and Technology, 2010;4(6):1345-1356. Sources of Research Support: NIH Grant R01DK082805 to LSF and ALM. Nothing to Disclose: LSF, ALM

Pub #	P1-499
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Type I Diabetes & Diabetic Complications (1:30 PM-3:3(PM)
Title	Urinary Conjugated Metabolites of α-Tocopherol (Vitamin E) in Children and Young People with Type 1 Diabetes Mellitus
Author String	G Sharma, D Muller, M Dattani, S O'Riordan, P Hindmarsh, K Mills UCL Institute of Child Health, London, UK; University Hospitals of Leicester, Leicester, UK
Body	Diabetes is a major risk factor for cardiovascular disease. Both absolute blood glucose concentrations and glucose variability have been implicated in atherosclerosis through effects on oxidative stress (OS). Developing easy to measure biomarkers of OS would help in determining in a prospective manner the impact of glycaemic control on OS and macrovascular disease in children and young people (CYP) with DM1. Two groups of metabolites of vitamin E (α-tocopherol) are described. The first, include α-tocopherylquinone, α-tocopheronic acid and α-tocopheronolactone (α-TL), which result from oxidation and opening of the chromanol ring of α-tocopherol. The second, (α-carboxy-ethyl- hydroxychroman (α-CEHC) and α-carboxymethyl-bytyl-hydroxychroman) result from the successive shortening of the phytyl side chain of α-tocopherol. The principal urinary metabolites of α-tocopherol are α-CEHC and αTL, both of which are excreted as their polar sulphate (S) and glucuronide (G) conjugates. We describe a liquid chromatography/tandem mass spectrometry method for the direct assay of the conjugate(vitamin E metabolites and compare urinary metabolite concentrations in 32 (15M) CYP with DM1 aged 12.9 years (range 7.8-18.4)and matched controls. The m/z transitions used for the S and G metabolites were 356.97>80.37 and 453.05>112.79 respectively. Within assay coefficients of variation (CVs) ranged from 0.88-3.73% (n=20) and between assay CVs 1.18-4.32% (n=20). Recoveries were [ge]90%. Two peaks, presumed to be isomers, were obtained for αTL-glucuronide (esignated αTL-G1 and G2). Compared to controls the vitamin E metabolite markers of OS (αTL-G1 1098, αTL-G2 562 and αTL-S 98 nmol/mmol) were elevated compared to controls (76, 34, 10 nmol/mmol respectively P [le] 0.001). In contrast although there was a slight increase in α-CEHC-G 126 verus 73 nmol/mmol) and α-CEHC-S (138 versus 57 nmol/mmol)this was only significant for α-CEHC-S (P=0.05). Vitamin E Markers of OS can be easily measured using this methodology and are elevated in patients with DM1.± Nothing to Disclose: GS, DM, MD, SO, PH, KM

Pub #	P1-500
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Type I Diabetes & Diabetic Complications (1:30 PM-3:3(PM)
Title	Advances in the Development of a Functional Bioartificial Pancreatic Construct
Author String	MM Corrado, MJ Beveridge, S Neelan, B Sorensen, NE Simpson University of Florida, Gainesville, FL; University of Florida, Gainesville, FL
Body	Introduction: Type 1 diabetes develops when the body's immune system destroys pancreatic [Beta]-cells. A novel approach to treat this form of diabetes involves implanting insulin-secreting [Beta]cells contained within a tissue-engineered pancreatic construct. We present here our recent results of using an implantable macro-construct comprised of insulin-secreting cells contained in alginate beads housed within a biocompatible ring. Methods: Female C3H/HeN mice were rendered diabetic by one i.v. injection of alloxan (75 mg/kg), and body mass and fasting blood glucose levels were monitored. After three consecutive days with a fasting blood glucose level >300 mg/dl, a bioartificial pancreatic construct was implanted into the recipient mouse's peritoneal cavity to reverse the alloxan-induced diabetic state. This construct was composed of various sized PDMS rings attached to one another, and its cavity was filled with alginate to house [Beta]TC-tet insulinoma cells entrapped in alginate beads. Mass, fasting glucose levels and length of survival were monitored. Results: 75 mg/dl alloxan induced profound hyperglycemia after one i.v. dose. Untreated, alloxan injected animals survive for only a few days. Upon receiving the construct therapy, the blood glucose of most diabetic mice returned to the normoglycemic range. Some diabetic mice remained at a hyperglycemic level, but all were able to maintain long-term survival (weeks to months), unlike those that did not receive any treatment. Conclusions: This study has demonstrated the viability of the bioartificial pancreas in vivo, and the feasibility of this therapeutic approach to control diabetes and prolong survival in recipient mice. Moreover, the ability to maintain long-term controlled hyperglycemia allows for studies that can investigate effects of diabetic hyperglycemia on the eyes, kidneys, and cardiovascular system, as well as a means to stabilize animals while being exposed to methods aimed toward regenerating pancreatic [Beta]-cells. This device has clinical applications, and may allow people afflicted with diabetes to live a normal lifestyle, one without daily insulin injections and finger pricking, and without the long-term deleterious effects of this disease. Work to increase the device longevity through better understanding the impact of the implantation on the recipient's immune system is ongoing. Sources of Research Support: NIH Grant RO1 DK47858 awarded to NES; Support for MC and BS by the Howard Hughes Medical Institute Science for Life Program. Nothing to Disclose: MMC, MJB, SN, BS, NES

Pub #	P1-501
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Type I Diabetes & Diabetic Complications (1:30 PM-3:3(PM)
Title	Metformin Improves Sirolimus-Induced Hyperglycemia and Normalizes Islet Insulin Content
Author String	C Clure, V Shivaswamy, B Griffin, C Reyelts, A Calleroz, C Borgeson, R Bennett, J Larsen, F Hamel Veterans Affairs Medical Center, Omaha, NE; University of Nebraska Medical Center, Omaha, NE
Body	Immunosuppressants contribute significantly to post-transplant diabetes mellitus (PTDM), which increases graft failure and mortality. We have shown that tacrolimus (TAC) and sirolimus (SIR) induce hyperglycemia in normal rats and SIR causes greater hyperinsulinemia than TAC and increases islet insulin content. Subsequently, we showed that metformin (MET) selectively improves hyperglycemia induced by SIR, but does not affect insulin resistance. We hypothesized that MET improves SIR-induced hyperglycemia by preservation of beta cells. To test this, we treated 4 groups of normal Sprague-Dawley rats with TAC, SIR, TAC+SIR or diluent (control) for 14 days compared with 4 more groups which received those same treatments plus MET. All rats had daily glucose measurements. On day 14, the rats were fasted overnight prior to an oral glucose tolerance test (OGTT) (1.5g/kg) and blood was collected at 0, 15, 30, 60 and 120 minutes for glucose and insulin measurements before sacrifice. Pancreata were harvested from all groups and were analyzed by immunohistochemistry. Pancreatic insulin and glucagon content were quantified using Image J software (NIH) and represented as mean integrated density. SIR and TAC+SIR had significantly higher mean random glucose (days 0-14) and MET lowered mean daily glucoses in SIR and TAC+SIR groups. SIR, TAC, and TAC+SIR had higher glucose response to the OGTT, compared to controls ($p<0.05$). SIR increased insulin concentration after the OGTT compared to controls ($p<0.05$). MET did not affect glucose or insulin responses to the OGTT. Pancreatic insulin content was higher in SIR compared to TAC+SIR ($p <0.05$) and trended higher than controls ($p=0.09$). MET significantly lowered the insulin content in SIR compared to SIR alone ($p<0.05$). Glucagon content was not significantly different among any of the treatment groups. In conclusion, MET improved overall mean glucose concentration over time after SIR and TAC+SIR treatment and normalized islet insulin content after SIR treatment. While the mechanism of these effects need to be further studied, this is the first study to show that MET can improve immunosuppressant-induced hyperglycemia and normalize islet insulin content to potentially prevent PTDM. Nothing to Disclose: CC, VS, BG, CR, AC, CB, RB, JL, FH

Pub #	P1-502
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Type I Diabetes & Diabetic Complications (1:30 PM-3:3(PM)
Title	Effect of Oligonucleotide IMT504 in a Type I Diabetes Model Induced by Multiple Low Doses of Streptozotocin in Mice
Author String	MS Bianchi, V Calvo, NA Chasseing, C Libertun, AD Montaner, VA Lux-Lantos Instituto de Biología y Medicina Experimental, Ciudad Autónoma de Buenos Aires, Argentina; Instituto de Biología y Medicina Experimental, Ciudad Autónoma de Buenos Aires, Argentina; Fundación Pablo Cassará, Ciudad Autónoma de Buenos Aires, Argentina
Body	IMT504, the prototype of the PyNTTTTGT class of oligonucleotides, stimulates mesenchymal stem cells both in vitro and in vivo (1). We have shown that the oligonucleotide IMT504 induces a marked recovery of single dose streptozotocin (STZ)-induced toxic diabetes in male rats that correlates with early expression of progenitor cell markers (2). Here, we evaluated the effect of IMT504 on a type I diabetes model induced by multiple low doses of STZ in mice. Male Balb/C mice (6-8 week-old) were injected with STZ ip (40mg/kg, diluted in citrate buffer) daily for 5 consecutive days or with citrate buffer as control (C). Normal glycemia (Gly) in the fed condition was 149±13 mg/dl. Animals which developed Gly levels [ge] 250 mg/dl were considered diabetics and injected daily with IMT504 doses (20mg/kg/day, sc) for 10 days (STZ-IMT) or saline as control (STZ) (day 1). Another 5 doses of IMT504 starting on days 21 and 36 were then administrated. A group of C mice were injected with the same IMT doses (C-IMT).Body weight was recorded and Gly was measured for a total of 66 days. At the end of the experiment, glucose tolerance tests (GTT) were performed (2g/kg BW glucose was injected ip, and glucose determined in tail blood samples). Four days later fasted animals were sacrificed, blood samples and pancreases collected for hormonal determinations and histological studies respectively. We observed that 20% of STZ mice (2/10) showed spontaneous reversion of the diabetic condition whereas IMT treatment induced a marked blood glucose decrease in 88% of STZ-IMT-treated mice (7/8) [day 66= Gly (mg/dl): C: 130±9 (n=6) vs STZ-IMT: 278±46, $p<0.01$, STZ-IMT vs STZ: 557±20, $p<0.01$]. GTTs showed a partial recovery in the STZ-IMT responsiveness [ANOVA: $p<0.001$, 0 min= C: 117±11, STZ-IMT: 164±9, STZ: 309±53, STZ vs C and STZ-IMT: $p<0.02$; 30 min= C: 292±51, STZ-IMT: 342±33, STZ: 488±36, C vs STZ: $p<0.02$; 120 min C: 133±15, STZ-IMT: 352±28, STZ: 472±52, C vs STZ and vs STZ-IMT: $p<0.02$]. Regarding body weight, IMT promoted a transient decrease in STZ mice. Besides IMT improved beta cell function in diabetic animals [HOMA beta cell= C: 66±29, STZ-IMT: 46±8, STZ: 13±5, ANOVA: $p<0.01$, STZ vs C and vs STZ-IMT: $p<0.03$]. Histomorphological analysis of pancreatic sections showed severe decreases in islets number from STZ mice, while a recovery was observed in islets from STZ-IMT animals, supporting our findings. IMT504 improves the diabetic condition in this model of type I diabetes. (1) Hernando IA et al., Stem Cells 2007; 25:1047 (2) Bianchi MS et al., Diabetologia 2010; 53:1184 Sources of Research Support: CONICET (PIP 363 2010); ANPCyT (BID PICT 2006 N[ordm]00200); ANPCyT (BID PICT 2007 N[ordm] 01050; Universidad de Buenos Aires (ME 038); Johnson & Johnson Argentina. Nothing to Disclose: MSB, VC, NAC, CL, ADM, VAL-L

Pub #	P1-503
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Type I Diabetes & Diabetic Complications (1:30 PM-3:3(PM)
Title	Characterization of Platelet Mitochondrial Function: Effect of Glucose
Author String	V Budharaju, BD Fink, JA Herlein, W Sivitz University of Iowa Hospitals and Clinics, Iowa City, IA; University of Iowa, Iowa City, IA
Body	**Background**: Although enucleated, platelets have abundant mitochondria. Mitochondrial function in platelets and the effect of glucose are not well understood. **Methods**: We used an adherent cell respirometer (Seahorse, Inc.) to assess mitochondrial function in intact human platelets and compared parameters to those observed in cultured endothelial cells. We also carried out limited studies comparing platelet mitochondrial function between control and type 1 diabetic subjects. **Results**: Platelet mitochondria demonstrated robust mitochondrial oxidative metabolism which was 4.3 fold greater than non-mitochondrial oxygen consumption. As compared to bovine endothelial (BAE) cells, basal platelet mitochondrial respiration was 93 ± 5% maximal as determined by chemical uncoupling whereas basal respiration in BAE cells was 65 ± 4% of maximal ($p < 0.001$, $n = 15$ platelet and 14 BAE preparations). Platelet mitochondrial respiration during ATP synthesis (oligomycin inhibitable oxygen consumption) accounted for 57 ± 7% of maximal mitochondrial respiration in platelets compared to 40 ± 3% in BAE cells (p < 0.05). Calculated [ldquo]apparent[rdquo] respiratory state for platelet mitochondria was 3.38 ± 0.09 compared to 3.69 ± 0.06 in BAE cells ($p < 0.01$). In limited studies, platelet oxygen consumption under basal conditions, during ATP formation, or during maximal uncoupling did not differ between platelets obtained from subjects with type 1 diabetes (n=11) and control subjects (n= 4). Moreover, adding glucose up to 22 mM to platelets during incubation *in vitro* did not alter basal, ATP-linked, or maximal respiration. Extracellular acidification rates relative to OCR were greater in platelets compared to BAE cells, but was not affected by diabetes or addition of glucose during incubation. **Conclusion**: Platelets manifest robust oxidative metabolism and, at least when incubate *ex vivo*, respire at nea maximal capacity. Platelet mitochondrial function is not affected by incubation at high glucose concentrations Sources of Research Support: Iowa Fraternal Order of the Eagles. Nothing to Disclose: VB, BDF, JAH, WS

Pub #	P1-504
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Type I Diabetes & Diabetic Complications (1:30 PM-3:3(PM)
Title	Attenuation and Fragmentation of Tight Junctions in *db/db* Mouse Myocardium
Author String	MR Hayden, T Chennamaneni, SV Bagree, SD Sowers, JR Sowers University of Missouri, Columbia, MO; University of Missouri, Columbia, MO; University of Missouri, Columbia, MO; Harry S Truman VA Medical Center, Columbia, MO
Body	Obesity and type 2 diabetes mellitus are now considered epidemic in childhood -adolescent and adult populations. Additionally, diastolic dysfunction plays and important role in diabetic cardiomyopathy. The young obese db/db mouse model was chosen to demonstrate myocardial ultrastructural remodeling as compared to its lean litter mates at 12 weeks of age. In addition to excessive lipid droplet accumulation in intermyofibrillar regions, mitochondrial biogenesis resulting in displacement of myocardial sarcomeres, and abnormal inner membranes and matrix of mitochondria, it was observed that endothelial tight junction(s) (TJ) were markedly remodeled. This novel TJ remodeling consisted of attenuation and fragmentation with open endothelial clefts, which may result in leaky capillaries in the myocardium. Abnormal remodeling of TJ within myocardial capillaries may result in increased myocardial toxicity, dysfunction, and edema of the extracellular matrix. Importantly, these changes may contribute to increased diastolic dysfunction and were observed concurrent with early diastolic dysfunction by ultrasound studies. Oxidative stress was also demonstrated in these models. Future studies regarding the individual protein changes of claudins, occludins, zona occludens, JAM-1 of tight junctions, and vascular endothelial (VE) cadherins-catenins of endothelial cells need to be undertaken in order to better understand the novel observational ultrastructural remodeling. Nothing to Disclose: MRH, TC, SVB, SDS, JRS

Pub #	P1-505
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Type I Diabetes & Diabetic Complications (1:30 PM-3:3(PM)
Title	The Role of TxNIP in the Development of Diabetic Nephropathy
Author String	A Shah, E Masson, L Xia, H Goldberg, C Whiteside, G Fantus University of Toronto, Toronto, Canada; University of Toronto, Toronto, Canada
Body	Thioredoxin-interacting protein (TxNIP) is an endogenous inhibitor of thioredoxin (Trx), a ubiquitous thiol oxidoreductase that regulates cellular redox status. TxNIP is upregulated by high glucose (HG) and has been shown to promote oxidative stress. We have found that Hcb-19 mice, which harbour a TxNIP gene mutation and lack TxNIP, are partially protected from streptozotocin(STZ)-induced diabetes. Since HG and reactive oxygen species (ROS) are key mediators of the microvascular complications of diabetes, we investigated the potential role of TxNIP in the pathogenesis of diabetic nephropathy (DN). Hcb-19 and C3H control mice were rendered equally diabetic with low dose STZ (Hcb19 received 2 x 5 day injections) and followed for 6 months. While Hcb-19 mice showed albuminuria (213.9±83.4 ug albumin/mg creatinine) in the non-diabetic state, in contrast to C3H (non-diabetic 28.4±16.8 ug/mg vs diabetic 385±184.4), there was no increase caused by diabetes (217.5±84.9). While immunohistochemical (IHC) analysis of glomeruli revealed no significant differences in $TGFb_1$ and collagen staining in non-diabetic Hcb19 vs C3H controls, after 6 months of diabetes glomerular accumulation of both were significantly increased in C3H ($p<0.01$ vs non-diabetic C3H) with minimal increase in Hcb19. To investigate the mechanism of protection, primary mouse mesangial cells (MC) were cultured from the 2 strains and exposed to HG. C3H MC exposed to HG (25 mM) for 3 h showed a significant increase in total cell ROS determined by DCF (2.29±0.13 fold, $p<0.001$) as well as MitoSox (mitochondrial ROS, 3.54±0.08 fold, $p<0.001$) fluorescence, while Hcb-19 MC had no response. Trx activity was decreased by HG only in C3H MC (0.31±0.07 of C3H NG, i.e. 69% decrease, $n=5$, $p<0.001$). Of interest lucigenin based NADPH oxidase assay revealed activation by HG only in C3H (2.21±0.28 fold, $p<0.05$) but not in Hcb19 MC. Recently, the mitochondrial-localized NADPH oxidase isoform, Nox4 has been implicated in HG-mediated ROS generation. Nox4 protein (immunoblots) was increased by HG in C3H total cell lysates (1.70±0.19 fold, $p<0.05$) and isolated mitochondrial fractions (2.14±0.26 fold, $p<0.05$), but not in Hcb19 MC. These data indicate that TxNIP is a critical component of the HG-ROS signaling pathway, apparently required for the induction of the mitochondrial NADPH oxidase isoform Nox4. Thus, TxNIP may be a promising target to prevent the complications of diabetes. Nothing to Disclose: AS, EM, LX, HG, CW, GF

Pub #	P1-506
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Type I Diabetes & Diabetic Complications (1:30 PM-3:3(PM)
Title	High Glucose-Induced Reactive Oxygen Species Production Is Mediated by c-Src in Mesangial Cells
Author String	KWK Lee, L Xia, HJ Goldberg, C Whiteside, G Fantus University of Toronto, Toronto, Canada; University of Toronto, Toronto, Canada; Mount Sinai Hospital, Toronto, Canada
Body	The pathogenesis of diabetic nephropathy (DN), a leading cause of end stage renal disease, remains incompletely understood. The pathologic changes caused by chronic exposure to high glucose (HG) require ROS (reactive oxygen species). Mitochondria as well as NADPH oxidase(s) appear to contribute to the generation of ROS in response to HG. In previous studies, we observed the activation of the Tyr kinase Src by HG and showed that in HG Src is required for EGFR transactivation, stimulation of MAPKs and synthesis of collagen IV in cultured rat mesangial cells (MC). Inhibition of Src in STZ-diabetic DBA2/J mice blocked albuminuria, mesangial matrix expansion and collagen IV deposition. Src has been reported to be both upstream and downstream of ROS. To determine its role in DN, MC were exposed to either normal glucose (NG 5mM) or HG (25 mM) and ROS measured using DCF fluorescence in the presence and absence of Src family kinase inhibitors, Dasatinib and AZD0530, as well as after transfection with Src-specific siRNA. After 3 h HG significantly induced ROS production (2.12 ± 0.04 fold vs. NG, $p<0.01$) that was markedly decreased by Dasatinib (1.15 ± 0.03 fold vs. NG), AZD0530 (1.02 ± 0.05 fold vs. NG), and Src siRNA (1.18 ± 0.03 fold vs. NG). Src has been reported to phosphorylate Vav2, a GEF (guanine nucleotide exchange factor) for the small GTPase Rac1, which is involved in NADPH oxidase activation. HG stimulated Vav2 Tyr172 phosphorylation (2.24 ± 0.29 fold vs. NG, $p < 0.05$) and Rac1 membrane localization (52 ± 2% in HG vs. 31 ± 4% in NG, $p < 0.05$) (a reflection of its activated GTP-bound state). Both were blocked by AZD0530 (Vav2 phosphorylation 0.97 ± 0.23 fold vs. NG; Rac1 membrane localization 33 ± 2%). In addition, Src siRNA blocked HG-induced Vav2 phosphorylation (0.77 ± 0.18 fold vs. NG). HG also stimulated the physical association of Src with Vav2 and Vav2 with Rac1 determined by co-immunoprecipitation. AZD0530 blocked Vav2-Rac1 association, indicating that the kinase activity of Src is required for Vav2-Rac1 signaling. MC contain Nox2 which is known to be activated by Rac1. Lucigenin-based NADPH oxidase activity was stimulated by 60% ($p<0.05$) by HG which was abrogated by AZD0530. These data indicate that HG stimulates Src which subsequently, via Vav2-Rac1, NADPH oxidase and the generation of a major proportion of ROS in MC. In light of our previous findings, Src-mediated ROS appears to be a major contributor to the pathologic changes seen in DN. Nothing to Disclose: KWKL, LX, HJG, CW, GF

Pub #	P1-507
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Type I Diabetes & Diabetic Complications (1:30 PM-3:3(PM)
Title	Effects of All-Trans-Retinoic Acid on Treatment of Type 2 Diabetic Neuropathy
Author String	JS Park, Y Lee, EH Lee, S Lee, J-H Jung, J Kim, JS Yoo, JS Nam, SA Kang, T-w Noh, MH Cho, KW Kim, CW Ahn, BS Cha, EJ Lee, SK Lim, KS Kim, HC Lee College of Medicine, Yonsei University, Seoul, Korea
Body	Neuropathy is one of the most common complications in diabetes mellitus. Nerve growth factor (NGF) is believed to regulate nervous system, neuronal differentiation, and regeneration of damaged nerves, and their role in diabetic neuropathy has been emphasing recently. Retinoic acid increases the endogenous expression of NGF. Our aim was to investigate effects of all trans retinoic acid (ATRA) in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, and on the expression of NGF and NGF Receptors $p75^{NGFR}$ and trkA in dorsal root ganglion (DRG) cells. Twenty OLETF were received 10 mg/kg/day ATRA *p.o.*, and 20 OLETF and 10 Long-Evans Tokushima Otsuka (LETO) rats were received cellulose as a vehicle once a day for 16 weeks. At the end of treatment, blood glucose and NGF in serum and sciatic nerve were measured. Neurometer current stimulus test was conducted to examine improvements of current stimulus thresholds. Nerve capillary density was measured in search of morphological changes secondary to neuropathy and regeneration. The effects of ATRA on neurite growth from DRG cells exposed to NGF were assessed and mRNA of NGF and NGF receptors $p75^{NGFR}$ and trkA in response to ATRA in DRG were measured by PCR and real-time PCR. Serum glucose decreased by ATRA treatment. NGF was decreased in ATRA-non-treated OLETF (683.71 ± 169.89 pg/mL in serum and 150.0 ± 28.8 pg/g in nerve) but increased in ATRA-treated OLETFc ompared to LETO (1423.5 $\pm$ 503.2 pg/mL in serum and 243.2 ± 41.9 pg/g in nerve) and in ATRA-treated OLETF (1855.2 ± 667.3 pg/mL in serum and 377.9 ± 61.5 pg/g in nerve) ($p<0.05$). Threshold increased significantly in OLETF and LETO, but decreased at 2000, 250 Hz in ATRA-treated OELTF compared to ATRA-non-treated OLETF. Nerve capillary density was decreased in ATRA-non-treate OLETF (173.2 $\pm$ 42.4 /mm^2) compared with LETO (262.78 $\pm$ 34.1 /mm^2). Neurite outgrowth and length in DRG increased in NGF and ATRA dose dependently. The high glucose (>30 mM) inhibited NGF dependent neurite formation. These results suggest that high glucose inhibits neurite formation. In DRG, ATRA regulated gene expression of NGF within 24 hr dose-dependently. But ATRA did not increase the expression of NGFR, $p75^{NGFR}$ and trkA mRNA levels. Our results have shown that ATRA increases serum and nerve contents of NGF in OLETF and influence nerve cell regeneration by promoting synthesis of NGF and suggest that ATRA has a potential therapeutic rol for diabetic neuropathy. Nothing to Disclose: JSP, YL, EHL, SL, J-HJ, JK, JSY, JSN, SAK, T-WN, MHC, KWK, CWA, BSC, EJL , SKL, KRK, HCL

Pub #	P1-508
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	HbA1c -- Evaluation of the DCA2000[reg] and Quo-Test Systems[reg]
Author String	T Azevedo, M Ferreira, A Giestas, AC Carvalho, S Pinto, I Palma Hospital Santo António - CHP, Oporto, Portugal; IPO-Coimbra, Coimbra, Portugal
Body	Introduction: HbA1c measurement is an excellent tool for the management of diabetic patients. There are many portable devices for measuring HbA1c. DCA2000[reg] system uses antibody affinity method and Quo-Test[reg] assay is a boronate affinity method. Objective: To compare the two portable devices for measuring HbA1c: DCA2000[reg] and Quo-Test[reg]. Methods: Finger-stick blood samples for measurement of HbA1c by DCA2000[reg] and Quo-Test[reg] systems and venous blood samples for laboratory measurement of HbA1c by HPLC were collect in 52 diabetic patients. The results obtained by each portable analyzer were compared individually with those reported by lab for accuracy and bias. Statistical analysis was performed using SPSS 18.00. Results: The mean HbA1c level was 8.08 ± 1.79 % (5.4-13.8) by DCA2000[reg], 7.78 ± 1.91 % (4.8-14.1) by Quo-Test[reg] and 8.11± 1.78% (5.1-13.5) by laboratory methods. We found a linear positive and statistically significant correlation between HbA1c values obtained by each of the portable devices and the laboratory values: for DCA2000[reg] analyzer, the Pearson[acute]s correlation coefficient was r=0.98 (p <0.001) and for Quo-Test[reg] analyzer was r=0,97 (p<0,001). Regarding the agreement between the results: for DCA2000 [reg] analyzer, the difference between its values and those reported by lab was on average 0.031% HbA1c lower for the portable device, 95% CI = [-0.138, 0.077], p = 0.568; for Quo-Test[reg] analyzer, the difference between its values and those reported by lab was on average 0.333% HbA1c lower for the portable device, 95% CI = [-0.467, -0.199], p<0,001. Thus, using the Quo-Test[reg] assay there was a systematic bias of 0,333% HbA1c towards lower values. Discussion and conclusions: In this study there was a positive correlation between the results of each portable device for the measurement of HbA1c (DCA2000[reg] and Quo-Test[reg]) and the lab results. We found better agreement between the results obtained by DCA2000[reg] system and lab than between the results obtained by Quo-Test[reg] analyzer and lab. For Quo-Test[reg] analyzer there was a systematic bias towards slightly lower values of HbA1c, which we think it can be easy to fix with calibration. Nothing to Disclose: TA, MF, AG, ACC, SP, IP

Pub # P1-509

Session Information POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)

Title The Effect of Sitagliptin on Serum Intact Proinsulin/Insulin Ratio (PI Ratio) of Type 2 Diabetes Patients

Author String M Sue, N Hiroi, T Watanabe, T Matsumoto, S Nakano, S Usui, K Iso, Y Tojo, K Kuboki, G Yoshino
Toho University School of Medicine, Ota-ku, Japan; Makita General Hospital, Ota-ku, Japan

Body

Serum intact proinsulin-insulin ratio (PI ratio), which elevates parallel with glucose tolerance status, is used as the parameter of pancreas β cell functions. It is the new topic as not only the convenient parameter of pancrease β cell function, but also as that of mechanism in insulin secretion.
DPP4 inhibitor inhibits resolution of GLP-1 by increasing insulin secretion and suppressing glucagon secretion. In addition, it is reported that DPP4 inhibitor promotes growth and proliferation of pancreas β cells by inhibiting apoptosis in vivo. In this study, we elucidated the effect of sitagliptin, a DPP4 inhibitor, on PI ratio of type 2 diabetes (T2DM) patients.

46 patients (22 male, mean age of 64.0 ± 11.7y) with poorly controlled T2DM treated with or without oral medication, were administrated 50 mg/day dose of sitagliptin. Fasting plasma glucose (FPG), HbA1c and PI ratio were measured before and 3 months after the administration. PI ratio was compared between abnormal high group with PI ratio more than 0.28, and control group with that ranged within 0.03 to 0.27. The statistica analysis was performed using paired t-test. $P < 0.05$ was significant.

Sitagliptin therapy significantly decreased FPG from 182.0 ± 53.3 mg/dL to 145.0 ± 42.9 mg/dL ($p < 0.001$), and HbA1c from 8.2 ± 1.0 % to 7.0 ± 0.7% ($p< 0.001$). Among all the patients, PI ratio was decreased significantly from 0.14 ± 0.08 to 0.11 ± 0.06 ($p < 0.01$). We analyzed the difference between the group of 5 patients with high PI ratio before sitagliptin therapy and the group of 41 patients with normal PI ratio. The high PI ratio group decreased significantly from 0.33 ± 0.07 to normal range of 0.20 ± 0.04 ($p < 0.05$), whose decrease rate was -38.2%. The normal PI ratio group decreased from 0.11 ± 0.05 to 0.09 ± 0.05 ($p < 0.05$) with decrease rate of -10.7%. There was no significance between decrease rate of two groups.

We revealed that sitagliptin significantly decrease FPG and HbA1c in T2DM patients. The decrease of PI ratio suggests the improvement in insulin secretion from pancreas β cell. Continuous hyperglycemia cause over activation of β cells and inhibit the activation of PC2 and 3, which effect insulin production, resulted in increase of proinsulin. The decrease of PI ratio represents normalization of PC 2 and 3 activation. In this study, we report the effect of sitagliptin in improving insulin secretion and normalizing abnormal insulin secreting mechanism.

Nothing to Disclose: MS, NH, TW, TM, SN, SU, KI, YT, KK, GY

Pub #	P1-510
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Diabetic Ketoacidosis and Venous Thromboembolism
Author String	J Goldman, F Matta, PD Stein College of Human Medicine, Michigan State University, East Lansing, MI; St Joseph Mercy Oakland Hospital, Pontiac, MI
Body	Diabetic ketoacidosis (DKA) has been associated with a persistent mortality rate of 3-4%, and venous thromboembolism (VTE) has been sporadically reported in such patients. Diabetes has been defined as a procoagulant state with predisposition to both thrombosis and attenuated fibrinolysis, and there is an increased relative risk for VTE in both diabetes mellitus type I (DM I) and diabetes mellitus type II (DM II)[3]. This study was undertaken to determine the prevalence and relative risk of VTE in DKA associated with both types of diabetes. Data from the National Hospital Discharge Survey were analyzed from 1979-2006. International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes were used to identify diseases. VTE was defined as the combination of pulmonary embolism and/or deep venous thrombosis. VTE was assessed among a total number of 907,198,000 patients including those with the diagnosis of DM I and DM II, with or without DKA that were discharged from short-stay nonfederal hospitals throughout the United States. DM I patients with VTE and DKA were 7,000 out of a subtotal of 1,377,000 (0.51%) (CI: 0.50-0.52) compared to DM I with VTE but no DKA or hyperosmolality which were 150,000 out of a subtotal of 10,713,000 (1.40%) (CI: 1.39-1.41). DM II patients with DKA and VTE were 5,000 out of a subtotal of 931,000 (0.54%) (CI: 0.53-0.56) compared to DM II with VTE but no DKA or hyperosmolality which were 738,000 out of a subtotal of 47,443,000 (1.56%) (CI: 1.56-1.56). Relative risk of VTE in DKA in hospitalized patients with DM I was 0.36 (CI: 0.35-0.37) and in those with DM2 it was 0.35 (CI: 0.34-0.36). Conclusion: Patients with diabetes mellitus type I and type II complicated by DKA have a lower risk for VTE The mechanism(s) underlying this decreased risk for VTE remain to be defined. 3 Stein, P.D., Goldman, J., Matta F. and Yaekoub, A.Y. Amer. Diabetes mellitus and risk of venous thromboembolism. Amer. J. Clin. Sci., 337: 259-264, 2009. Nothing to Disclose: JG, FM, PDS

Pub #	P1-511
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Hyperinsulinemia and Increased HOMA-IR in Pediatric Patients with Moderate Chronic Renal Insufficiency
Author String	MC Maggio, S Maringhini, D Montaperto, C Corrado, G Corsello University of Palermo, Palermo, Italy; ARNAS - Palermo, Palermo, Italy
Body	Patients with Chronic Renal Insufficiency (CRI) show an increased risk of cardiovascular disease, especially in pediatric onset. Hyperinsulinemia and insulin resistance, as well as inflammation and malnutrition, are well-known risk factors of cardiovascular disease. We studied 31 pediatric patients (19 M; 12 F), age:12.1±4.47, with CRI in conservative treatment. Following the Schwarz formula, the patients were divided in 3 groups: mild CRI:16(51,6%), moderate CRI:14(45,1%), severe CRI:1 (3,3%). We considered clinical and biochemical data (BUN, creatinin, proteins, albumin, Hb, cholesterol, HDL, triglycerides, CRP, ferritin), auxological parameters (stature, weight, bone age, pubertal stage, testicular volume or ovary echographic diameters, BMI), glucose and insulin levels (fasting and post-prandial glucose, insulin, c-peptide, HOMA-IR, HOMA B%), IGF-1. We compared all the data with a control group of 30 healthy children matched for gender and age. BMI was 20,53±5,07, with no significant difference between M and F; 24 pz (77%) (14M,10F) had a BMI <85[deg]C and 7 (23%) (2F; 5M) >85[deg]C. T0 insulin was 13,16±18,05; glycaemia T0: 91,81±18,38; glycaemia T60': 118,96±26,28; glycaemia T120': 115,13±35,04; HOMA-IR: 3±4,14. IGF-1 was 346±211,5. 2 pz (6%) had glycaemia T0' >126; 7 pz (23%) had glycaemia T60'>126; 7 patients (23%) had glycaemia T120' [ge]140. 10 (32%) pz had HOMA-IR >2,5. We report a statistically significant direct correlation of HOMA-IR vs BUN (r=0,671; p[le]0,002), HOMA-IR vs creatinin (r=0,676; p[le]0,002); insulinemia vs BMI (r=0,416; p=0,011); IGF-1 vs proteins (r=0,510; p=0,011). HOMA-IR was directly correlated with BMI (r=0,32; p=0,057), but without a statistically significance. No correlations were found between nutritional (albumin; Hb; ferritin) and inflammatory indexes (CRP); furthermore CRP did not present a statistically significant correlation with insulinemia and HOMA-IR. In our mild-moderate CRI patients we found high levels of glycaemia, insulinemia and HOMA-IR markers of insulin resistance. Children with higher BUN and creatinin levels showed higher HOMA-IR; higher BMI centile was relieved in children with higher insulinemia and HOMA-IR. The early occurrence of these risk factors in young patients, also in moderate involvement of kidney function, has a significant impact on cardiovascular prognosis. An integrate follow-up of these patients have to maintain an adequate BMI and HOMA-IR, in order to ameliorate long-term prognosis. Nothing to Disclose: MCM, SM, DM, CC, GC

Pub #	P1-512
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Prevalence of Diabetes Mellitus and Metabolic Syndrome in Prostate Cancer Patients Given Androgen Deprivation Therapy
Author String	MLCR Arkoncel, M Sagun, FR Arkoncel, C Jimeno, MC Lapitan University of the Philippines Philippine General Hospital, Manila, Philippines; University of the Philippines Philippine General Hospital, Manila, Philippines
Body	Context. Androgen deprivation therapy (ADT) is commonly used in the treatment of advanced, recurrent and metastatic prostate cancer. However, the resulting hypogonadism has adverse consequences. Objective. To compare the prevalence of diabetes mellitus (DM) and metabolic syndrome in prostate cancer patients with or without ADT. Design. Cross sectional analytic study. Setting. Tertiary referral center. Patients. Prostate cancer patients from the Integrated Surgical Information System database of the Philippine General Hospital from 2004-2010. Intervention. Patients who received hormonal therapy (ADT group - continuous monthly GnRH agonist injection for at least 6 months or bilateral orchiectomy at least 6 months prior) were compared to those who did not receive hormonal therapy (non-ADT group). Main Outcome Measures. Based on history, physical examination and laboratory results, patients with DM and metabolic syndrome were identified using the American Diabetes Association Standards of Medical Care in Diabetes 2010 and IDF Definition of Metabolic Syndrome, respectively. Results. The prevalence of DM in the ADT group is 42% and 19% in the non-ADT group ($p = 0.0460$). The probability of having DM is 2.17x higher among prostate cancer patients who received ADT compared to those who did not. The prevalence of metabolic syndrome in the ADT and non-ADT group is 37% and 28%, respectively ($p = 0.4620$). Conclusions. Prostate cancer patients have become an important emerging population of medically at risk older men. Our study showed that the prevalence of DM is significantly higher among the ADT group and a trend towards greater prevalence of metabolic syndrome in the same group.These men may benefit from counselling, screening and closer monitoring for the development of these metabolic complications. Sources of Research Support: Philippine Society for the Study of the Aging Male (PhiSSAM); Philippine Urological Association (PUA). Nothing to Disclose: MLCRA, MS, FRA, CJ, MCL

Pub #	P1-513
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Fasting Metabolite Profiles and Insulin Resistance in Children and Adolescents
Author String	SE McCormack, O Shaham, C Clish, VK Mootha, SK Grinspoon, A Fleischman Massachusetts General Hospital, Boston, MA; Children's Hospital Boston, Boston, MA; IBM, Haifa, Israel; MIT and Harvard, Cambridge, MA; Massachusetts General Hospital, Boston, MA; Harvard Medical School, Boston, MA
Body	**Background:** Understanding the pathophysiology of obesity-associated insulin resistance in children and adolescents is of critical public health importance. Metabolite profiling may offer new insights into complex biological processes such as insulin action. **Methods:** 69 subjects ages 8 - 18 years were enrolled in a comprehensive cross-sectional assessment of glucose homeostasis. Fasting laboratory studies were performed. (Initial observations from this cohort have been published.)(1) The concentrations of 60 metabolites were measured using liquid chromatography-tandem mass spectrometry.(2) The relationship between HOMA-IR, and the concentration of each of these metabolites was measured using Spearman's correlation analysis. P values were adjusted to account for multiple comparisons.(3) **Results:** 43% of subjects had a BMI [ge] 95th %ile; 58% were male. Subjects had a mean HOMA-IR of 1.84 ± SD of 2.04. Glutamate (Spearman's Rho 0.54, adjusted p value < 0.001), 3-hydroxyanthranilic acid (0.43, 0.04), and alanine (0.34, 0.05) are positively correlated with HOMA-IR, while citrulline (-0.46, 0.002) and glycerol (-0.36, 0.03) demonstrate an inverse relationship with HOMA-IR. **Discussion:** Glutamate may act a source of intermediates for gluconeogenesis, including alanine. Glutamate could be produced from metabolism of branched-chain amino acids. Concentrations of these have been found to be elevated in obese adults, and a factor containing glutamate was associated with HOMA-IR.(4) Our subjects with BMI >95th percentile had higher concentrations of branched-chain amino acids as well (p=0.0047). Increasing glycerol concentration, suggestive of ongoing lipolysis, was associated with greater insulin sensitivity in our subjects. This relationship persisted after controlling for age and BMI Z-score, and in the subset of subjects with BMI < 85th %ile. Our result may reflect a physiologic response to fasting in growing children, perhaps mediated by counter-regulatory hormones, in contrast to findings in insulin-resistant adults.(5) 3-hydroxyanthranilic acid (3-HAA) is a tryptophan metabolite that has pro-inflammatory effects. Citrulline is a component of the arginine-nitric oxide pathway; low citrulline levels could indicate endothelial dysfunction. **Conclusions:** Metabolite profiling demonstrates the potential association of HOMA-IR with gluconeogenic intermediates and lipid metabolism in insulin-resistant children and adolescents. Further studies are warranted (1) Fleischman A, Kron M, Systrom DM, Hrovat M, Grinspoon SK. Mitochondrial function and insulin resistance in overweight and normal-weight children. J Clin Endocrinol Metab. 2009;94(12):4923-30. (2) Shaham O, Slate NG, Goldberger O, Xu Q, Ramanathan A, Souza AL, et al. A plasma signature of human mitochondrial disease revealed through metabolic profiling of spent media from cultured muscle cells. Proc Natl Acad Sci U S A. 2010;107(4):1571-5. PMCID: 2824369. (3) Hochberg Y, Benjamini Y. More powerful procedures for multiple significance testing. Stat Med. 1990;9(7):811-8. (4) Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metabol. 2009;9:311-326. (5) Shaham O, Wei R, Wang TJ, Ricciardi C, Lewis GD, Vasan RS, et al. Metabolic profiling of the human response to a glucose challenge reveals distinct axes of insulin sensitivity. Mol Sys Biol. 2008;4:214. Sources of Research Support: NIH Grant 5K23DK80658 awarded to AF; NIDDK/P30-DK040561 (Pilot and Feasibility Grant; Harvard Clinical Nutrition Research Center) awarded to AF. Disclosures: SKG: Investigator, Theratechnologies; Consultant, Serono; Theratechnologies. Nothing to Disclose: SEM, OS, CC, VKM, AF

Pub #	P1-514
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Clinical Experience with U-500 Insulin: Risks and Benefits
Author String	A Boldo, R Comi Dartmouth Hitchcock Medical Center, Lebanon, NH
Body	Objective: To describe our clinical experience with U-500 insulin in insulin-resistant patients, including their change in glucose control, weight, insulin dose and hypoglycemia episodes. Methods: The charts of 53 patients that had U-500 insulin in their medication list in the last 2 years were identified and reviewed. HbA1c, weight and insulin dose were measured prior to U-500 insulin introduction, at 6 months and at the last clinic visit on U-500 insulin. Hypoglycemia, number of injections daily, reason for introduction and discontinuation of U-500 insulin were recorded. Results: The main reasons charted for introduction of U-500 insulin were high volume and poor diabetes control. The mean HbA1c decreased from 10.1% prior to U-500 insulin to 9.1% at 6 months and 8.6% at the last follow up (mean follow up was 36.6±24 months). Weight increased by a mean of 6.8kg and insulin dose increased by a mean of 0.44units/kg at the last charted visit. There was a significant increase in percentage of patients experiencing non-severe hypoglycemia episodes from 13% prior to the introduction of U-500 insulin to 42% at the last charted visit. There were no severe episodes of hypoglycemia due to U-500. The number of daily injections of insulin decreased significantly from a mean of 4(±1.4) injections per day prior to introduction of U-500 insulin to 3.3(±1.4) injections per day at the last visit. Of the 53 patients, 16 patients ultimately discontinued the use of U500 insulin. The most common reasons for discontinuation were increase frequency of hypoglycemia episodes and no insurance coverage or high cost for the patient. Conclusion: Patients with uncontrolled severely insulin resistance diabetes can be satisfactorily treated with U-500 insulin with the potential to improve glycemic control. An increase in weight and insulin/kg dose was observed as well as increase in non-severe hypoglycemia episodes. Nothing to Disclose: AB, RC

Pub #	P1-515
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Glucagon in Therapeutic Hypothermia
Author String	MW Call, DL Day, KD Mathews, D Collingridge, G Thomsen, J Ehrenkranz Intermountain Medical Center, Murray, UT; LDS Hospital, Salt Lake City, UT
Body	Therapeutic hypothermia (TH) is reduction of core body temperature to 32 to 34[deg]C for 12 to 24 hours. TH has been shown to improve survival and neurologic outcomes in patients who remain comatose after resuscitation from cardiac arrest.(1)(2) Hyperglycemia is common during TH and is thought to be due to catecholamine release and/or suppression of insulin secretion.(3) Animal studies demonstrate an increase in plasma glucagon and glucose in rats cooled to 19[deg]C for 20 hours.(4) Increased glucagon secretion raises blood glucose concentrations.(5) A rise in glucagon during TH could contribute to the hyperglycemia frequently seen with TH. This study was designed to determine if glucagon levels increase in humans during TH. Patients admitted to Intermountain Medical Center for TH following cardiac arrest between April and August 2010 were enrolled. Patients were maintained at a core temperature between 32 and 34[deg]C for 24 hours as measured by esophageal probe. Serum glucose and glucagon levels were obtained at four points during the admission: 1) prior to TH initiation, 2) two hours after the patients had reached a core temperature less than 34 [deg]C, 3) immediately prior to re-warming, 4) six hours after patients had reached a core temperature greater than 36[deg]C following re-warming. Seven patients were enrolled: 5 male and 2 female. The mean patient age was 64.6 years (range: 59 to 74 years). Mean glucagon levels for the four samples drawn were: 79.3 ng/L, 93.3 ng/L, 68.3 ng/L, and 106.7 ng/L respectively (range: <33 to 199 ng/L). There were no significant differences in glucagon concentration among any of the time points (Friedman one-way repeated measure of variance by rank $[chi]^2[3] = 4.54$, $p = 0.208$). Corresponding mean glucose levels were: 153 mg/dL, 139 mg/dL, 113.6 mg/dL, and 108.9 mg/dL (range: 62 to 240 mg/dL). All patients received insulin as required during TH to maintain blood glucose concentrations between 110 and 180 mg/dL. No patient required more than 3 units per hour while hypothermic. Three patients survived to discharge, four died from neurological injury. This analysis found no association between serum glucagon levels and induction or maintenance for 24 hours of hypothermia. In contrast to findings in animals, hypothermia may not cause glucagon secretion in humans. These results are limited by the small number of patients studied and by lack of inclusion of insulin resistant or significantly hyperglycemic patients. (1) HACASTG NEJM 2002;346:549-556 (2) Bernard, Gray et al. NEJM 2002;346:557-563 (3) Varon, Acosta Chest 2008;133;1267-1274 (4) Hoo-Paris, Jourdan et al. Am J Physiol 1991;260:R480-485 (5) Gromada, Franklin et al. Endocrine Reviews 2007;28:84-116 Sources of Research Support: Intermountain Medical Center Sorensen Heart & Lung Center. Nothing to Disclose: MWC, DLD, KDM, DC, GT, JE

Pub #	P1-516
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Autonomic Dysfunction in Asian Indian Type 2 Diabetes Mellitus Patients and Its Relation with Body Fat Content, Distribution and Insulin Resistance
Author String	P Punjabi, P Mathur, RC Gupta, SK Mathur, S Lalwani, I Mathur SMS Medical College, Jaipur, India; SMS Medical College, Jaipur, India; Mahatma Gandhi National Institute of Medical Sciences, Jaipur, India; Birla Institute of Scientific Research (BISR), Jaipur, India; SS Institute of Medical Sciences, Davengere, India; SMS Medical College, Jaipur, India
Body	Aim: To study autonomic dysfunction in Asian Indian type 2 diabetic patients by heart rate variability and it's relation with body fat content, distribution and insulin resistance. Subjects and Methods: Subjects: 33 T2DM patients aged (46.96 ± 8.90 yrs), M: F ratio 24:9 and 33 healthy controls aged (44.08 ± 9.15 yrs). M: F ratio 19:14. Methods: Short term heart rate variability (HRV) was measured by impedence plethysmograph recording of pulse wave in distal superficial arteries. Time domain and Frequency domain analysis of HRV was carried out. Time domain parameters (SDNN, rMSSD, pNN50) and frequency domain parameters (Total Power, LF power, HF Power, LF (nu), HF (nu), LF/HF Ratio) were determined. Body fat content and distribution was estimated by Dual energy X-ray absorbtiometry (DEXA). Insulin Resistance was assessed by Homeostasis Model Assessment (HOMA-R). Statistical methods: Student t test was used to compare mean value of variables in diabetics and controls. Multiple regression analysis was employed for correlations between parameters of autonomic dysfunction and Independent variables (weight, W;H Ratio, BMI, HOMA-R and parameters of Body fat content and distribution by (DEXA) in diabetics. Results: Parameters rMSSD, pNN50, Total power, LF Power, HF Power were statistically significantly lower in diabetics as compared to controls. Total power showed negative correlation with Weight and BMI ($r = -.44$; $p<.05$) and ($r=-.43$; $p<.05$) respectively. Frequency domain parameter HF (ms2) showed negative correlation with Weight, BMI and trunkal fat (gm %)($r=-.40$; $p<.05$) ($r= -.047$; $p<.05$) ($r=-.040$;$p<.05$) respectively. HF (nu) was negatively correlated with BMI ($r=-.43$; $p<.05$) whereas positive correlation was observed between LF (nu) and BMI (($r=.40$; $p<.05$). Conclusion: T2DM is associated with overall reduction in autonomic activity however, body fat content influences relative modulation of sympathetic and parasympathetic activity. Contrary to most previous reports, insulin resistance as well as W: H ratio had no influences on autonomic activity. Nothing to Disclose: PP, PM, RCG, SKM, SL, IM

Pub #	P1-517
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Micronutrient Antioxidants and Cardiovascular Risk in Patients with Diabetes Mellitus: A Systematic Review of Observational Studies
Author String	JC Almeida, RA Sarmento, FM Silva, G Sbruzzi, BD Schaan Federal University of Rio Grande do Sul, Porto Alegre, Brazil; Medicine School of Federal University of Rio Grande do Sul, Porto Alegre, Brazil; University Foundation of Cardiology, Porto Alegre, Brazil
Body	Several factors are involved in the development of cardiac complications in patients with diabetes mellitus (DM), including marked oxidative stress, but clinical trials evaluating the efficacy of antioxidants in preventing cardiovascular events have yielded conflicting results. **Aim:** To systematically review the role of antioxidant micronutrients in the development or presence of cardiovascular outcomes in patients with DM. **Methods:** We performed a search (Medline, Embase and Scopus) from January 1949 to November 2010 of observational studies that evaluated the role of micronutrient antioxidants in cardiovascular outcomes in patients with DM. All steps (reading titles and abstracts, eligible studies selection, data extraction, and quality scoring) were performed by two independent reviewers. The references of the articles included in the review were consulted to identify other potentially eligible studies. **Results:** We initially identified 12,454 articles, 27 were selected to read the full text, of which 5 were eligible for this review: 3 case-controls and 2 cohort studies (follow-up ranging from 7 to 14 years). One study was conducted in patients with type 1 DM, another study included patients with type 2 DM, and 3 studies did not specify the type of DM. Different antioxidant micronutrients were assessed across studies, the forms of measurement of these also are different from each other, as well as the outcomes evaluated, precluding the possibility of performing a metanalysis of the data obtained. Vitamin C was assessed from the intake (diet and/or supplementation), chromium and selenium were quantified in a toenail sample, and α-tocopherol and zinc were measured in patient serum. High intake of vitamin C supplements was associated with increased risk of cardiovascular disease, coronary artery disease (CAD), and stroke in one study (RR between 1.69 and 2.37), whereas low serum concentrations of α-tocopherol provided a protection of 30% for CAD in another. Among the minerals examined in 3 other studies (zinc, selenium and chromium), only an inverse association between serum zinc and CAD was observed (RR=1.70, 95% CI 1.21 to 2.38). **Conclusion:** Information about intake of micronutrient antioxidants and cardiovascular risk in individuals with DM is very scarce and heterogeneous. Therefore it is not yet possible to establish whether there is an association of cause and effect and/or to identify which antioxidant micronutrients are involved in this relationship. Nothing to Disclose: JCA, RAS, FMS, GS, BDS

Pub #	P1-518
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Diffusion Tensor Imaging: Radiological Findings in Middle-Aged US Veterans with Type 2 Diabetes Mellitu
Author String	JC Chapman, JG Welden, PM Sullivan, LA Roselli, TH Nassif, NM Myers, JH Pincus, MR Blackman, ES Nylen Veterans Affairs Hospital, Washington, DC; Veterans Affairs Hospital, Washington, DC; Veterans Affairs Hospital, Washington, DC; Veterans Affairs Hospital, Washington, DC

Body

Background: The prevalence of diabetes mellitus in the U.S. was nearly 24 million in 2007 (1). Most of these individuals (>95%) have type 2 diabetes mellitus (DM2). Diabetes is present in nearly 20% of the U.S. Veteran population (2), which is nearly 3 times the rate of that in the general U.S. population. Brain magnetic resonance imaging (MRI) studies have shown cortical and/or subcortical atrophy and/or cerebral small vessel disease (CSVD) in patients with DM2 (3,4), the latter usually resulting from damage to the vasculature and manifesting as lacunar infarcts and/or diffuse ischemic changes (leukoaraiosis). The samples in some of these studies had mean ages in the mid-sixth and seventh decade of life. As aging increases the incidence of these types of radiological findings (5,6), it is difficult to disentangle the effects of aging from the effects of DM2 on neuroanatomy. In order to control for age, we initiated a study of DM2 in middle aged Veterans. Furthermore, given the considerable white matter involvement in prior studies, we decided to employ diffusion tensor imaging (DTI). DTI methods examine patterns of molecular water diffusion that are thought to reflect axonal integrity.

Methods: We compared brain structure in male Veterans with DM2 versus non-diabetic sex-, age- and education-matched control subjects with DTI. Using a prospective, parallel group design, 5 cases and 9 controls were recruited. Cases had been diagnosed with DM2 by the Endocrine Service at the Washington, DC Veterans Affairs Medical Center (DC VAMC), had exhibited prior poor glucose control, but at the time o study, demonstrated well controlled glucose. The control group had no evidence of DM2 as demonstrated by laboratory measures. MRI images were acquired on a 3.0T Philips Achieva scanner.

Results: No significant group differences were found in age or education. Significant differences in FA and ADC were found bilaterally in the fornix, the body of the corpus callosum and the left genu of the corpus callosum.

Discussion: The fornix is a fiber bundle that transmits signals from the hippocampus to the mammillary bodies of the hypothalamus and has been implicated in memory dysfunction, a common cognitive finding in patients with DM2. Additionally, reduced FA in regions of the corpus callosum has been recently reported in patients with Type 1 Diabetes (7).

Conclusion: The current study provides preliminary data sufficient for further study.

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Sources of Research Support: Grant from the Institute for Clinical Research, Inc.

Nothing to Disclose: JCC, JGW, PMS, LAR, THN, NMM, JHP, MRB, ESN

Pub #	P1-519
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Serum Insulin-Like Growth Factor (IGF)-I and IGF-Binding Protein (IGFBP)-3 in Children with Diabetes: Are They Possible Markers for Glycemic Control in Diabetes?
Author String	MS Kim, S Arunachalam, S-Y Kim, PH Hwang, D-Y Lee Chonbuk National University Hospital, Jeonju, Republic of Korea; Chonbuk National University Hospital, Jeonju, Republic of Korea
Body	**Purpose**: Numerous studies suggest that growth factors may relate to the diabetes and its complications. However, it has not sufficiently reported about the relationship of serum IGF-1 and IGFBP-3 level with glycemic control or its complication in children with diabetes. The aim of this study was to investigate the hypothesis that serum IGF-1, IGFBP-3 levels may be marker for glycemic control, and they correlate with other clinical and laboratory parameters in children under 18-year-old with diabetes **Method**: We compared overnight fasting serum levels of IGF-I, IGFBP-3, age and body mass index(BMI) of diabetic subjects(n=87) with those of healthy children(n=152). Diabetic subjects were further divided into type 1 diabetes (n=69) and type 2 diabetes (n=18). And we examined the correlation between serum IGF-1/IGFBP-3 levels and clinical and laboratory data in children with diabetes. **Results:** Diabetic group (both type 1 and type 2) showed at a significantly older age (12.9 ± 4.1 years old) with a higher BMI (20.0 ± 4.4 kg/m^2) and higher IGFBP-3 level (4857.0 ± 1432.9 ng/mL) compared with controls (11.0 ± 4.1 years old, 18.5 ± 3.4kg/ m^2 , 2646.5 ± 652.0 ng/mL respectively; P<0.01). On contrary, IGF-1 level remained same in both diabetic and controls. And IGFBP-3 level correlated positively with IGF-1 age, BMI, blood pressure, serum C-peptide, and HbA1c in all diabetic subjects. In type 1 diabetes IGF-1 level was significantly lower (222.7 ±115.6 ng/mL) but higher IGFBP-3 level (4590.9±1411.1 ng/mL) compared with controls (293.3±171.9 ng/mL, 2646.5±652.0 ng/mL respectively; P<0.01). But age and BMI were similar in type 1 diabetes and controls. Importantly, IGF-1 and IGFBP-3 correlations were similar in all diabetes group patterns. But type 2 diabetes showed significantly higher BMI (24.8±3.4 kg/m^2) and IGFBP-3 level (5888.8±1064.4 ng/mL) compared with controls. But age and IGF-1 level were similar. IGF-1 correlated positively with IGFBP-3 and negatively with HbA1c whereas the other parameters showed no correlation. **Conclusion:** The IGF-IGFBP system is altered in diabetes patients. Our results, suggest the role of circulating IGF-I and IGFBP-3 in diabetic patients. Hence, the levels of circulating IGF-I and IGFBP-3 could be used as diagnostic markers of glycemic index. Nothing to Disclose: MSK, SA, S-YK, P-HH, D-YL

Pub #	P1-520
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Differentiating Type 1 and Type 2 Diabetes Mellitus at Time of Diagnosis in Hispanic-American Pediatric Patients Using Age, Gender, and BMI z-Score
Author String	P Jossan, N Keller, JN Braden, G Gildengorin, S Bhatia, S Teran, JA Noble Children's Hospital and Research Center Oakland, Oakland, CA
Body	***Background***: With the current rise in childhood obesity, physicians must consider both type 1 (T1D) and type 2 (T2D) diabetes mellitus as diagnostic possibilities when confronted with a child with new-onset diabetes. Because the management and therapy of the two disease processes are very different, the time between patien presentation and eventual availability of investigative laboratory results is one that expends excessive energy, time, and money when the diagnosis is not clear. ***Objective***: The purpose of this study was to formulate a mathematical model using only age, gender, and BMI z-score that could help physicians differentiate between T1D and T2D at initial presentation in Hispanic-American pediatric patients. ***Methods***: Data of 105 Hispanic-American patients diagnosed with T1D or T2D between 1998-2009 at Children's Hospital and Research Center Oakland (Oakland, California) were reviewed for age, gender, and BMI z-score at time of initial presentation of diabetes onset. Multivariate logistic regression analysis was used to create the mathematical model. ***Results***: In Hispanic-American patients age 2-18 years old, there was no difference in proportion of male or female sex between T1D or T2D ($p = 0.677$). T2D was associated with higher BMI z-scores (mean BMI z-score 0.510 ± 1.26 in T1D, 2.313 ± 0.344 in T2D, $p<0.001$) and older age ($8.52y \pm 3.64y$ in T1D, $12.86y \pm 2.31y$ in T2D; $p<0.001$). A mathematical model to predict the probability that a patient has T2D at initial presentation was generated with 92% sensitivity and 90% specificity. ***Conclusion***: This study suggests that a model based on age, gender, and BMI z-score may be formulated to help guide the physician diagnosing diabetes at the time of initial presentation, without waiting for biochemical markers. This diagnostic tool will be able to aid with appropriate allocation of resources as well as to guide initial and future management of Hispanic-American pediatric patients by elucidating more quickly their specific diabetes diagnoses. Preliminary use in the field has been promising. Such a model warrants a larger subject pool and application of prospective data to further evaluate sensitivity and specificity. Nothing to Disclose: PJ, NK, JNB, GG, SB, ST, JAN

Pub #	P1-521
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Depressive Symptoms in Diabetic Patients
Author String	AF OliveiraFilho, WE Chagas, RC Martiniano, IV Meira LIma, AB Nunes Municipal Health Consortium, Cuite, Brazil; Federal Univesity of Rio Grande do Norte, Natal, Brazil
Body	OBJECTIVE: To determine the prevalence of depressive symptoms and the relationship between depressive symptoms and metabolic control in diabetic individuals from rural community. METHODS: We have studied 190 diabetic individuals aged 10-83 years (median age 57.73 years) in a community clinic setting. Depressive symptoms were assessed using Beck Depression Inventory. Glycemic levels. HbA1c, fasting lipid profile, BMI, and blood pressure, were measured on each participant. Diabetes-related health behaviors were assessed by questionnaire, including social, demographic, economic, anthropometric, clinical history, and chronic complications aspects. RESULTS: The prevalence of depressive symptoms (score > or =10) was 67.74%. Depressive symptoms were more prevalent among women with diabetes than men, regardless of age group. The Beck Inventory data were able to detect depression mild to severe in 72.2% diabetic women and in 53.3% diabetic men. There was a relationship between depressive symptoms and worsening of metabolic control, but we could not demonstrate relationship concerning depressive symptoms and educational or financial degree. The frequency of depressive symptoms was correlated with the presence of chronic complications of diabetes, especially peripheral neuropathy. CONCLUSIONS: Data supported the association of depressive symptoms to bad metabolic control in diabetic individuals. The high prevalence rates of depression indicate psychiatric intervention to precocious detection and treatment, since these factors consist important protective mechanisms against depression influence in outcome and chronic complications. Furthermore, the depressive state involves hormonal changes potentially implicated in the worsening of diabetic control. It may be necessary to include psychiatric approach in diabetic care, besides the modification of lifestyle to achieve beter glycemic control in this population. Sources of Research Support: Fapesq/CNPq 010/04. Nothing to Disclose: AFO, WEC, RCM, IVML, ABN

Pub #	P1-522
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	The Effects of Caffeinated and Decaffeinated Coffee on Sex Hormone-Binding Globulin and Endogenous Sex Hormone Levels: A Randomized Controlled Trial
Author String	NM Wedick, CS Mantzoros, EL Ding, AM Brennan, B Rosner, EB Rimm, FB Hu, RM van Dam Harvard School of Public Health, Boston, MA; Beth Israel Deaconess Medical Center, Boston, MA

Body

Context: Findings from observational studies suggest that sex hormone-binding globulin (SHBG) and endogenous sex hormones may be mediators of the putative relation between coffee consumption and risk of type 2 diabetes.
Objective: To evaluate the effects of caffeinated and decaffeinated coffee on SHBG and sex hormone levels.
Design: After a two-week run-in phase with caffeine abstention, we conducted an 8-week parallel-arm randomized controlled trial conducted between May 2006 and September 2008. **Setting:** General greater Boston area community
Participants: Healthy adults (n=42) who were regular coffee consumers, nonsmokers, and overweight [body mass index 25-35 kg/m^2]. **Intervention:** Five 6-ounce cups of caffeinated or decaffeinated instant coffee or water (control group) per day consumed with each meal, mid-morning, and mid-afternoon. **Main Outcome Measures:** SHBG and sex hormones [i.e., testosterone, estradiol, dehydroepiandrosterone sulfate].
Results: No significant differences were found between treatment groups for any of the studied outcomes at the Week 8 visit. At week 4, we did not observe an effect of coffee intake and SHBG levels in men, although a borderline significant increase for decaffeinated coffee was observed among women compared with consuming no coffee. At week 4, we also observed several differences in hormone concentrations between the treatment groups. Among men, consumption of caffeinated coffee increased total testosterone and decreased total and free estradiol. Among women, decaffeinated coffee decreased total and free testosterone and caffeinated coffee slightly decreased free testosterone.
Conclusions: Our data do not indicate a consistent effect of caffeinated coffee consumption on SHBG levels in men or women. This is the first randomized controlled trial investigating the effects of caffeinated and decaffeinated coffee on SHBG and sex hormones and our findings necessitate further examination in a larger intervention trial.

Harvard Catalyst Human Research Center Laboratory Support Award; Boston Obesity Nutrition Research Center pilot and feasibility grant (grant #: 2005-P-000377/2); National Institutes of Health - National Center for Research Resources grant M01-RR-01032 (Harvard Clinical and Translational Science Center) and grant number UL1 RR025758. The Mantzoros Lab is also supported by the National Institute of Diabetes and Digestive and Kidney Diseases grants 58785, 79929 and 81913, and AG032030.

Disclosures: ELD: Study Investigator, Patent Pending on SHBG and Type 2 Diabetes. Nothing to Disclose: NMW, CSM, AMB, BR, EBR, FBH, RMvD

Pub #	P1-523
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Incidence of Postpartum Diabetes and Glucose Intolerance among Women with Gestational Diabetes Mellitus Seen at University of Santo Tomas Hospital -- A Preliminary Study
Author String	AY Sia-Atanacio, A Andag-Silva, EC Cunanan University of Santo Tomas Hospital, Manila, Philippines
Body	**Context:** Gestational diabetes mellitus (GDM) is associated with both chronic insulin resistance and impaired insulin secretion, both of which are also involved in type 2 diabetes. Thus, women with gestational diabetes mellitus are at increased risk to develop Type 2 diabetes. The incidence of postpartum glucose intolerance in the Philippines is not known. **Objective:** To determine the incidence of postpartum diabetes and/or glucose intolerance among women with GDM delivered at University of Santo Tomas Hospital (USTH) Charity and Pay Division and to compare the risk factors present among women with and without glucose intolerance postpartum. **Methods:** A prospective cohort study was performed from October 2009 to October 2010 at USTH. Women with gestational diabetes who fulfilled the inclusion and exclusion criteria were followed up at 6 weeks to 1 year postpartum using 75g oral glucose tolerance test. Clinical variables such age, height, weight before and after pregnancy, OGTT values, family history and obstetric history including mode of therapy used were recorded and compared. **Results:** The incidence of diabetes and impaired fasting glucose/impaired glucose tolerance postpartum (IFG/IGT) was 11% and 48% respectively. Women who developed postpartum glucose intolerance were more obese before pregnancy (p=0.016), had higher 2^{nd} hour and 3^{rd} hour glucose value on 100g OGTT (p=0.003, p=0.006) and had a greater number of positive OGTT values (p=0.012). Earlier onset of GDM during pregnancy (p=0.005) is also significantly associated with the development of postpartum glucose intolerance. Despite reminders through calls and text messages for follow-up 75g OGTT testing, 30% of women were still lost to follow-up, indicating the poor compliance to follow-up among these women. **Conclusion:** The incidence of postpartum glucose intolerance among women with GDM is high. This underscores the need for clinicians to be more vigilant in the postpartum monitoring of GDM patients in order to prevent associated health risks in these women. Strategies to implement compliance to postpartum glucose testing must be formulated to increase rates of follow-up testing among these women. Sources of Research Support: Philippine Society of Endocrinology and Metabolism Diabetes grant. Nothing to Disclose: AYS-A, AA-S, ECC

Pub #	P1-524
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Long-Term Benefits of an Ad-Libitum Non-Ketogenic Low-Carbohydrate Diet on Diabetes Control: Observations in the Clinical Setting
Author String	N Torbay, R Nawar American University of Beirut, Beirut, Lebanon; The Weight Care Clinic, Dubai, United Arab Emirates
Body	**Introduction**: The advantages of weight loss in obese diabetics have been established in a number of studies. We are not aware of any studies that have investigated the long-term effects of an ad-lib non-ketogenic low-carbohydrate diet on diabetics. The aim of this retrospective study is to assess effects of such a diet on glucose metabolism markers and lipid profile after one year. **Methods**: Twenty-six recently diagnosed diabetic patients (18 males, 8 females; mean age 52.8±9.4 yrs, mean BMI 35.4±6.3 kg/m^2, mean duration of diabetes: 0.7 ± 1.3 years) reporting to our clinic, were instructed on an ad-libitum non-ketogenic low-carbohydrate diet and maintained it for at least a year, were included in the study. Patients were encouraged to exercise and maintain a healthy lifestyle. No restrictions on use and type of fat were made. The diets provided 130-150g of carbohydrate per day to avoid ketosis. Weight and blood samples were obtained at baseline and at 1 year. **Results**: The mean changes observed between baseline and one year respectively were: a significant decrease in mean BMI (35.4±6.3 vs. 31.7±5.3 kg/m^2; $p<0.001$), fasting blood sugar (162.9±83.0 vs. 117.6±22.2 mg/dl; $p=0.013$), HbA1c (8.5 % ±2.5 vs. 6.2 % ±0.5 ; $p=0.001$), triglycerides (217.1±185.2 vs. 135.6±53.4 mg/dl; $p=0.015$), and HOMA1 (6.2±3.2 vs. 4.0±1.7; $p=0.009$). A significant increase was observed in HDL levels (48.4±17.0 vs. 54.7±13.6; $p=0.003$). Fasting insulin and cholesterol levels decreased at one year and were almost significant ($p=0.06$ and 0.09 respectively). No significant change was observed in LDL or on medication intake after 1 year. **Conclusion**: Independent of the amount of calories consumed and types of fat used, a low-carbohydrate non-ketogenic diet is an effective tool for weight loss and metabolic control in diabetic individuals. Nothing to Disclose: NT, RN

Pub #	P1-525
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Biochemical, Lifestyle and Psychosocial Factor Characteristics of Newly Diagnosed Type 2 Diabetics: Baseline Data from the Korea National Diabetes Program Cohort Study
Author String	MC Choi, YJ Lee, SO Chin, SY Rhee, S Chon, YC Hwang, IK Jeong, S Oh, KJ Ahn, HY Chung, JT Woo, SW Kim, JW Kim, YS Kim Kyung Hee University School of Medicine, Seoul, Korea
Body	Type 2 diabetes is currently considered a worldwide epidemic. It is an important public health concern with significant human, social, and economic implications associated with diabetes. During past few years, the profile of the newly diagnosed type 2 diabetic patients is changing. The aim of this study is to describe the clinical characteristics of newly diagnosed drug-na[iuml]ve type 2 diabetic patients in Korea and compare these with previously published western large cohort studies. Baseline data of newly diagnosed patients enrolled to the Korea National Diabetes Program (KNDP) cohort study conducted by 12 sites in Korea were used. Of 4,256 patients recruited to the KNDP cohort, the data of 728 newly diagnosed drug-na[iuml]ve type 2 diabetics were analyzed. Biochemical data and standardized surveys on lifestyle and psychosocial stress were collected. The Brief Encounter Psychosocial Instrument (BEPSI) score was used to evaluate the grade of psychosocial stress of the patients. A total of 728 patients with a mean age of 51.8 ± 11.0 years with 419 males and 309 females were included. Mean HbA1c was 8.2 ± 2.4% and was significantly higher in men (8.5 ± 2.5 vs. 7.9 ± 2.1%; P <0.001) and significantly differed by increasing age (P <0.001). Mean body mass index (BMI) was 25.4 ± 3.3 kg/m^2 and did not differ by gender. HDL-cholesterol was significantly higher in women (48.6 ± 13.1 vs. 44.0 ± 11.3 mg/dL; P <0.001). Triglyceride level was significantly higher in men (200.1 ± 175.3 vs. 157.7 ± 110.1 mg/dL P <0.001). The prevalence of metabolic syndrome was 48.3% in men, and 54% in women, respectively. The percentages of patients who exercised level of mild physical activity (3-4 times/week) and moderate physical activity (>5 times/week) were 17.4 and 27.6%, respectively, and did not differ by gender. The psychosocial stress assessed by the BEPSI score were moderate level and not different in both gender (1.9 ± 0.9 vs. 1.9 ± 0.9; P = 0.986). The mean age, HbA1c, BMI, and blood pressure in this study were lower compared to those in previously published western cohort studies. In conclusion, this study suggests that the profile of the newly diagnosed type 2 diabetics in Korea might be changed during past years, with lower mean age, HbA1c, and lower prevalence of obesity compared to the western countries. Nothing to Disclose: MCC, YJL, SOC, SYR, SC, YCH, IKJ, SO, KJA, HYC, JTW, SWK, JWK, YSK

Pub #	P1-526
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Diabetes Mellitus and Quality of Life in Hospitalized Patients
Author String	AC Costa, Jr, MP Miranda, JC Moura, CF Martins, PS Oliveira, EFS Cerqueira, RA Mendes, CC Coelho, VC Mota, ECO Naliato UNIFESO (Serra dos [Oacute]rgãos University Center), Teresópolis, Brazil
Body	Several studies have shown that quality of life (QoL) is decreased in individuals with diabetes. The presence of diabetic complications and comorbidities has an additional negative impact on diabetics' QoL. Objectives: To compare QoL in hospitalized patients with and without diabetes and comorbidities using a generic instrument (the SF-36 questionnaire). Patients & Methods: This cross-sectional study analyzed SF-36 scores of 84 hospitalized patients (age = 42.2 ± 19.1 years, body mass index = 26.2 ± 5.1 kg/m[sup2], duration of hospitalization = 11.3 ± 9.9 days) betwee April/2010 and October/2010. Most patients were women (58.3%). The prevalence of hypertension, diabetes and ischemic heart disease (IHD) was 31%, 19% and 14.3%, respectively. The leading causes of hospitalization were: obstetric disorders (24.4%), cardiovascular disorders (19.5%), and infection (18.3%). SF-36 scores of diabetics and non-diabetics were compared. Group comparisons of SF-36 scores were also performed for the other main comorbidities. SF-36 scores and other clinical data were simultaneously analyzed in order to identify correlations. Results: No difference was found between SF-36 scores of men and women. Diabetics had lower physical functioning (46.3 ± 23.0 vs 66.3 ± 28.5, $p < 0.01$), general health (52.0 ± 22.6 vs 64.6 ± 20.1, $p = 0.04$) and social functioning scores (39.8 ± 19.5 vs 55.9 ± 29.5, $p = 0.04$) than non-diabetics. In addition, diabetics had a higher prevalence of hypertension (62.5% vs 25.0%, $p < 0.01$) and ischemic heart disease (IHD, 31.3% vs 10.3%, $p = 0.04$) than non-diabetics. Patients diagnosed with hypertension also had lower physical functioning (50.6 ± 25.9 vs 68.2 ± 28.1, $p < 0.01$), general health (55.7 ± 19.3 vs 65.3 ± 21.4, $p = 0.03$) and social functioning scores (42.1 ± 25.5 vs 57.9 ± 28.6, $p = 0.02$). Moreover, physical functioning, general health and social functioning scores were lower in patients with IHD (44.2 ± 17.6 vs 65.6 ± 28.9, 48.8 ± 11.0 vs 64.4 ± 21.6, 31.3 ± 16.4 vs 56.4 ± 28.5, respectively, $p < 0.01$). In the multivariate analysis, hypertension was considered the main influence on the physical functioning score ($p < 0.01$), while IHD, the main influence on the general health ($p = 0.01$) and the social functioning scores ($p < 0.01$). Conclusion: The strongest determinants of reduced QoL in this group of hospitalized patients with diabetes were cardiovascular complications/comorbidities. Nothing to Disclose: ACC, MPM, JCM, CFM, PSO, EFSC, RAM, CCC, VCM, ECON

Pub #	P1-527
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Relationship between Plasma Parathyroid Hormone Levels and Glomerular Filtration Rate in Indian Diabetics with Chronic Kidney Disease
Author String	A Sinha, A Shrivastav, S Chowdhury, S Mukherjee, D Dutta Institute of Post Graduate Medical Education and Research, Kolkata, India
Body	Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) worldwide. Secondary hyperparathyroidism (SHPT) is a common, important complication of chronic renal insufficiency (CRI) and ESRD. Despite current guidelines, knowledge of the epidemiology of metabolic bone disease in the population with less severe CKD remains limited. Characterizing the burden of, and outcomes associated with, alterations in bone and mineral metabolism may have important clinical implications. Aims: To compare the performance of Cockcroft Gault (CG) formula and Modification of Diet in Renal Disease (MDRD) equation against isotopic GFR (using 99mTC) measurement, for estimation of GFR among patients with diabetes. Relationship between serum iPTH levels and GFR as estimated by isotope study, CG and MDRD and other metabolic parameters like Ca and Phosphate. Material and methods: The study group consisted of 80 consecutive diabetic patients attending Diabetic Clinic of SSKM Hospital, Calcutta, India and willing to participate in the study. No subjects were treated by dialysis at the time of the study. Patients with vit D deficiency (<30 ng/dl) were excluded from the study. Results: Both sexes (46 men and 34 women)were represented in study population.Mean ± SD HbA1c was 8.42 ± 1.2 %.Mean isotopic GFR was 37.18 ± 17.22 ml [bull] min[minus]1 [bull] 1.73 m[minus]2.GFR by isotope method correlated with GFR calculated by MDRD method (r = 0.823 , p = 0.00) and with CG method (r = 0.719 , p = 0.000).Mean phosphate in our population is 2.64 ± .59 mg/dl. Mean iPTH is 131.128 ± 96.23 pg/ml.Mean corrected Ca is 8.83 ± 1.40 mg/dl. There is a good correlation with 99mTC GFR with iPTH (r = -0.577) as well as with MDRD(r = -0.585). There is a moderate correlation of Phosphate with isotope GFR(= -0.202)and MDRD(r = -0.376),but poor correlation with corrected calcium. When defined by CKD stages correlation was maximum in CKD stage 4(r = -0.819) compared to stage 3(r = -0.736) and stage 5(r= -0.571). Conclusion: GFR calculated by both MDRD and CG formula correlate well with Isotope scan. However, MDRD has bette correlation in diabetic patient. There is a good negative correlation of iPTH with 99mTC GFR as well as with MDRD. Maximum correlation of iPTH with 99mTC GFR is found in CKD stage 4 compared to stage 3 and 5 in diabetic patients.Since many patients cannot afford PTH estimation in our country, GFR estimation may help select those patients who would benefit maximally from such estimations. Nothing to Disclose: AS, AS, SC, SM, DD

Pub #	P1-528
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Glycemic Index, Glycemic Load and Risk for Type 2 Diabetes: A Meta-Analysis
Author String	ZT Madhun, DD Garrison, JH Romeo, TZ Madhun, JM Ybarra, HF Saadi Case Western Reserve University, Cleveland, OH; Baldwin-Wallace College, Berea, OH; Cleveland State University, Cleveland, OH; Instituto de Cardiologia Avanzada y Medicina, Barcelona, Spain; UAE University, Al Ain, United Arab Emirates
Body	Diabetes mellitus type 2 has been a growing epidemic across all ages. The challenge to prevent diabetes became a primary public health issue, and the means to achieve it in the most cost-efficient manner possible is under intense investigation. The glycemic index (GI) and glycemic load (GL) have been primary targets of study to ascertain predisposition to diabetes. We set up to collect observational studies from 1981 to 2010 and according to a set of of stringent inclusion and exclusion criteria, 11 cohorts were used for GI analysis and 13 cohorts were used for GL analysis. The adjusted data were pooled using the random effect model. GI was 1.1((lower limit of 1.04, upper limit 1.29, 95% confidence level, P-value of 0.006, subjects with high GI are at 16 % increased risk for developing diabetes) and GL was 1.07 (lower limit of 1.03, upper limit of 1.10, 95% confidence level, p-value of < 0.0001, subjects with high GL are at 7 % increased risk for developing diabetes). Funnel plots did not show publication bias. The meta-analysis could not answer why there was a disproprtionate increased risk for diabetes with GI when compared to GL. Diets with elevated GI and GL may lead to post-prandial hyperglycemia, which is a known risk factor for cardiovascular disease. In similar cohorts, it has been shown that insulin resistance is not related to to elevated GI and GL. Thus, post-prandial hyperglycemia is one of the important factors in the natural history and progression to diabetes. Further research is needed involving large interventional studies and educational public health campaigns to to provide data to indicate if diet with low GI and GL are capable of reducing the risk for developing diabetes and cardiovascular disease. If adopting a strategy of low GI and GL is proven to be successful, then enormous savings will accrue for the health care plan budgets and most importantly a marked reduction of mortality and morbidity will be expected. Sources of Research Support: Unrestricted grant from the Figgie Family Charitable Foundation Inc. Nothing to Disclose: ZTM, DDG, JHR, TZM, JMY, HFS

Pub #	P1-529
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Assessment of Parameters Concerning the Difference between the Methods of Blood Glucose Measurements
Author String	D Kul, S Isik, D Berker, U Ozuguz, E Abayli, S Guler Ministry of Health, Ankara Numune Research and Training Hospital, Ankara, Turkey; Ministry of Health, Ankara Numune Research and Training Hospital, Ankara, Turkey
Body	**Objective:** It is well known that microvascular and macrovascular complications of diabetes mellitus (DM) can be effectively prevented with tight glycemic control. Self monitoring of blood glucose with a glucometer is very helpful to achieve strict goals. **Methods:** In this study, 188 patients with type 2 DM and 27 normoglycemic individuals were included. Fasting and postprandial venous serum glucose (SG) levels assayed by standard biochemical methods are compared to capillary blood glucose (CBG) and venous blood glucose (VBG) values by a single type glucometer in samples obtained simultaneously. Total cholesterol (TC), LDL-C, HDL-C, fasting and postprandial triglyceride (TG), TSH, sodium, hematocrit, creatinine, and HbA1c levels were also analyzed. **Results:** Fasting and postprandial CBG measurements revealed higher results higher than those of SG by a mean of 12.4 ±13.9 mg/dl [range= (-33) - 97.0)] and 17.2±18.0 mg/dl [range= (-40.0)-74.0], respectively. The VBG measurements were also higher than SG as a mean value of 10.0 ±13.7 mg/dl [range= (-54) - 82.0] in fasting and 9.1±17.6 mg/dl [(-69.0) - 76.0] postprandially. There was a statistically significant compatibility between fasting SG-CBG, SG-VBG, and VBG-CBG levels (Kappa= 0.678, 0.686 and 0.850, respectively; $p<0.001$ for all). Postprandial measurements were also significantly compatible (Kappa=0.669, 0.761 and 0.793, respectively; $p<0.001$ for all). HbA1c, LDL-C and HDL-C levels were negatively correlated with the difference between CBG and SG ($r=-0.205$, $p=0.005$; $r=-0.165$, $p=0.025$ and $r=-0.219$, $p=0.003$), while sodium and creatinine levels were correlated positively ($r=0.157$, $p=0.031$ and $r=0.201$, $p=0.006$). TSH and HbA1c levels were negatively correlated with the difference between VBG and SG, while sodium and creatinin levels were correlated positively ($r=-0.165$, $p=0.024$; $r=-0.289$, $p<0.001$; $r=0.185$, $p=0.011$ and $r=0.231$, $p<0.001$). **Conclusion:** The difference between the results of measurements conducted with glucometer and measurements conducted in laboratory are affected by various factors. If personal glucose monitoring is planned for patients with DM, the glucometer to be used must be checked personally by the patients with laboratory measurements. Nothing to Disclose: DK, SI, DB, UO, EA, SG

Pub #	P1-530
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Glucose Tolerance Status Is a Better Predictor of Diabetes and Cardiovascular Outcomes Than Metabolic Syndrome
Author String	CF de Souza, MB Dalzochio, FA de Oliveira, CR Neumann, JL Gross, CB Leitao Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil; Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil
Body	**OBJECTIVE:** To evaluate the importance of oral glucose tolerance test (OGTT) in predicting diabetes and cardiovascular disease (CVD) in patients with and without Metabolic Syndrome (MetS) from a low-risk population treated in a primary care unit. **RESEARCH DESIGN AND METHODS:** A prospective cohort study was conducted with subjects regularly attending the primary care unit of Hospital de Clinicas de Porto Alegre. Participants underwent a 75 g OGTT. MetS definition was based on the criteria of IDF/AHA/NHLBI-2010. **RESULTS:** Participants mean age was 61±12 years (males: 38%; whites: 67%). Of the 148 subjects included 127 (86%) were followed for 36±14 months. Subjects were classified into four groups based on baseline OGTT: 29% normal (n=43), 28% impaired fasting glucose (IFG; n=42), 26% impaired glucose tolerance (IGT; n=38) and 17% diabetes (n=25). MetS prevalence was lower in normal group (28%), intermediated in IFG (62%) and IGT (65%) groups and higher among subjects with diabetes (92%; P <0.001). The incidence o diabetes increased along with the stages of glucose metabolism disturbance (normal: 0%, IFG: 16%, IGT: 28%; P=0.004). No patient with normal OGTT developed diabetes, regardless the presence of MetS. Diabetes at baseline was the major determinant of CVD occurrence (normal: 0%, IFG: 4%, IGT: 0%, diabetes: 24%; P=0.001). In Cox-regression analysis, only the 2 h OGTT results were associated with diabetes (OR=1.03; 95%CI 1.01-1.06; P <0.001) and CVD development (OR=1.013; 95%CI 1.002-1.025; P=0.024). **CONCLUSIONS:** In this sample of subjects undergoing diabetes screening, the OGTT results were better predictors of diabetes and CVD than the MetS status. Sources of Research Support: Fundo de Incentivo a Pesquisa (FIPE) of Hospital de Clinicas de Porto Alegre. Nothing to Disclose: CFdS, MBD, FAdO, CRN, JLG, CBL

Pub #	P1-531
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Prevalence of Glomerular and Tubular Proteinuria in a Mexican Population of Type 2 Diabetes Patients
Author String	E Sabath, D Montero, I Aviles, P Garcia-Solis, JC Solis-S, HL Hernandez-Montiel, L Robles-Osorio Medical School, Universidad Autonoma de Queretaro, Queretaro, Mexico; Hospital General de Queretaro, Queretaro, Mexico
Body	Diabetic nephropathy is the most frequent cause of chronic renal failure in Mexico and is a growing health issue in the country. Few studies have addressed the prevalence and characteristics of diabetic nephropathy (DN) in type 2 diabetes mellitus (T2DM) patients and there is no information about tubular proteinuria in this population. Is the aim of this study to evaluate the prevalence and characteristics of DN in urban and suburban population of central Mexico. We enrolled 111 adult patients with T2DM, 24 (21.6%) males and 87 (78.4%) females. Age of patients at baseline ranged from 26 to 73 years and BMI was 28.5 ± 5.2. The mean time from diabetes diagnosis was 8.12 ±7.6 years. Treatment type: diet alone in 9.0%; oral agents 87.4% and 3.6% insulin. Prevalence of hypertension was high, 56% were taking antihypertensive drugs (66% were on ACE inhibitor). Mean HbA1c was 8.9%, 35% with HbA1c less than 7%. Spot urine samples to calculate for albumin/creatinine ratio and α1-microglobulin as a marker of tubular injury were taken. GFR less than 60 ml/min was found in 14% of patients. Microalbuminuria was found in 23% of patients and macroalbuminuria in 14.5%. Forty two percent of patients had increased level of α1-microglobulin. Univariate correlations showed no correlation between urinary α1-microglobulin excretion and age, sex, uric acid, albumin excretion and HbA1c. In conclusion these findings showed a high prevalence of renal disease in this population, with more patients showing tubular injury manifested for increased levels of α1-microglobulin; glycemic control is poor. This sample shows a high risk population to develop chronic renal failure. (1) Villalpando S et al., Salud Publica Mex 2010; 52 suppl 1:S19-S26 (2) Hong CY et al., Diab Care 2003;26:338-342 (3) Gilbert RE et al., Kidney Int 1999;56: 1627-1637 Sources of Research Support: Fondos Mixtos CONACYT-Estado de Querétaro. Nothing to Disclose: ES, DM, IA, PG-S, JCS-S, HLH-M, LR-O

Pub #	P1-532
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Predictive Markers of Glucose Intolerance in Obese Korean Children and Adolescents
Author String	HS Lee, JS Hwang Ajou University School of Medicine, Suwon, Korea
Body	**Objective:** Obesity prevalence in Korea has been increasing during recent decades. Childhood obesity is associated with an increased likelihood for having impaired glucose tolerance (IGT), dyslipidemia, and diabetes. The goal of study was to determine the prevalence of IGT and to assess the validity of glycated hemoglobin A1c (HbA1c) as a screening test for IGT in obese children and adolescents. **Research design and methods:** We studied 74 obese and overweight children (body mass index >85th percentile for age and gender) 4-17 years of age referred to the endocrine clinic at Ajou University Hospital in Korea. Arthropometric parameters and biochemical tests were performed. All subjects underwent HbA1c and oral glucose tolerance test. Multivariate logistic regression was used to determine independent predictors of IGT. **Results:** The prevalence of IGT was 9% in children (4-10 years of age) and 18.9% in adolescents (11 to 17 years of age). Type 2 diabetes was identified in 8 adolescents (10.8%). HbA1c was identified as a significant predictor for an IGT (P<0.001). Based on the receiver operating characteristic (ROC) curve, a HbA1c level of 5.7% had optimal sensitivity and specificity of 72.4% and 67.9%, respectively. **Conclusion:** Obesity associated with an increased risk of IGT. A HbA1c value of 5.7% should be used as a screening tool to identify children and adolescents with IGT. Nothing to Disclose: HSL, JSH

Pub #	P1-533
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Effect of Coffee Consumption on the Glycemic Index of Khalas Dates Tested in Healthy and Diabetic Subjects
Author String	JM Al Kaabi, B Al-Dabbagh, HF Saadi, S Gariballa, J Yasin Faculty of Medicine and Health Sciences UAE University, Al Ain, United Arab Emirates
Body	**Background:** The consumption of dates along with coffee is a deeply rooted habit in Arabs. This study was designed to determine the effect of coffee consumption on the glycemic index of a common variety of dates (khalas) tested in healthy subjects and individuals with type 2 diabetes mellitus. **Methods:** Composition analysis was carried out for khalas dates (tamer stage) and the weight of the dates flesh equivalent to 50 g of available carbohydrate was calculated. The study subjects were 13 healthy participants with a mean age 40.2±6.7 years and 10 participants with type 2 diabetes mellitus controlled on lifestyle measures and/or metformin with mean age 40.8± 5.7 years and with HbA1c of 6.6± 0.7%. Each subject was tested with 50 g of glucose (3 days), 50 g equivalent of available carbohydrates of the dates without coffee (1 day) and with coffee (1 day). Capillary glucose was measured over 2 hrs for the healthy subjects at 0, 15, 30, 45, 60, 90 and 120 min and over 3 hrs for the diabetic subjects at 0, 30, 60, 90, 120, 150 and 180 min. The Glycemic indices were determined as the ratios of the incremental areas under the response curves for the dates alone and dates plus coffee and were compared to glucose ingestion. **Results:** Mean glycemic indices of the khalas dates were 55.1± 27.9 and 62.4± 31.2 among healthy individuals without and with coffee consumption respectively (P=0.433). Corresponding mean glycemic indices among individuals with type 2 diabetes were 53.0± 18.9 and 41.5± 17.2 (P=0.045). **Conclusion:** Our results show that the consumption of khalas dates with coffee tends to lower the mean glycemic index of khalas dates among individuals with type 2 DM. Further studies are needed to confirm the above findings and to assess the control of hyperglycemia in subjects with diabetes when different varieties of dates are consumed with coffee. Nothing to Disclose: JMAK, BA-D, HFS, SG, JY

Pub #	P1-534
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Self-Monitoring of Blood Glucose in Type 2 Diabetes Mellitus: Effect on Glycemic Control in Non-Insulin-Treated Patients
Author String	B Madeo, S Scaltriti, S Romano, C Diazzi, EG Marwan, ARM Granata, ER Cavani, V Rochira Department of Medicine, Endocrinology & Metabolism, Geriatrics, NOCSAE of Baggiovara, University of Modena & Reggio Emilia, Modena, Italy; Department of Medicine, Endocrinology & Metabolism, Geriatrics, NOCSAE of Baggiovara, University of Modena & Reggio Emilia, Modena, Italy
Body	**INTRODUCTION:** The efficacy of Self Monitoring of Blood Glucose (SBMG) on glycaemic control in non insulin-treated (NIT) type-2-diabetes-mellitus (T2DM) is still controversial. In this paper we evaluated the influence of SMBG on glycaemic control in patients with T2DM with poor glycaemic control treated with oral antidiabetic agents. **METHODS:** 30 NIT-T2DM patients with poor glycaemic control and glycated haemoglobin (HbA1c)>7% under treatment with oral hypoglycaemic agents attending our Diabetes Clinic were randomized as follows: *SMBG Group* (patients currently using SBMG without a previous training to interpret the result); *Control Group* (patients who were asked to increase the dosage at visit 1 for a better control of diabetes). The patients were evaluated after 3 and 6 months and HbA1c and fasting plasma glucose (FPG) were monitored. 15 subjects (7 females and 8 males) aged 65±7.7years were randomized in the SMBG Group and 15 (6 females and 9 males) aged 62.7±9.7years in the Control Group. **RESULTS:** HbA1c levels significantly dropped after 3 (7.7±0.7%) and 6 (7.5±0.7%) months from baseline (8.4±0.6%) in the SMBG Group ($p<0.001$); HbA1c levels significantly dropped after 3 (8.0±1.1%) and 6 (7.7±1.0%) months from baseline (8.5±1.0) also in the Control Group ($p<0.05$) but without a significant difference between the two Groups when compared at the same time (3 or 6 months) of control. The FPG progressively decreased in both Groups in a significant way from baseline in SMBG Group just after 3 months, while in the Control Group 6 months were necessary for reaching significance; no significant difference between the two groups was found when compared each other at 3 and 6 months.**Conclusions:** The SMBG is as effective as increasing the dosage of oral antidiabetic therapy in improving the glycaemic control in NIT-T2DM subjects with previous poor glycaemic control even without a detailed training for interpreting the results. Nothing to Disclose: BM, SS, SR, CD, EM, ARMG, ERC, VR

Pub #	P1-535
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Clinical Risk Scoring is Not Useful in Screening Patients with Pre-IGT (Hyperinsulinemic State): The Importance of 2nd Hour Insulin Measurement in OGTT
Author String	IRC Nisce, LM Asis University of Santo Tomas, Espana, Manila, Philippines
Body	**Context:** Pre-diabetes is a condition where blood glucose level is neither normal nor in the diabetic range. Pre-prediabetes (pre-IGT) is a state of normal 1st and 2nd hour blood glucose level after a 75g OGTT with elevated stimulated 2nd hour insulin level and evidence of development of tissue damage. The challenge for physicians is identifying these high risk individuals in the pre-prediabetic state. **Objective:** To develop and determine usefulness of risk factor scoring to identify Filipinos at risk of developing type 2 DM. **Design and method:** Patients' records evaluated for type 2 DM at an endocrine clinic (1999-2010) were reviewed. Groups were based on 2nd hour glucose and insulin levels after a 75g OGTT using ADA guidelines type 2 DM, impaired glucose tolerance (IGT), and normal. A pre-IGT category (normal 2nd hour glucose but elevated insulin) was added. A score (point) was assigned based on frequency of occurrence of these risk factors: BMI [ge] 23kg/m^2 (7), family history of DM (6), age >40 (5), male gender (4), hypertension (3), if female, PCOS (2) and history of GDM (1). Lifestyle changes and pharmacologic therapy (insulin sensitizer for pre-IGT and IGT, plus sulfonylurea in type 2 DM) were started. **Results:** Of the initial 258 patients, 116 qualified for analysis (71% females). The prevalence of pre-IGT, IGT and DM were 44%, 18% and 21%, respectively. In the pre-IGT group, there was significant difference in the 2nd hour insulin level compared to normal (91.7 vs 20.4uIU/mL, p=0.0002), with the same observation seen in IGT and type 2 DM groups compared to normal. Interestingly, the risk score between normal and pre-IGT group was not significant (11.2 ± 6.5 vs 11.4 ± 5.8, p=0.92) implying the absence of clinical clues in the pre-IGT state. Conversely, risk score between normal vs. IGT and type 2 DM were statistically significant. In the IGT group (n=21), 15% progressed to type 2 DM after 2 years inspite of intervention with their baseline risk score not statistically different to the type 2 DM group. Inclusion of PCOS and GDM resulted to a significant difference in the risk score of the pre-IGT group in females vs. general subjects (11.4 ± 5.8 vs. 11.8 ± 5.8, p=0.002). The same trend is observed in the IGT category (p=0.05). **Conclusion**: Pre-IGT state can only be diagnosed by determination of the 2nd hour insulin level. The risk scoring system identifies only individuals at risk to develop type 2 DM if they are already in the IGT state with a score of [ge] 7. Nothing to Disclose: IRCN, LMA

Pub #	P1-536
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Sustainable Glycemic Control with Combination Insulin Therapy (Biphasic Insulin Plus Rapid-Acting Insulin Mimicking Physiologic Insulin Secretion in Type 2 Diabetic Patients
Author String	NER Laplano, LM Asis University of Santo Tomas Hospital, Manila, Philippines
Body	CONTEXT: Individualized insulin regimens targeting optimal glycemic control resulted to significant reductions in microvascular and macrovascular complications in type 2 diabetic patients. Combination therapy with premixed insulin (NPH plus short-acting insulin) plus rapid-acting insulin mimics biphasic physiologic basal requirement and prandial response to glucose load. We have previously shown that this combination regimen reduced HbA1c by 17.5% (9.07 vs. 7.48) over a 6-month period, with sustained reduction of up to one year (7.48 vs. 7.44) with no significant hypoglycemic episodes after patient education on automatic snacking. OBJECTIVE: This study aims to determine sustainability of glycemic control of more than one year in type 2 diabetic patients who are on combination premixed plus rapid-acting insulin. METHODOLOGY: In this descriptive retrospective analysis, we determined sustainability of glycemic control by comparing HbA1c levels at baseline and on subsequent consults, and noted interval period of each follow-up visit from time of target glycemic control. RESULTS: We reviewed records of 327 patients in an outpatient endocrinology specialty clinic. Fifty-one patients (36 females, 15 males; mean age at consult: 55.8 years; mean duration of DM: 8.9 years) achieved target HbA1c after a mean duration of 9.7$\pm$7 months on combination insulin therapy (from 9.7$\pm$1.4% to 7.2$\pm$0.8%). Serial HbA1c determination (n=44) showed further reduction (from 7.2% to 6.8%) after a mean duration of 12.3$\pm$1.8 months. After a mean follow-up interval of 47.5$\pm$12 months, target glycemic goal (mean at 6.56%, n=13) was still sustained. Further extension of follow-up demonstrated a remarkable 61.7 months mean sustainability of glycemic control (mean at 6.8%, n=6), showing an overwhelming 30% HbA1c reduction from baseline. With patient compliance on automatic snacking, there were no hypoglycemic episodes noted. CONCLUSION: The use of combination pre-mixed plus rapid-acting insulin mimicking physiologic insulin secretion showed an overwhelming sustainability of optimal glycemic control (9.7 vs. 6.8 or 30% reduction from baseline) for more than 5 years (mean 61.7 months). Nothing to Disclose: NERL, LMA

Pub #	P1-537
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Are Our Diabetic Patients Well Controlled?
Author String	J Mesquita, A Varela, F Correia, C Arteiro, D Braga, M Ferreira de Almeida, J Tiago Guimaraes, J Luis Medina, D Carvalho São João Hospital, Porto, Portugal; São João Hospital, Porto, Portugal
Body	**Introduction:** Diabetes mellitus (DM) is a chronic disease associated with lipid, blood pressure and coagulation abnormalities that requires continuous medical follow-up not only with regard to glycemic control, but also with regard to blood pressure and lipid profile. **Aims:** To assess glycemic (glycated haemoglobin - A1c, fasting glycaemia - FPG) and lipid control (plasma total cholesterol - Chol, plasma HDL cholesterol - HDL, plasma LDL - LDL and plasma triglycerides - TGs levels) of diabetic patients followed in an outpatient Endocrinology clinic. To determine the evolution of the previous parameters and the percentage of patients in whom the therapeutic goals (A1c<6.5%, FPG<110mg/dL, LDL<100mg/dL, TGs>150mg/dL, HDL>40mg/dL in men and HDL>50mg/dL in women) were reached. **Methods:** We evaluated 1635 diabetic patients followed because of DM, from 2004 to 2009. All of them had at least four analytical studies conducted in the Department of Clinical Pathology of São João Hospital, during this period of time. **Results:** Patients were followed up for an average period of 3.3 years and had analytical monitoring with a mean frequency of 6.7±1.8 months; 733 (44.8%) were female. In the initial evaluation they had mean age of 52.5±15.4 years, mean FPG of 148.9±67.4mg/dL (N:75-115mg/dL), A1c=7.5±2.1% (N:4-6%), Chol=191.3±43.2mg/dL (N<200mg/dL), HDL=48.9±14.5mg/dL (N>60mg/dL), LDL=120.5±33.1mg/dL (N<130mg/dL) and TGs=157.3±17.9 mg / dL (N<150mg/dL). In the last evaluation, they had FPG=137.6±70.9mg/dL, A1c=7.1±1.6%, Chol=187.5±44.8mg/dL, HDL=49,9±14.2mg/dL, LDL=118.1±33.9mg/dL and TGs=149.6±208.3mg/dL. Regarding glycemic control, at the end of this period of time, 38.3% had A1c<6.5% and 35.3% had FPG<110mg/dL. Regarding lipid profile, 30.2% had LDL<100mg/dL, 60.3% had TGs<150mg/dL, 53.2% of men had HDL>40mg/dL and 42.8% of women had HDL>50mg/dL. **Conclusions:** Despite a tendency to an improvement of glycemic and lipid control, even with a follow-up of diabetic patients by endocrinologists, the percentage of patients who reached the targets set by national and international guidelines was low. Nothing to Disclose: JM, AV, FC, CA, DB, MFdA, JTG, JLM, DC

Pub #	P1-538
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	The Relationship between Glycated Haemoglobin Result and Dietary Choices
Author String	EL Tng, C Chong, LW Cho Changi General Hospital, Singapore; Changi General Hospital, Singapore
Body	**Objective:** Dietary preference is a core factor that influences glycaemic control but it is also the most difficul element to change. We aim to optimise dietary counselling for people with diabetes by studying the relationship between their lifestyle preferences like sweetened drink consumption, frequency of eating out and glycated haemoglobin (HbA1c) levels. **Methods:** A retrospective, self-reported questionnaire on demographics, lifestyle activities, dietary choices and health belief was administered to patients attending the Diabetes Centre, Changi General Hospital, Singapore, between July to November 2010. Results were analysed with SPSS[trade] 12.0.1 for Windows [trade]. A two-tailed $P<0.05$ was considered to indicate statistical significance. **Results:** Fifty one patients took part in the study (age 48.33±16.01 years [mean±SD]). The youngest patient was 18 years old and the oldest patient was 84 years old. There were 30 males and 21 females (male:female ratio 1.43). There were 33 Chinese, 8 Malays, 7 Indians and 3 Eurasians. The mean (±SD) HbA1c was 7.97±1.66%. Frequent soft drink and fruit juice consumption is correlated with poorer HbA1c levels (P=0.012). Frequent outside meal consumption is correlated with poorer HbA1c levels (P=0.009). Patients who consumed one or less outside meal per day consumed sweetened drinks on an average of 2.48 times per week while those who consumed two or more outside meals per day consumed more sweetened drinks (average of 2.93 times per week). Eurasians most frequently drink soft drinks and fruit juices (mean=3.67 times per week), followed by the Chinese (mean=2.84 times per week), the Malays (mean=2.67 times per week) then the Indians (mean=1.86 times per week). Unemployed patients drink the most soft drinks and fruit juices (mean=5 times per week), followed by professionals (mean=3 times per week), retirees (mean=2 times per week), manual workers (mean=1.86 times per week) then housewives (mean=1 time per week). **Conclusion:** Frequent sweetened drink consumption and outside meals are positively correlated, and both lead to poorer glycaemic control. We recommend that dietary counselling be individualised according to the patients' demographic characteristics. Sweetened drink consumption should be actively sought in demographi groups with frequent sweetened drink and/or outside meal consumption. Such patients should be educated on dietary and sweetener alternatives and reading of food labels so they may make healthier food choices. Nothing to Disclose: ELT, CC, LWC

Pub #	P1-539
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Continuous Glucose Monitoring in Individuals with Pre-Diabetes
Author String	A Choudhary, Z Antal, MG Vogiatzi Weill Cornell/New York Presbyterian Hospital, New York, NY

Body

BACKGROUND: Pre-diabetes in children is a rising health concern, given the current epidemic of childhood obesity. Research in recent years has shown that acute glycemic excursions are more likely to result in chronic vascular complications than persistent hyperglycemia.(1)Determining the prevalence and severity of glycemic excursions in patients with pre-diabetes is, therefore, of interest. We hypothesized that an oral glucose tolerance test (OGTT) may not be representative of the overall glycemia in patients with pre-diabetes, who may experience many episodes of pathologic glucose excursions on a daily basis. Continuous glucose monitoring (CGM) using a glucose sensor measures interstitial glucose at frequent intervals (at least 288 measurements a day), and may better reveal the glycemic status of a patient with pre-diabetes.
METHODOLOGY: This is a prospective study to monitor CGM in individuals with impaired fasting blood glucose (Blood glucose: 100-125 mg/ dl) and /or impaired glucose tolerance (Blood glucose >140 mg/dl at 2 hours during an OGTT). CGM was used for at least 72 hours in these patients. Finger-stick glucose levels were checked four times/day for calibration of the sensors. The parameters that were measured were mean blood glucose and glycemic excursions >140 mg/dl using the sensor. HbA1c levels were also measured.
RESULTS: So far, CGM was performed in 4 patients (3 females: 1 male) ages 11 to 18 years. One subject had both impaired fasting and impaired glucose tolerance, and three subjects had normal fasting but impaired glucose tolerance. The HbA1C ranged from 5.9 to 6.4%. CGM showed a mean blood glucose of 113.5 mg/dl with the range of 83-162 mg/dl in all subjects. There was a variability in the percentage of time the subjects experienced glucose excursions : < 70 mg/dl: 0-3%, 70-140 mg/dl : 77-100% and > 140 mg/dl : 0-23 %. The subject with both impaired fasting and impaired glucose tolerance had the highest HBA1c, highest mean blood glucose and the highest glycemic excursion by CGM. The mean blood glucose and glycemic excursion by CGM in the other subjects appeared to be related to the HBA1c levels.
CONCLUSION: Although we have a limited number of subjects, these preliminary results suggest that the HbA1C levels correlate with mean blood glucose and glycemic excursions as measured by CGM. However, further studies are needed to prove this. This pilot study will also help us evaluate the role of CGM in individuals with pre-diabetes.

1. Hirsch, Irl, Brownlee,Michael:should minimal blood glucose variability become the gold standard of glycemic control? Journal of diabetes and Its complications 19(2005) 178-181

Nothing to Disclose: AC, ZA, MGV

Pub #	P1-540
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Quality of Life and Satisfaction in Children Using Continuous Glucose Monitoring System (CGM)
Author String	A Shah, K Chadha, S Fourtner Women and Children's Hospital of Buffalo, Buffalo, NY; University at Buffalo, Buffalo, NY
Body	BACKGROUND: Satisfaction of pediatric patients and their parents using continuous blood monitor system (CGM) has not been extensively investigated. The effects on the quality of life of a pediatric patient and their parents are analyzed with this survey. METHODS: All children who had used CGM, either in the past or presently, were asked to complete a patien satisfaction survey. Institutional review board approved the survey form, consent and assent forms. All families agreed to participate after consent. Older children completed the form while parents completed the survey for young children. Records were reviewed for HbA1C data and to confirm date of use. RESULTS: 7 surveys were completed. The two sensors in use were Guardian REAL-time (n=3) and Dexcom (n=4). All of the children were wearing the sensor continuously when in use. 42% (3/7) of the children stopped using the sensor completely; all of these were using the Guardian REAL time and all of them were above the age of 13 years. The children who are still using the sensor are all below the age of 9 years. 57% (4/ 7) of patients reported that the sensor did not meet their expectations, though 5 out of 7 claimed it helped thei diabetes control. 57% (4/7) of patients felt it helped them detect an episode of hypoglycemia prior to symptoms occurring and in 71% (5/7) patients the sensor detect hyperglycemia prior to being symptomatic. 86% (6/7) of the patients experienced false alerts for an abnormal blood sugar. 5 out of the 7 patients said they would recommend the sensor to other people. The average HgbA1c prior to usage of the CGM was 7.9% and 8.3% after using the CGM. CONCLUSION: While children and their families do report problems with CGM systems majority are content enough to continue use. There appears to be more satisfaction with the Dexcom CGM system although the sample size at present is small. Higher continuation rates are seen in younger children. Future direction for this study is to complete surveys on all CGM users in our population and correlate CGM with glycemic control. Nothing to Disclose: AS, KC, SF

Pub #	P1-541
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Air Pollution Effect on Variation of Glycosylated Hemoglobin A (HbA1c) Level in Diabetic Patients
Author String	F Mousavi, SA Jahed, A Rajab, AK Nikousokhan Tayar, R Tabatabaei, G Kashi, R Khajehkazemi Islamic Azad University Tehran Medical Branch, Tehran, Islamic Republic of Iran; Iranian Diabetic Society, Tehran, Islamic Republic of Iran; International Diabetic Federation, Tehran, Islamic Republic of Iran; Islamic Azad University Research & Sciences, Tehran, Islamic Republic of Iran; Islamic Azad University Tehran Medical Branch, Tehran, Islamic Republic of Iran; Kerman University of Medical Sciences, Kerman, Islamic Republic of Iran; Islamic Azad University Tehran Medical Branch, Tehran, Islamic Republic of Iran
Body	Introduction: air pollution and its effects on human health had become a major concern of many healthcare providers and decision makers. Effects of air pollution on functionality of neural, cardiovascular, gastrointestinal, and endocrinal systems have drawn majority of attentions to themselves in past recent years. In This study effect of air pollution on variation of Glycosilated Hemoglobin A (Hba1c) level in diabetic patients have been evaluated which is a unique study in Iran and Middle East region. Materials and methods: Tehran capital of Iran faced special condition of air pollution in September-January 2010-11. Pollution Standard Index (PSI) which is a cumulative scale for quality of inhaled air was used to report pollution extent in mentioned period and exact period 12 months before that time. A retrospective cohort study has been carried out on 330 patients diagnosed with diabetes mellitus for at least 12 months referring to 3 endocrinal care clinics. A questionnaire consisting of 7 questions in two demographic and personal healthcare sections has been deployed. In second section respondents answered to questions regarding their Glycosilated Hemoglobin A (HbA1C) levels which reflected their diabetes control level on September-January 2010-11. Afterwards, patients' HbA1C recorded on September-January 2009-10 have been taken out from their personal healthcare profile. Chief reason for setting a 12 month review point was to eliminate effect of seasonal variation of HbA1C on this study especially in diabetic type I group. Descriptive statistics and paired T-test analysis has been carried out for result commentation. Results: respondents have been divided to 2 main groups; group one, 108 patients (53.7% female and 46.3% male) with diabetes mellitus type I and mean age of 17.22 years. Group two, 222 patients (58.6% female and 41.4% male) with diabetes mellitus type II and mean age of 53.91 years. In group 1 in mentioned annual period HbA1C increased from 7.71 to 7.75. However, the difference was not statistically significant (P =0.828). In group 2 in mentioned annual period HbA1C increased from 7.06 to 7.08 which was not statistically significant (P =0.798). Conclusion: air pollution effect did not varied HbA1C in a 1 year period significantly. Nothing to Disclose: FM, SAJ, AR, AKNT, RT, GK, RK

Pub #	P1-542
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Paternal Somatic Mosaicism of KCNJ11 Mutation Causing Permanent Neonatal Diabetes with iDEND Syndrome in Offspring: SEARCH for Diabetes in Youth Study
Author String	R Kanakatti Shankar, LK Gilliam, C Pihoker, S Ellard, AT Hattersley, D Standiford, LM Dolan Cincinnati Children's Hospital Medical Center, Cincinnati, OH; University of Washington Medical Center, Seattle, WA; University of Washington, Seattle, WA; Peninsula Medical School, University of Exeter, Exeter UK

Body

Background: Heterozygous activating mutations in the KCNJ11 gene encoding the Kir6.2 subunit of the K-ATP channel cause Permanent Neonatal Diabetes (PNDM). Since Kir6.2 is expressed in the central nervou system and the pancreas, about 20% of patients have varying degrees of neurological symptoms termed the DEND (developmental delay, epilepsy and neonatal diabetes) syndrome. In the absence of seizures, it is termed intermediate (iDEND). Sulfonylureas (SUs) are usually more effective than insulin in achieving glycemic control in these patients. For G53D and some other mutations in the KCNJ11 gene associated with the iDEND syndrome, SU therapy has been shown to improve neurological symptoms as well. Germline mosaicism has been reported in unaffected parents for other KCNJ11 mutations, but to our knowledge, this is the first report of a somatic (and inferred germline) mosaicism of the G53D mutation in a non-diabetic parent who may have mild neurological manifestations of iDEND.

Case: We report a male infant born to parents of Philippino and Spanish descent, with no family history of diabetes, who presented with diabetic ketoacidosis at 3.5 months of age. He was diagnosed with type 1 diabetes, and was treated with insulin. Delays in motor and language milestones were noted along with mild hypotonia and motor stereotypies. Identification of a G53D missense mutation in the KCNJ11 gene at 8 years of age confirmed PNDM and iDEND syndrome. He was transitioned from insulin to glyburide, resulting in excellent glycemic control (HbA1c decreased from 7.3% to 6%, normal 3.5-6.3%), but no significant improvement in motor skills. Testing for the mutation in his parents revealed that his father was mosaic for th G53D mutation, found in 20% of peripheral blood lymphocytes. The father's fasting and 2h post-prandial blood glucose levels were normal on an oral glucose tolerance test, but he did have a history of attention deficit hyperactivity disorder (ADHD) and learning difficulty.

Conclusion: We report somatic (and inferred germline) mosaicism for the G53D mutation in the KCNJ11 gene in a non-diabetic father, with iDEND syndrome in the child. Treatment with glyburide did not dramatically improve neurological symptoms as previously reported for this mutation. Parental mosaicism is important to recognize since the risk of diabetes in future offspring may be as high as 50%. Genetic testing and counseling should be offered, even in the absence of a family history of diabetes.

Sources of Research Support: SEARCH for Diabetes in Youth is funded by the Centers for Disease Control and Prevention and supported by the National Institute of Diabetes and Digestive and Kidney Diseases. The monogenic diabetes ancillary study was supported by the Juvenile Diabetes Research Foundation.

Nothing to Disclose: RKS, LKG, CP, SE, ATH, DS, LMD

Pub #	P1-543
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Effects of High-Dose Cholecalciferol on Metabolic Function in Diabetes: A Randomized Control Trial
Author String	S Elkassaby, LC Harrison, N Mazzitelli, J Wentworth, PG Colman, S Fourlanos Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; Royal Melbourne Hospital, Melbourne, Australia
Body	BACKGROUND Vitamin D nutrition is dependent primarily on cutaneous synthesis in response to UVB radiation. Even in sun abundant countries, D deficiency is common. In addition to vitamin D receptors, pancreatic beta cells also contain 1-alpha-hydroxylase, which converts vitamin D to its active form, 1,25(OH)2D3. Studies in mice and cross-sectional studies in humans suggest beneficial outcomes of vitamin D supplementation on beta-cell function. However, randomised controlled trials of vitamin D supplementation in humans with diabetes are lacking. AIM To determine the effect of oral cholecalciferol (vitamin D3) on metabolic function in people with diabetes. METHODS In a double blind placebo-controlled trial, 50 adults with recent-onset diabetes (within 12 months of diagnosis were randomised to 6000 iu/day of cholecalciferol (n=26) or placebo (n=24) for 6 months. Inclusion criteria included age of diabetes onset 30-60 years, non-insulin requiring, HbA1c < 8% and serum 25D3 28-85nmol/l. The primary outcome was insulin secretion assessed by a glucagon stimulation test [delta C-peptide (DCP)]. Secondary outcome measures were insulin resistance by HOMA-IR, plasma glucose, HbA1c and blood pressure. RESULTS Serum 25D3 and 1,25D3 increased significantly in the D3 treatment arm (median 25D3 baseline 59 nM, 3 months 150 nM and 6 months 128 nM). At 3 months, median DCP was significantly higher in the treatment group compared to placebo [0.89 nM (IQR 0.51-1.40) vs. 0.65 nM (IQR 0.44-1.06); p = 0.04]. At 6 months, there was no significant difference in DCP between the groups. HbA1c decreased significantly at 3 months in the treatment group [baseline 6.2% (IQR 5.9-6.6) vs. 5.9% (5.7-6.5); p = 0.02]. Post-prandial glucose decreased significantly at 3 months in the treatment group compared to placebo [7.2 mM (IQR 5.6-7.8) vs. 7.7 mM (IQR 5.6-7.8); p = 0.03]. There was no significant change in HOMA-IR or blood pressure. CONCLUSION Oral cholecalciferol treatment resulted in modest but transient effects on beta-cell function and glycaemia. Further larger randomised controlled trials of vitamin D3 are required in diabetes populations. Sources of Research Support: Walter and Eliza Hall Institute of Medical Research; National Health and Medical Research Council of Australia; Bob Munro Foundation; Royal Australasian College of Physicians (Basser family scholarship). Nothing to Disclose: SE, LCH, NM, JW, PGC, SF

Pub #	P1-544
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Impact of Dietary Macronutrient Composition on Insulin Sensitivity, Fasting Glucose, and Beta-Cell Response in Healthy, Overweight, Men and Women
Author String	BA Gower, LLT Goree, PC Chandler-Laney, AC Ellis, K Casazza, WM Granger University of Alabama at Birmingham, Birmingham, AL; University of Alabama at Birmingham, Birmingham, AL
Body	BACKGROUND: Elevated fasting glucose is thought to impair glucose-stimulated insulin secretion (GSIS). Reduction in dietary fat may reduce fasting glucose by improving insulin sensitivity. However, reduction in dietary carbohydrate (CHO) may improve beta-cell function by minimizing the demand for insulin secretion. Thus, it is not clear whether a lower-fat diet or a lower-CHO diet would be most beneficial for glucose contro and beta-cell function among individuals with, or at risk for, elevated fasting glucose. OBJECTIVE: This study was conducted to examine the effects of a higher-CHO/lower-fat diet and a lower-CHO/higher-fat diet on insulin sensitivity, fasting glucose, and beta-cell response to glucose. METHODS: Participants were 69 overweight, non-diabetic, men and women; 42 were normal glucose tolerant (NGT, fasting glucose <100 mg/dL), and 27 had impaired fasting glucose (IFG, fasting glucose $\geq$100mg/dL). For 8 weeks, participants were provided with all food, and received either a higher-CHO/lower-fat diet (55% CHO, 18% protein, 27% fat) or a lower-CHO/higher-fat diet (43% CHO, 18% protein, 39% fat). Although energy intake was calculated to be eucaloric, most participants experienced some weight change (average of -1.68 kg); thus analyses were adjusted for weight change. Insulin sensitivity and beta-cell response to glucose (basal, PhiB; dynamic, PhiD; and static, PhiS) were calculated by mathematical modeling using glucose, insulin, and C-peptide data obtained during a liquid meal tolerance test. RESULTS: After 8 weeks, participants on the higher-CHO/lower-fat diet tended to have higher insulin sensitivity (P=0.123); this effect was significant within NGT (P<0.05). In all participants combined (P<0.05) and within IFG (P<0.001), the higher-CHO/lower-fat diet was associated with lower fasting glucose after 8 weeks. Within IFG, fasting glucose at baseline and the change in fasting glucose over the intervention were associated with PhiD (-0.53, P<0.01) and the change in PhiD (-0.42, P<0.05), respectively. CONCLUSION: Results are compatible with the hypothesis that a higher-CHO/lower-fat diet 1) has beneficial effects on insulin sensitivity; 2) reduces fasting glucose; and 3) leads to improved GSIS via reduction in fasting glucose. If confirmed, these results may have an impact on dietary recommendations for overweight individuals with and without IFG. Sources of Research Support: R01DK67538, M01RR00032, UL1RR025777, P30DK56336, P60DK079626. Nothing to Disclose: BAG, LLTG, PCC-L, ACE, KC, WMG

Pub #	P1-545
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Agreement between the Diagnostic Criteria for Diabetes Mellitus in Mexican Patients
Author String	D Espinoza-Peralta, FJ Gomez-Perez, S Hernandez-Jimenez, P Almeda-Valdes, MDR Letona-Barillas Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico City, Mexico
Body	Background: Historically, type 2 diabetes mellitus (T2DM) diagnosis has been based mainly on plasma glucose levels, either fasting (FPG) or after an oral challenge with 75 grams of glucose (OGTT). Recently, the use of glycated hemoglobin (A1c) has been recommended by the ADA and EASD for the diagnosis of T2DM The degree of agreement between these diagnostic tools has not been previously explored in Mexico. Methods: Cross-sectional, retrolective and descriptive study of subjects with neither a previous diagnosis of diabetes nor other illness or drugs that could affect glucose homeostasis. Subjects were submitted to an OGTT and A1c measurement on the same day. We estimated the agreement between FPG, OGTT and A1c with Kappa statistic. A1c was measured by a using ion-exchange, high-performance, liquid chromatography (BioRad). Serum glucose was measured by an enzymatic method in Synchron CX equipment. Results: We screened 1247 subjects, 133 (98 women and 35 men) fulfilled the inclusion criteria. The degree of agreement beyond chance for T2DM was k=0.272 for FPG and OGTT ($p<0.001$), k=0.212 for A1c and FPG ($p=0.001$), and for A1c and OGTT k=0.227 ($p=0.005$). Interestingly, there was a subgroup of patients (19.35%) with diagnosis of DM with A1c criteria, but normal by both FPG and OGTT criteria. Conclusions: We found that the agreement between the three diagnosis criteria is low. Previous studies have reported a variable performance of A1c in general healthy-considered population. In our context, including ambulatory patients from a reference hospital, A1c diagnoses a larger number of patients with T2DM. We couldn[acute]t determine which of these patients were false positives, this data needs further investigation. Based on this information it is not possible to recommend the use of A1c for the diagnosis of T2DM in our population. In addition, many laboratories in our country do not have standardized methods to estimate A1c, this also should be considered before extending the recommendation of using A1c as a criteria for diagnosis T2DM in Mexico. Nothing to Disclose: DE-P, FJG-P, SH-J, PA-V, MDRL-B

Pub #	P1-546
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Prevalence of BlaCTX-M in Diabetic Foot Infections: A Hospital-Based Study
Author String	Z Mohammad, A Malik, J Ahmad, S Mohd Faculty of Medicine, Aligarh Muslim University, Aligarh, India; Faculty of Medicine, Aligarh Muslim University, Aligarh, India
Body	The development and spread of resistance to β lactams is a major clinical concern which is mainly due to production of various β lactamases. One of them, extended spectrum β-lactamases (ESBLs) are plasmid mediated enzymes capable of hydrolyzing broad spectrum cephalosporins and monobactams causing resistance to antimicrobials and may exhibit co-resistance to many other classes of antibiotics resulting in limitation of therapeutic options. The inappropriate use of antibiotics results into treatment failure leading to amputation of foot in many cases. 162 [T2DM: 134(82.7%); T1DM: 28(17.3%)] diabetic foot ulcer (DFU) patients were studied for drug resistance during January 2009 to September 2010. Ulcer samples were collected for aerobic bacterial culture and sensitivity testing by standard methods. The Enterobacteriaceae isolates resistant to any one of the 3rd generation cephalosporins were studied for ESBL production. A total o 127(78.3%) Enterobacteriaceae members were isolated. The most common isolate was E coli 71(55.9%) followed by Klebsielle sp 33(25.9%), Proteus sp 13(10.2%), Acinetobacter sp 8(6.2%) and Morganelle morganii 2(1.5%). 109(85.9%) isolates were found to be resistant to 3rd generation cephalosporins of which ESBL production was observed in 87(79.8%) isolates. The molecular detection of CTX-M type ESBL (which showed 100% specificity and sensitivity in control strains) was done in resistant E coli and Klebsiella sp isolates by PCR. 63.6% of Klebsiella spp. isolates were found positive for CTX-M followed by E coli (43.6%). Forty seven (29%) DFU patients were positive for CTX-M type ESBL. The CTX-M positive status was associated with poor glycemic control (HbA1c >8%) in 37(78.7%, P<0.001), osteomyelitis 22(46.8%, P<0.004), neuropathy 27(57.4%, P<0.05) and previous antibiotic use 34(72.3%, P<0.05) but not with patients characteristic, ulcer type, type of diabetes, duration of ulcer and duration of hospital stay. The prevalence of ESBLs among the members of Enterobacteriaceae constitutes a major threat to currently available β lactum therapy leading to amputation in DFUs. There is an urgent need to minimize the misuse of available antibiotics. Nothing to Disclose: ZM, AM, JA, SM

Pub #	P1-547
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Glucose Metabolism Study in Patients with Functioning Transplanted Pancreas Using Continuous Glucose Monitoring System
Author String	MW Lauria, JM Figueiro, B Aramani, MD Sanches, AM Lana, A Ribeiro-Oliveira, Jr Felicio Rocho Hospital, Belo Horizonte, Brazil; New York Presbyterian Hospital, New York, NY; UFMG, Belo Horizonte, Brazil
Body	<u>Objective</u>: To compare glucose profiles in patients with type 1 diabetes treated with simultaneous pancreas/kidney transplantation (SPKT) or pancreas transplantation alone (PTA) to those under intensive insulin therapy (IIT) and to non-diabetic healthy controls (HC) by means of continuous glucose monitoring system (CGMS), assessing CGMS effectiveness as a predictor of graft dysfunction (GD). <u>Research Design and Methods</u>: Over 3 days, 40 subjects were connected to CGMS: 12 with SPKT, 10 with PTA, 10 HC and 8 IIT. All transplanted patients had a normal oral glucose tolerance test and were in use of a tacrolimus-based immunosuppressive regimen. Glucose control was evaluated by the 72-hr-mean glucose concentration (MGC) and variability. After 5 years, baseline data were reassessed comparing patients who ha had GD to those without GD. <u>Results</u>: MGC and variability were higher in the IIT than the other groups. PTA and SPKT had higher MGC than the HC. Comparisons between transplanted groups revealed higher MGC in the PTA than the SPKT (IIT= 7.8± 3.7 vs PTA = 5.6 ± 1 vs SPKT = 5.4 ± 1 vs HC = 5.2 ± 0.6 mmol/l; $p < 0.05$ for all comparisons). MGC at baseline CGMS were higher among those patients with GD in comparison to those without GD in five years (6 ± 1.2 vs 5.4 ± 0.9 mmol/l; $p < 0.05$). <u>Conclusions</u>: CGMS demonstrated the benefits of pancreas transplantation in terms of glucose control but revealed differences among transplanted patients and healthy controls. Furthermore, CGMS measures were found to be GD predictors. Nothing to Disclose: MWL, JMF, BA, MDS, AML, AR-O

Pub #	P1-548
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Adults with Type 1 Diabetes Followed in a Pediatric *vs.* Adult Endocrine Practice
Author String	S Subaran, J Quintose, G Gopalakrishnan Alpert Medical School of Brown University, Providence, RI
Body	**Objective:** The transition of patients with Type 1 Diabetes Mellitus from pediatric to adult endocrinology is complex. Our goal is to evaluate factors that influence transition to adult endocrinology and to determine whether there is a difference in the clinical care of adult patients followed in the pediatric versus adult endocrine practice. **Method:** In this retrospective chart review, we identified 187 Type 1 diabetic patients between the ages of 18-22 years followed for at least one year duration in our pediatric endocrine practice. Forty-one of these patients were eventually transitioned to the adult endocrine practice. The clinic chart of each patient was reviewed. Medical and laboratory data from each visit was recorded. Pediatric patients were subdivided into three groups: referred to adult clinic, remained in pediatric clinic and lost to follow-up. Comparisons between groups were evaluated using analysis of variance, paired t-tests and chi square tests. **Results:** The average age of the 187 pediatric patients was 19.1 years with a7.5 year average duration of diabetes. Majority were male gender (56%). For the 41 adult patients, the average age was 21 years with 51% male gender. Patients remaining in the pediatric clinic were more likely to have a lower HbA1C (8.6 vs. 9.7 and 9.9 respectively; $P<0.05$) and to have more frequent office visits in one year (3.9 vs. 3.2 and 2.5 respectively; $P<0.05$) when compared to those referred to the adult clinic and those lost to follow-up. Comparison between the 187 pediatric charts and the 41 adult charts showed significantly more documentation of influenza (54% vs. 9%) and pneumonia (39% vs. 1%) vaccinations, foot exam (83% vs. 1%), opthalmologic exam (78% vs. 42%) and inquiries about the use of alcohol (79% vs. 8%) and tobacco (91% vs. 8%) in the adult versus the pediatric endocrine practice ($p<0.05$). **Conclusion:** Pediatricians are more likely to retain adult patients with better glycemic control. The adult endocrine practice was more likely to address factors that influence complications of diabetes. It is well known that prevention of diabetic complications is critical to long-term morbidity and mortality. This study demonstrates the need for a more comprehensive evaluation and prevention of potential diabetic complications by pediatricians. Nothing to Disclose: SS, JQ, GG

Pub #	P1-549
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Cognitive Function Is Influenced by Ghrelin Gene Variants and Metabolic State
Author String	M Mora Porta, ML Mansego, J Chaves, E Palomera, G Diaz, X Buquet, M Serra-Prat, M Puig-Domingo Hospital Clínic of Barcelona, Barcelona, Spain; Hospital Clínico Universitario de Valencia, Valencia, Spain; Hospital de Mataró, Mataró, Spain; Hospital Clínico de Valladolid, Valladolid, Spain; Hospital de Mataró, Mataró, Spain; Hospital Germans Trias i Pujol, Badalona, Spain
Body	**Context**: Cognitive state and brain volume have been related to body mass index (BMI), abdominal fat, waist hip ratio, components of metabolic syndrome (MS) and ghrelin. Genetic variations within the ghrelin gene have been recently associated to MS. **Objectives:** We investigated cognitive state evaluated by Mini-Mental State Examination (MMSE) in relatior to MS components (ATP-III criteria) and six SNPs of ghrelin gene in non-institutionalized individuals aged 7(years or older. **Design, participants and methods:** 280 subjects (137 men /143 women, age 77.1±5.9) participating in the Mataró aging study (a population-based study) were included. Individuals were phenotipically characterized by anthropometric variables, lipids, glucose, blood pressure, and MMSE. SNPs -501AC (rs26802), -994CT (rs26312), -604GA (rs27647), R51Q (rs34911341), M72L (rs696217) and L90G (rs4684677) of the ghrelin gene were studied. Genotypes were determined by polymerase chain reaction and SNapshot minisequencing. **Results**: Mean MMSE score was 28.9 (SD: 5.5); 16.8% of individuals had cognitive impairment (defined as MMSE <24). Mean BMI was 28.1 (SD: 4.1) kg/m2; 30.9% were obese (BMI[ge]30 kg/m2). Mean waist circumference was 101.5 (SD: 11.6) cm; 67.3% had central obesity (pathological waist circumference) and 50.0% had MS. Cognitive impairment was significantly associated with age ($p<0.001$), female gender ($p=0.025$), low educational background ($p<0.001$) and glucose impairment or diabetes ($p=0.045$). MMSE score was significantly correlated with BMI ($p=0.014$, $r=-0.147$), Barthel scale ($p=0.023$, $r=0.136$), Geriatric Depression Scale (GDS) ($p=0.006$, $r=-0.163$) and Mini Nutritional Assessment (MNA) ($p=0.020$, $r=0.143$). M72L and L90G SNPs were associated to MMSE score: in M72L , CC genotype 29.4±5.3 vs CA 27.4±6.1, $p=0.043$, after adjusting for age, gender, GDS, educational level and diabetes, $p=0.218$; in L90G, AA genotype 29.3±2.3 vs AT 27.2±6.5, $p=0.068$, after adjusting for age, gender, GDS, higher education and diabetes, $p=0.007$). Cognitive impairment was associated to L90G (34.8% in AT genotype vs 14.0% in AA, $p=0.016$, after adjusting for age, gender, GDS, educational level and diabetes $p=0.003$, OR 5.9 -CI: 1.8-19.5-) **Conclusion**: Hyperglycemia or diabetes is associated to cognitive impairment. L90G Ghrelin gene variant seems to be associated to cognitive impairment in old Spanish community dwelling individuals. Disclosures: MP-D: Advisory Group Member, Merck BV. Nothing to Disclose: MMP, MLM, JC, EP, GD, XB, MS-P

Pub #	P1-550
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Insulin Resistance and Pancreatic Beta-Cell Function in *Helicobacter pylori* Seropositive Subjects in the National Health and Nutrition Examination Survey 1999-2000 Cohort
Author String	LR Malamug, R Karnchanasorn, RA Samoa, KC Chiu City of Hope National Medical Center, Duarte, CA; Harbor-UCLA Medical Center, Torrance, CA
Body	Infection has been thought to play a role in type 2 diabetes (T2D), in which insulin resistance (IR) plays a key role. *Helicobacter pylori* (H. pylori) has been suggested to be associated with the development of IR. However, most of the studies involved only Asian and European cohorts with relatively small sample sizes. Our aim was to determine the role of H. pylori infection in IR and T2D in an American cohort. We examined data from 4136 non-Hispanic white (NHW), non-Hispanic black (NHB), and Mexican Americans (MA) aged 18 and over from the NHANES 1999-2000 cohort. H. pylori status was defined by the titer of H. pylori antibody. We calculated the odds ratios for states of glucose tolerance (normal glucose tolerance vs. abnormal glucose tolerance, including diabetes) based on the H. pylori status by gender and ethnicity. Based on the homeostatic model assessment (HOMA), we calculated IR (HOMA-IR) and beta cell function (HOMA-B) in non-diabetic subjects. We also compared HOMA-IR and HOMA-B in non-diabetic subjects based on the H. pylori status. The results were adjusted for age and BMI, and also for additional covariates, including poverty index, education, alcohol consumption, tobacco use, and physical activity. The H. pylori status was a risk factor for abnormal glucose tolerance in NHW males (P=0.001), NHW females (P=0.0003), NHB males (P=0.03) and MA females (P=0.0004), but not in NHB females (P=0.12) and MA males (P=0.05). As the mean age of H. pylori positive subjects was older, no association was found after adjustment for age and BMI, and also after adjustment for all covariates. No difference in HOMA-IR was found in all ethnic and gender groups based on the H. pylori status. HOMA-B differed only in MA females based on H. pylori status (P=0.002). However, after adjustment for age and BMI and also adjustment for all covariates, no difference was found in either HOMA-IR or HOMA-B in all ethnic and gender groups except for HOMA-IR in NHB females (P=0.03 and P=0.04, respectively). H. pylori infection was not a risk factor for abnormal glucose tolerance. Although a marginally significant difference in HOMA-IR was noted in NHB females, H. pylori did not play a significant role in insulin sensitivity and beta cell function. We concluded that H. pylori infection does not play a major role in the pathogenesis of T2D. To our knowledge, this is the very first large scale study of the role of H. pylori in T2D in the United States. Nothing to Disclose: LRM, RK, RAS, KCC

Pub #	P1-551
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Endothelin-1 and Endothelin-A Receptor Immunoreactivity Is Increased in Patients with Diabetic Nephropathy
Author String	CM Zanatta, FV Veronese, MS Loreto, DA Sortica, VN Carpio, MI Edelweiss, VD Silva, TG Lopes, LP Klassman, FC Schacher, F Bondar, JL Gross, LH Canani Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil; Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil; Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil; Hospital São Lucas, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil; Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil
Body	**Background.** Endothelin-1 (ET-1) is associated with progression of renal disease, acting as vasoconstrictor and growth factor for mesangial cells. ET-1 might have a role in the development of diabetic nephropathy (DN). In normal human kidney, ET-1 and endothelin receptor A (ETRA) are more markedly expressed in vessels, and less markedly in glomerular structures. The aim of this study was to determine the level of ET-1 and ETRA expression in patients with DN as compared to IgA nephropathy (positive comparison group) and to normal kidney tissue. **Methods**. Cross-sectional study comprising 13 patients with type 2 diabetes mellitus and DN, 10 patients with proteinuric IgA nephropathy and 13 samples of normal kidney from tumor nephrectomies. Demographic and selected data were collected from medical charts. The distribution and intensity of ET-1 and ETRA expression in renal biopsies were determined by immunohistochemistry. **Results.** Patients with DN and IgA nephropathy on biopsy had markedly increased staining for ET-1 in endothelial cells of glomerular capillaries and peritubular capillaries as compared to controls ($p < 0.001$). ETRA staining was also more intense and more diffuse in DN and IgA nephropathy than in controls ($p=0.019$). A positive correlation was observed between proteinuria and ET-1 expression ($r=0.634$, $p=0.027$). **Conclusion.** The higher expression of ET-1 and ETRA found in both DN and IgA nephropathy suggests a potential role for the endothelin system in DN as well as in other non-diabetic glomerular diseases. Nothing to Disclose: CMZ, FVV, MSL, DAS, VNC, MIE, VDS, TGL, LPK, FCS, FB, JLG, LHC

Pub #	P1-552
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Delayed P300 Latency and Voltage in 85 Patients with and without Hypometabolic PET Scans
Author String	ER Braverman, K Perrine, UJ Damle, PK Reddy, PK Reddy, K Blum Weill Cornell College of Medicine, New York, NY; PATH Foundation NY, New York, NY; University of Florida College of Medicine & McKnight Brain Institute, Gainesville, FL
Body	**Introduction:** PET scan identified hypometabolism is a well-documented, though expensive, indicator of mild cognitive impairment (MCI) and likely progression to dementia. Dementia is the fourth leading cause of death in the United States and predictors of PET hypometabolism could be cost-effective in early diagnosis. Electrophysiological decline is a known antecedent to memory loss, so a comparison of P300 latency and voltage to PET scan results could elucidate the processes of cognitive decline. **Objective:** Compare electrophysiological status from P300 latency and voltage with brain PET hypometabolism findings. **Design:** Patients presenting with memory and attention complaints received a comprehensive medical evaluation, including P300 latency and voltage measurements, neuropsychological testing, and FDG PET scans. 44 patients were identified as having hypometabolism on brain PET scans. The patients with hypometabolism were compared to a larger sample (n=41) without hypometabolism on measures of P300 latency and voltage. **Results:** Patients with hypometabolism did not differ significantly in age (mean age=58.7) compared to those with normal metabolism (mean age=55.8). An analysis of covariance comparing the two groups on P300 measures covarying for age showed significantly slower P300 latencies (346ms, p=.003) and significantly lower P300 voltage (3.25mV, p=.0005) for patients with hypometabolism vs. patients with normal metabolisn (323ms, 5.0mV). **Conclusion:** Brain PET hypometabolism patients exhibit slower brain speeds more consistent with individuals 20 to 30 years older (since P300 latency decline occurs at 7 to 10ms/decade and the difference between the two groups was 23ms). Dementia and microcellular brain disease may occur very early. Many individuals with an average 40 to 50 year old's processing speed may have more advanced MCI than expected Brain deterioration may progress in the following order: microcellular, electrophysiological (P300 and qEEG) metabolic (PET), and finally anatomical (MRI), with atrophy and hydrocephalus. Delays in P300 latency and lower voltage may predict hypometabolism in PET scans and be cost-effective for individuals who cannot afford PET. We urge additional studies in larger populations to confirm these important results. Neuropsychiatric evaluation of the dementing process may need to begin at age 40 or earlier to have an impac on dementia. Disclosures: KB: Chairman of the Board and Chief Scientific Officer, LifeGen Inc, Reward Deficiency Solutions Inc. Nothing to Disclose: ERB, KP, UJD, PKR, PKR

Pub #	P1-553
Session Information	POSTER SESSION: CLINICAL - Miscellaneous Topics in Diabetes (1:30 PM-3:30 PM)
Title	Hyperbaric Oxygen Therapy Increases Insulin Sensitivity in Individuals with and without Type 2 Diabetes
Author String	LK Heilbronn, IM Chapman, DC Wilkinson University of Adelaide, Adelaide, Australia; Royal Adelaide Hospital, Adelaide, Australia; Royal Adelaide Hospital, Adelaide, Australia

Body

Background: Clinicians have reported the need to reduce doses of hypoglycemic agents to prevent hypoglycemia following hyperbaric oxygen (HBO) therapy in individuals with type 2 diabetes mellitus (DM2). This anecdotal evidence is supported by two recent studies showing that HBO reduces fasting glucose by more than 20%.

Objective: To determine whether peripheral insulin sensitivity is increased in patients undergoing hyperbaric oxygen therapy.

Design: Prospective intervention study.

Setting: Hyperbaric Oxygen Unit, Royal Adelaide Hospital, Australia

Patients: Non obese, patients without diabetes (n=5) and obese patients with type 2 diabetes (n=5), presenting for wound healing therapy by daily HBO, were recruited. Patients with current or recent systemic infections, or on oral corticosteroids, were excluded. Medications were not changed during the study.

Measurements: Fasting blood glucose and insulin sensitivity (hyperinsulinemic euglycemic clamp) was measured on 3 occasions. The last 2-hours of each clamp was performed either in ambient air (at baseline), or during the third and twentieth treatment with HBO (100% oxygen and 2 atmospheres of pressure).

Results: Peripheral insulin sensitivity was significantly increased by hyperbaric oxygen therapy in the whole cohort ($p=0.04$). Further analysis revealed that this increase was significant at both the 3rd treatment ($+37.3 \pm 12.7\%$, $p = 0.02$) and 20th treatment ($+40.6 \pm 12.6\%$, $p = 0.009$). There was no group effect, although splitting the dataset into those with and without type 2 diabetes showed that significance was reached only in DM2 ($p=0.008$), with 4 out of 5 non-diabetics improving insulin sensitivity following HBO ($p=0.1$). The reduction in fasting glucose following HBO was not statistically significant.

Limitations: Interpretation is limited by the small group size and the use of patients being treated for wound healing, although the early increase in insulin sensitivity preceded significant wound healing.

Conclusion: Insulin sensitivity increases within a few days of starting hyperbaric oxygen treatment, and this increase is maintained for at least 20 sessions. This increase in insulin sensitivity is equivalent to that observed following moderate weight loss. The mechanisms underlying the insulin sensitising effect of HBO remain unclear.

Nothing to Disclose: LKH, IMC, DCW

Pub #	P1-554
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	Cerebral Infarction with Transient Visual Loss in Juvenile Diabetic Ketoacidosis
Author String	SY Kim, BT Lim, HS Lee, HS Lee, JS Hwang Bundang Jesaeng General Hospital, DMC, Seongnam, Korea; Ajou University School of Medicine, Ajou University Hospital, Suwon, Korea
Body	Diabetic ketoacidosis (DKA) is a metabolic disorder caused by insulin deficiency and is the most important cause of mortality and morbidity in children with type 1 diabetes mellitus. Acute neurological complications related to DKA include cerebral edema, cerebral infarction, brain herniation, cortical venous thrombosis and rarely, cerebral hemorrhage. Cerebral infarction is rare in juvenile DKA. We report a girl with a newly diagnosed type 1 diabetes mellitus who presented with juvenile DKA and developed cerebral infarction with transient visual loss. A 3-year-old previously healthy girl with polydipsia and polyuria lasting for a week was transferred to the emergency room from a local hospital. On initial examination, she was drowsy, had dried lips and tongue witl decreased skin tugor and weight loss of about 1kg. Her estimated fluid deficit was at 7%. She also had tachypnea with Kussmaul's respiration and tachycardia. A full neurologic exam to look for focal neurological deficit could not be done due to her stuporous mental status. Her blood pressure and body temperature was normal. Her laboratory data showed hemoconcentration (WBC of 30100 cells/[mu]L, Hemoglobin of 14.8 g/dL, Hematocrit of 43.3%, Platelet of 476000/ [mu]L), hyperglycemia (serum glucose of 630 mg/dL), metabolic acidosis (pH of 7.088, serum bicarbonate of 1.7 mmol/L, base excess of -25.7 mmol/L, arterial carbon dioxide of 5.7mmHg), glycosuria (urine glucose 3+), ketonuria (urine ketone 4+). She was diagnosed with DKA and was admitted into the intensive care unit. She was treated with appropriate fluid hydration, continuous insulin drip, and electrolyte replacement. About 13 hours after admission, the DKA was controllec and her mental status was improved. Her blood sugar was controlled by subcutaneous insulin injection but she could not recognize her parents. An ophthalmologic examination revealed that her pupils were reacting and her fundi were normal. An Emergency cranial CT scan revealed cerebral edema and subcortical infarction of both occipital lobes. She was promptly treated with mannitol to lower the intracranial pressure and supportive care was continued. Coagulation studies revealed that her protein C activity (61%) was deceased. Her vision gradually recovered and she completely regained her vision after 1 week. Unfortunately a follow-up image could not be performec due to her financial problem. (1) Carl GF et al., Endocr Res(in eng)2003;29:73-82 (2) Glaser N et al., N Engl J Med(in eng)2001;344:264-9 Nothing to Disclose: SYK, BTL, HSL, HL, JSH

Pub #	P1-555
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	Diabetic Ketoacidosis with Cerebral Hemorrhage and Alpha Coma in an Adolescent Female
Author String	ZF Shoar, C Dunne, WR Yorns, Jr, G Rezvani St Christopher's Hospital for Children, Drexel University College of Medicine, Philadelphia, PA; St Christopher's Hospital for Children, Drexel University College of Medicine, Philadelphia, PA
Body	Background: Diabetic ketoacidosis (DKA) is one of the most common complications of type 1 diabetes in children. Neurological complications including seizures and coma can be secondary to cerebral edema, hemorrhagic, or ischemic events. Alpha coma (AC), an alpha-frequency EEG rhythm, is a rare finding in comatose patients and is generally associated with a poor prognosis. We report an adolescent with severe DKA, intraparenchymal cerebral hemorrhage, and AC, who had rapid resolution of neurological symptoms. Clinical Case: A 17-year old female with a 4-year history of poorly controlled type 1 diabetes was seen in the ED for change in mental status. At presentation she was unresponsive and severely dehydrated with Kussmau respirations. She experienced a 3-second seizure upon arrival and another 2-minute seizure which was treated with lorazepam and fosphenytoin, and she required intubation. IV fluid and mannitol were administered and an insulin drip started. She remained unresponsive to pain with absent oculocephalic reflexes but reactive pupils. Initial laboratory findings included pH 6.69, sodium 131 mmol/L, potassium 10.9 mmol/L, chloride 88 mmol/L, carbon dioxide 3 mmol/L, glucose 1622 mg/dL, calcium 8.6 mg/dL, phosphate 18.5 mg/dL. CT of the brain showed acute intraparenchymal hemorrhage in the right parietal cortex and left pineal region with extension into the adjacent quadrigeminal plate cistern, but no cerebral edema. EEG showed diffuse alpha frequency of 8-12 Hz with an amplitude of 20-30 [micro]V with frontal predominance. Within 24 hours the DKA and electrolyte abnormalities resolved, she regained consciousness and was extubated. Twenty-four hours after the initial event MRI and MRA of the brain demonstrated multiple 1-2 cm hemorrhages in various areas of the cortex in addition to those previously identified on CT. MRV showed normal vasculature. Repeat EEG at 48 hours showed a normal rhythm and her neurologic exam returned to baseline. Conclusion: To our knowledge, this is the first patient reported with intraparenchymal hemorrhage and AC caused by DKA. Patients with DKA are predisposed to vascular thrombosis, but intraparenchymal hemorrhage secondary to DKA is rare and the cause is unknown. Our findings indicate that, although AC typically portends a poor prognosis, the outcome may be better when it is caused by DKA. Nothing to Disclose: ZFS, CD, WRY, GR

Pub #	P1-556
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	A Case Report of New-Onset Type 1 Diabetes Mellitus with Diabetic Ketoacidosis, Deep Vein Thrombosis and Pulmonary Embolism -- A Rare Presentation
Author String	SR Chandratre, G Babar Children's Mercy Hospital, University of Missouri at Kansas City, Kansas City, MO
Body	Introduction: Patients with Diabetes Mellitus have a hypercoagulable state that may increase their risk for deep vein thrombosis(DVT). Although a prothrombotic state is described in type 1 diabetes mellitus(T1DM) in general and diabetic ketoacidosis(DKA) in particular, there are relatively few reports of clinically obvious DVT and no reports of pulmonary embolism complicating T1DM and DKA. Clinical Case: We report a case of a 14 year old caucasian female who presented with a 3 day history of right leg swelling, pain and difficulty in ambulation. She had no respiratory compromise and was maintaining saturations of 97-98% on room air. There was no appreciable weight loss, polyuria, polydipsia and polyphagia. She was started on oral contraceptives (OCP) drospirenone 3 mg/ethinyl estradiol 20 mcg(YAZ [reg]) about 2 months ago. There was no family history of thrombotic disease, diabetes or any other endocrine disorder. Physical exam showed moderate dehydration and physical signs of DVT. Doppler ultrasound of lower extremity showed occlusive DVT within the right popliteal vein extending to the external iliac vein. Angiographic CT scan of chest indicated left pulmonary embolus. Laboratory investigations showed elevated PT, INR, PTT, D dimers, fibrinogen, low antithrombin 3, negative serum HCG, elevated white blood count and hematocrit. Basic metabolic profile showed a sodium of 130 mEq/L(normal:135-145 mEq/L), serum bicarbonate of 10 mEq/L(normal:20-30 mEq/L) blood glucose of 280 mg/dl(normal:60-110 mg/dl) with elevated anion gap of 17 mEq/L(normal:7-14 mEq/L). Urinalysis indicated 3+ ketones, 3+ glucose(normal:no glucose,no ketones) with specific gravity of 1.04(normal:1.005-1.035). She had elevated insulin antibodies (Ab), elevated islet cell autoAb:ICA-512/IA-2 Auto Ab, normal glutamic acid decarboxylase auto-Ab and Hemoglobin A1c of 11.9% (normal:4-6%). She had normal insulin C-peptide, thyroid hormones and negative celiac screen. Factor V Leiden, prothrombin gene(factor II) variant and 20210G>A mutation was negative. She was treated with intravenous fluids, insulin and heparin. Conclusion: The profound hypovolemic state due to DKA and hypercoagulability risk secondary to the usage of OCP as well as type 1 diabetes mellitus with DKA caused deep vein thrombosis and pulmonary embolus. To the best of our knowledge, this is the first reported case of new-onset type 1 diabetes mellitus presenting with this combination of DKA, deep vein thrombosis and pulmonary embolism. Nothing to Disclose: SRC, GB

Pub #	P1-557
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	Reversal of Severe Post-Gastric-Bypass Hyperinsulinemic Hypoglycemia with Dietary and Pharmacologic Interventions
Author String	ED Geamanu, KE Foster-Schubert, DE Cummings University of Washington, Seattle, WA; University of Washington, VA Puget Sound Health Care System, Seattle, WA; University of Washington, Diabetes & Obesity Center of Excellence, VA Puget Sound Health Care System, Seattle, WA
Body	**Background.** Severe hyperinsulinemic hypoglycemia developing long after Roux-en-Y gastric bypass (RYGB) is a rare complication that sometimes necessitates pancreatectomy. Here we present a case of this condition treated successfully with dietary intervention or acarbose. **Clinical Case**. An obese 56-year-old man had a biliopancreatic diversion to promote weight loss. He lost 275 pounds but developed malabsorption requiring pancreatic enzymes. Consequently, the operation was converted to a RYGB, 2 years after the first surgery. One year later, following an 80-pound weight regain, he developed frequent episodes of postprandial hypoglycemia, with 6 instances of lost consciousness and 3 hospital admissions for hypoglycemia over the next 2 years. To examine this problem, he received, on successive mornings, three isocaloric mixed macronutrient test meals: high-carbohydrate, then high-protein-fat, then high-carbohydrate plus acarbose 100 mg. Plasma glucose and insulin levels were measured at 0, 15, 30, 60, 100, and 150 min after each meal. Following the high-carbohydrate meal, glucose peaked at 265 mg/dL (15 min postprandial) and insulin at 380 mU/l (30 min postprandial). 100 min after the meal, he became diaphoretic, with glucose of 41 mg/dL and insulin of 13mU/l. By 150 min post-meal, glucose and insulin had normalized. In contrast, after the isocaloric low-carbohydrate, high-protein, high-fat meal, postprandial glucose and insulin levels remained comparatively stable at 86-120 mg/dL and 14-36 mU/l, respectively. On Day 3, the patient consumed an equivalent high-carbohydrate meal as on Day 1, immediately preceded by acarbose 100 mg. Glucose peaked at 15 min (229 mg/dL) and insulin at 30 min postprandial (477 mU/l). Unlike on Day 1, however, no postprandial hypoglycemia occurred (glucose levels were 83, 84, and 80 mg/dl at 60, 100, and 150 min, respectively). The patient was discharged on acarbose 100 mg before meals. During the ensuing year, he has experienced no hypoglycemic events or blood glucose values <65 mg/dL. **Conclusion.** Life-threatening hyperinsulinemic hypoglycemia developing long after RYGB is a rare complication with poorly understood mechanisms. We avoided postprandial hypoglycemia either by using a low-carbohydrate meal or by administering acarbose before a high-carbohydrate meal. The clinical outcomes with these simple manipulations were dramatic, suggesting that easy dietary and/or pharmacological interventions can be valuable in this setting. Nothing to Disclose: EDG, KEF-S, DEC

Pub #	P1-558
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	The Discovery of Persistent Neonatal Diabetes in a Mother after the Birth of a Diabetic Neonate
Author String	N Malhotra, D Khurana, R Bargman Nassau University Medical Center, East Meadow, NY
Body	Background: We report the case of a 25 yo woman with a diagnosis of type 1 diabetes who delivered a baby with neonatal diabetes (ND) and was subsequently weaned off insulin to sulfonylurea. Case: 25 yo female with a diagnosis of type 1 diabetes gave birth to a full term, SGA baby girl. The mother was managed with insulin since diagnosis of diabetes at age one. She had inadequate glycemic control with frequent hypoglycemia. She tested negative for GAD 65, islet cell and anti-insulin antibodies. On day 2, the infant was found to be hyperglycemic and over the next few days the sugars continued to climb reaching a maximum of 350 mg/dl. Insulin was<2 IU/ml, C-peptide 0.7 ng/ml. The family history and the early onset of diabetes in the infant were suggestive of ND, so a trial of glyburide of 0.2 mg/kg was attempted. She responded well with BG dropping to 60mg/dl within a few hours. The dose was titrated to 0.02mg/kg/dose to achieve BG in the low 100s. Genetic testing for the KCNJ11 mutation was sent and is pending. The diagnosis of ND responsive to sulfonylurea in the baby suggested that the mother may have the same condition since commonly ND is inherited in an autosomal dominant fashion. We wished to get genetic testing done for the mother but she planned to return to a third world country within the month and was unable to pay for the test. We admitted the mother to the hospital for a trial of glyburide in a controlled environment. She responded remarkably and was weaned off insulin within 2 days. She was discharged home on glyburide and is doing well. Discussion: Ramsay reported the first case of ND in 1926(1). Almost half of all patients with ND have a mutation in the KCNJ11 gene encoding the Kir6.2 subunit of K-sensitive ATPase channels. Gloyn et al have demonstrated insulin secretion in response to IV Tolbutamide, suggesting that some patients with KCNJ11 mutations may respond to oral sulfonylurea therapy(2). The glycemic response in our patient was superior on glyburide than insulin with near normalization of postprandial values without hypolgycemia. Conclusion: We would like to stress the importance of questioning the diagnosis of Type 1 diabetes and getting genetic testing in appropriate candidates. Also, patients unable to afford testing may be given a trial of sulfonylureas in an observed setting. This approach will improve glycemic control and quality of life of this diabetic population. (1) Ramsay, W. R. (1926); Trans. Amer. Paed. (2) Gloyn AL;N Engl J Med 2004; 350:1838-49. Nothing to Disclose: NM, DK, RB

Pub #	P1-559
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	Gross Insulin Resistance Secondary to Use of Large Doses of Diuretic
Author String	C Wlodek, A Lulsegged Princess Royal University Hospital, Greater London, UK; Princess Royal University Hospital, Greater London, UK
Body	A 43 year old type 2 diabetic male was admitted for glucose control despite very high doses of insulin. He wa also on Lasix (furosemide), the dose of which had been increasing since hospitalisation in 2005 for alcoholic hepatitis and oedema. By August 2010 he was taking Lasix 750mg for idiopathic oedema and a total of 450 units of insulin, yet HbA1c was 15%. His BMI was 42, he had mild pitting oedema to the mid shins but no evidence of pulmonary oedema. Heart failure, hypoalbuminaemia and nephrotic syndrome were excluded. Over 4 weeks, the dose of Lasix was gradually discontinued. He was fluid restricted and advised a low carbohydrate diet. Insulin doses were adjusted according to BMs and his requirements diminished dramatically to a total of 90 units daily - an 80% reduction and he lost 8kg. Background Diuretics impair glucose tolerance, however the effects behind this phenomenon are unclear. One mechanism may be secondary to a reduction in serum potassium since there is a significant correlation between the degree of diuretic - induced hypokalaemia and increased plasma glucose. Furthermore potassium supplementation or potassium sparing agents attenuate a rise in plasma glucose (1). A recent study demonstrated increased hepatic fat content after thiazide treatment, which correlated with the magnitude of insulin insensitivity (2). This is associated with insulin resistance at the level of the liver and skeletal muscle. Zhou et al. (2008) proposed that increased inflammation or oxidative stress with diuretics alters adipocyte activity since inflammatory markers were higher in patients taking diuretics (3). Although Lasix classically has less impact on glucose homeostasis several reports confirm that it deteriorates glycaemic control, possibly related to peripheral tissue insulin resistance (4). Conclusion Although one has to be careful in implicating diuretics in glucose intolerance and insulin resistance, due to the well established association between these metabolic abnormalities and hypertension (5), in the case of our patient we demonstrated a link between his Lasix use, for oedema, and insulin resistance. This case not only confirms the link between Lasix and poor glycaemic control in a diabetic patient but also highlights the importance of judicious prescribing. His weight loss probably contributed to improved insulin resistance. We demonstrated, under close clinical supervision, that he did not require Lasix and thus improved his diabetic control. (1) Zillich AJ, Garg J, Basu S, Bakris GL, Carter BL (2006). Thiazide diuretics, potassium, and the development of diabetes: a quantitative review. Hypertension 48: 219-224 (2) Carter BL, Einhorn PT, Brands M, He J, Cutler JA, Whelton PK, Bakris GL, Brancati FL, Cushman WC, Oparil S, Wright JT (2008). Thiazide-induced dysglycemia: call for research from a working group from the national heart, lung and blood institute. Hypertension 52: 30-36 (3) Zhou MS, Schulman IH, Jaimes EA, Raij L (2008). Thiazide diuretics, endothelial function, and vascular oxidative stress. J Hypertens 26: 494-500 (4) Dimitriadis G, Leighton B, Parry-Billings, Tountas C, Raptis S and Newsholme EA (1998). Furosemide decreases the sensitivity of glucose transport to insulin in skeletal muscle in vitro. European Journal of Endocrinology 139: 118-122 (5) Ramsay LE, Yeo WW, Jackson PR (1992). Diabetes, impaired glucose tolerance and insulin resistance with diuretics. European Heart Journal 13 (Suppl G): 68-71 Nothing to Disclose: CW, AL

Pub #	P1-560
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	Use of Hemodialysis in a Patient with Hyperosmolar Hyperglycemic State and Rhabdomyolysis -- Case Report and Literature Review
Author String	B Alfonso, B Medvedovsky, D Rapoport, T Araki, A Dubrow, L Poretsky Beth Israel Medical Center, New York, NY; Beth Israel Medical Center, New York, NY; Beth Israel Medical Center, New York, NY

Body

Case Presentation

A 63-year-old man with past medical history of hypertension and hyperlipidemia presented with one week of generalized weakness, immobilization, polyuria, polydipsia, and vomiting. His medications included simvastatin, almodipine and hydrochlorothiazide.

On presentation he was afebrile, BP 140/60 mm of Hg, pulse 114 BPM. Physical exam revealed lethargy and dry mucous membranes. Laboratory values were notable for glucose 1527 mg/dL, sodium 152 meq/L, bicarbonate 17 meq/L, urea nitrogen 61 mg/dL, creatinine 1.8 mg/dL, anion gap 34, phosphorus 6.2 mg/dL, lipase 3120 u/L, amylase 329 u/L, creatine kinase 9560 u/L. He was diagnosed with hyperosmolar hyperglycemic state with ketoacidosis and treated with hydration and intravenous insulin. Despite glycemic improvement, the kidney function and the electrolyte imbalance worsened. Sodium peaked at 191 meq/L; creatinine reached 5.8 mg/dl and phosphorus decreased to 1.6 meq/L. Hypernatremia improved with free water repletion. Hemodialysis was initiated on hospital day 4 and continued for 3 weeks. Patient was discharged on insulin therapy, without dialysis. Currently his HbA1c is 6% and renal function is normal.

Discussion

HHS is a life threatening condition involving multiple organs. Rhabdomyolysis is classically described in crush injuries and medication use. It has rarely been reported in HHS, leading to increased mortality in the setting of acute kidney injury (AKI). Electrolyte shifts have been proposed as a mechanism for rhabdomyolysis in HHS (1). In addition to HHS, our patient had two other risk factors for rhabdomyolysis: statin use and low serum phosphate. Hypophosphatemia is thought to cause rhabdomyolysis by depleting ATP, leading to the inability of muscle cells to maintain the membrane integrity (2). Elevated serum concentrations of sodium, chloride, glucose, blood urea nitrogen and uric acid were associated with the occurrence of rhabdomyolysis in the hypophosphatemic state.

In this case the early recognition of rhabdomyolysis, followed by prompt fluid resuscitation and hemodialysis led to a full recovery.

Conclusion

HHS with ketoacidosis is a life-threatening condition. A rarely described complication is rhabdomyolysis leading to AKI. Prompt recognition and treatment is crucial in preserving the kidney function and improving mortality and morbidity associated with hyperosmolar state.

(1) Rosa EC et al., Ren Fail 1997;19(2):295-301.
(2) Knochel JP et al., J Clin Invest 1978;62(6):1240-1246.

Nothing to Disclose: BA, BM, DR, TA, AD, LP

Pub #	P1-561
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	Insulin Pump Therapy in Neonatal Diabetes Mellitus
Author String	SE Hyun, HW Chae, AR Kwon, WJ Lee, JH Kim, H-S Kim, E-G Yoo, DH Kim College of Medicine, Yonsei University, Seoul, Korea; College of Medicine, CHA University, Sungnam, Korea; So-hwa Children's Hospital, Seoul, Korea
Body	Background: Neonatal diabetes mellitus (NDM) is a rare disease requiring insulin treatment. Its treatment is primarily focused on maintaining adequate glycemic control and avoiding hypoglycemia. Obtaining satisfactory weight gain and catch-up growth is also important because many of these newborns exhibit intrauterine growth retardation (IUGR). Continuous intravenous insulin infusion is the preferred initial treatment, but long-term installation of an intravenous line is often intolerable in newborns with IUGR. Intermittent subcutaneous insulin injection is often tried, but hypoglycemia is frequent and sometimes inevitable. Although the insulin pump therapy is widely used in adult and childhood practice, there is no consensus on the use of insulin pumps in NDM. We report two cases of neonatal diabetes mellitus treated with insulin pump therapy; one is transient, and the other is assumed to be permanent. Clinical case: Case 1: A 10-day female infant was referred to us with IUGR and poor weight gain. Hyperglycemia was noted on admission, and continuous intravenous insulin infusion was started. However, the patient's serum glucose levels fluctuated widely, and maintaining the intravenous route became difficult within the following weeks. Continuous intravenous insulin infusion (CSII) with an insulin pump was introduced on the 25th day of life, and good glycemic control was achieved without hypoglycemia or any other adverse effects. Molecular analysis revealed a paternal disomy of chromosome 6, suggesting transient NDM, and insulin was discontinued at 5 months of age. Case 2: This female infant was born at 38weeks of gestation weighing 2040g to a mother with gestational diabetes. At 2 months of age, she was taken to emergency department for acute dyspnea, due to diabetic ketoacidosis. Hyperglycemia was observed and HbA1c was checked 12.6%. In spite of continuous intravenous insulin infusion, blood glucose levels had fluctuated until CSII was applied to this infant. She attained good glycemic control and satisfactory weight gain from then on. CSII is being continued in outpatient clinic over a year, and gene study related with the PNDM is in progress. Conclusion: We suggest that CSII is a safe and effective mode of treatment for NDM. Early adoption of insulin pump therapy may also shorten the length of hospital stays in patients with NDM. Nothing to Disclose: SEH, HWC, ARK, WJL, JHK, H-SK, E-GY, DHK

Pub #	P1-562
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	A Case of Insulin Autoimmune Syndrome Discovered with Disturbance of Consciousness
Author String	M Mouri, K Kubo Hiroshima Prefectural Hospital, Hiroshima, Japan
Body	A 71-year-old Japanese man was found to lose consciousness. His family called his home doctor. The blood glucose was low(<30 mg/dl)[sbquo] intravenous glucose administration restored his consciousness. He was brought to our emergency hospital to determine the cause of hypoglycemia. He had no personal history of intake of hypoglycemic agents. He had never received medications which are known to elicit antibodies to insulin[sbquo] such as insulin[sbquo] alpha-mercaptopropionyl glycine[sbquo] methimazole or alpha lipoic acid(ALA) et al. On admission[sbquo] insulinoma was suspected[sbquo] but contrast material-enhanced CT scan showed no evidence of tumors. The level of serum immunoreactive insulin(IRI) was 1241 [micro]IU/ml (normal range 0-18.7 [micro]IU/ml)[sbquo] and high titers of insulin autoantibodies(>90 %)(normal range<0.3 %) were found. Basal C-peptide level was 1.02 ng/ml. Scatchard plot analysis showed a large capacity-low affinity tendency for high affinity sites of insulin autoantibodies. The diagnose of insulin autoimmune syndrome(IAS) was made. His HLA genotype contains DRB1[lowast]0406. He was advised to fractionate his meals[sbquo] and since then he has not experienced hypoglycemic attacks[sbquo] but titers of insulin antibodies are still high. IAS has been mainly reported in Japan[sbquo] and is a quite rare cause of hypoglycemia in other countries. But most IAS patients have spontaneous remission without any positive treatment[sbquo] IAS need to be taken into account in hyperinsulinemic hypoglycemia. Nothing to Disclose: MM, KK

Pub #	P1-563
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	Effects of Fiber Supplementation on Glycemic Excursions and Incidence of Hypoglycemia in Children with Type 1 Diabetes
Author String	NS Nader, AL Weaver, SK Eckert, A Lteif Mayo Clinic, Rochester, MN; Mayo Clinic, Rochester, MN; Mayo Clinic, Rochester, MN
Body	Objective: Nutritional therapy is an important component of diabetes management. Fibers are known to slow down the absorption of carbohydrates after a meal. The aim of this study was to determine whether the addition of a fiber supplement to the diet of pediatric patients with type 1 diabetes has an effect on the magnitude of glucose excursions and/or the incidence of hypoglycemia. Methods: The study was a prospective, interventional, observational study with a within-subject cross over design. 10 children, diagnosed with type 1 diabetes for at least two years, were recruited. The study was divided into two phases. In the first phase, children were asked to follow their usual meal plan. In the second phase of the study, subjects continued to follow the same meal plan except fiber was added to the diet using a powder supplement (wheat dextrin). Each subject received a total of 20 grams of fiber/1000 Kcal/day. In each phase, subjects wore a continuous glucose monitoring device (CGMD) for three days and kept food records. Data was collected on glucose concentrations from the CGMD. The blood glucose excursion level following each meal was compared between the two phases of the study by fitting a repeated measures regression model. The incidence of hypoglycemia was also compared between the two phases by fitting a logistic regression model with GEE methodology. Results: The mean blood glucose excursion after meals was not significantly different between the two phases (overall mean, 70 vs. 80.6 mg/dL, phase 1 vs. 2; p=0.17). There was also no statistical difference in the incidence of hypoglycemia during the two phases of the study (9.4% vs. 12.0%; p=0.55). There was a strong negative correlation between the amount of fiber supplemented and the mean maximum post-prandial blood sugar after the lunch and breakfast meals (Spearman rank correlation coefficient = -0.86 for lunch and -0.76 for breakfast). Conclusion: Although our study did not show an overall decrease in glucose excursion or incidence of hypoglycemia with fiber supplementation, we did find a strong negative correlation between the amount of fiber added during the supplemental phase and the mean maximum post-prandial blood sugar after the lunch and breakfast meals. We speculate that different types of fiber may have different effects on blood glucose with wheat dextrin having a greater dampening effect, so those who added the most wheat dextrin to their diet saw the greatest effects. Nothing to Disclose: NSN, ALW, SKE, AL

Pub #	P1-564
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	An Extreme Paradoxical Effect on Weight and Glycemic Control When Using Exenatide To Treat a Patient with Steroid-Associated Diabetes
Author String	L Spurr, A Solomon Princess Alexandra Hospital, Harlow, UK
Body	**Introduction** Diabetes mellitus occurring in association with supraphysiological endogenous or exogenous glucocorticoids represents a therapeutic challenge. Newer agents, especially subcutaneously delivered GLP-1 analogues that may improve glycaemia and bring about weight loss would appear therapeutically attractive (1). **Clinical Case** A 39 year old woman developed diabetes whilst taking prednisone (initially 30mg, then 20mg long term) for sarcoidosis. Before starting steroids her weight was 103kg (BMI 39), fasting glucose 4.4 mmol/l and HbA1c 5.3% (34 mmol/mol). A progressive increase in fasting glycaemia and HbA1c occurred over 3 years, necessitating oral treatment using metformin and gliclazide. During that time, when acutely unwell, the patient developed lactic acidosis, thus metformin was therefore discontinued. 6 years after the diabetes was diagnosed, insulin was initiated and titrated upwards culminating with 40 units of NPH twice daily plus 30 units x3 daily short-acting insulin, achieving an HbA1c of 8.2% (66 mmol/mol) at that time, whilst still on 20mg of prednisone. This regimen was associated with weight gain up to 125kg. At that point, obstructive sleep apnoea was developing, weight loss was clearly required, and therefore the dose of insulin was reduced in combination with significant efforts to optimise diet and take gentle exercise. With these changes, she experienced some weight loss (116 kg), but also worsening of glycaemic control (HbA1c 9.1%).The use of a GLP-1 agonist, exenatide was then considered. Despite careful counselling to continue insulin at an appropriate dose with the exenatide, she substituted the insulin for the exenatide and thus had 3 months' of exenatide monotherapy. The exenatide (5 mcg bd for one month then 10 mcg bd) resulted in both a marked weight loss of 14% (116 kg to 100 kg) but a simultaneous severe worsening in glycaemic control (HbA1c 9.1%, 76 mmol/mol to 11.6%, 103 mmol/mol). The patient is now on a combination of exenatide twice daily and Levemir (insulin detemir) and has improving glycaemia, stable weight and is feeling well. **Clinical Lessons** This case illustrates that can be a wide variation in responsiveness to GLP-1 analogues such as exenatide for treating diabetes (2). This may be particularly the case if taken alone. It also provides an instructive insight into further research required that could assist in refining optimal treatment regimens for patients with steroid-associated diabetes. (1) van Raalte DH et al., Diabetes Care 2011; Jan 7. [Epub ahead of print] (2) Davis SN et al., Diabetes Care 2007; 30: 2767 Nothing to Disclose: LS, AS

Pub #	P1-565
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	Concurrent Diagnosis of Type 2 Diabetes and Necrotizing Pancreatitis in an Obese Adolescent Male
Author String	C Dunne, F DeLuca, G Rezvani St Christopher's Hospital for Children, Philadelphia, PA
Body	**Background:** while transient hyperglycemia is frequently reported in children with acute pancreatitis, pancreatitis-related diabetes mellitus (DM) has a low incidence in the pediatric population. We describe a 12-year old patient who presented with pancreatitis and concurrent new-onset DM. **Clinical case:** A 12 year old obese male presented to the local ED with a 4-day history of stabbing, non-radiating abdominal pain and multiple episodes of non-bloody, non-bilious emesis. There was no history of trauma. Laboratory evaluation revealed leukocytosis with neutrophilia and blood glucose of 509 mg/dl. His family history was negative for DM, pancreatitis or lipid metabolism. An abdominal CT revealed findings consistent with cholelithiasis and necrotizing pancreatitis. After being transferred to the pediatric intensive care unit, hyperglycemia was confirmed (436 mg/dl), and amylase/lipase were noted to be elevated (349 U/L, normal <100 U/L; 252 U/L, normal, 0-60 U/L, respectively). All viral studies were negative, HbA1C was 6.0 % and his pH was 7.37. IA-2, Anti islet cell and GAD 65 antibodies were negative. During his initial hospital stay, the patient required insulin to remain euglycemic; however, he was discharged 2 weeks later without insulin and resolution of pancreatitis. 2 weeks later, the patient was re-admitted for abdominal pain, elevated amylase and lipase, and hyperglycemia. During this 2nd hospital stay (36 days), his persistent hyperglycemia again required the use of insulin (as much as 1.2 Units/kg/day). An MRI/MRCP demonstrated a developing pancreatic pseudocyst, which was surgically excised, and at discharge he still required use of basal/bolus insulin (0.4 Units/kg/day). 4 months after his initial presentation, he was admitted for abdominal pain and hyperglycemia. HbA1C was 10.7 %, and he admitted to have discontinued insulin treatment and blood glucose monitoring. Labs demonstrated mild hepatic inflammation but no s/s of pancreatic inflammation. Patient was restarted on SQ insulin, and abdominal pain subsided. **Conclusion:** DM is a rare complication of acute pancreatitits in children. Most reported cases of pancreatitis-related DM occur in individuals with multiple risk factors ie metabolic disturbances, medication use or family history(1). Our patient was a healthy teenager with negative relevant family history. Our findings indicate that a complete evaluation of glucose metabolism may be warranted in a patient with a single episode of pancreatitis. (1) Raman VS et al., The Journal of Pediatrics 2010 (in press); Hyperglycemia and Diabetes Mellitus in Children Nothing to Disclose: CD, FD, GR

Pub #	P1-566
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	Sitagliptin (Januvia) Induced Acute Hepatitis -- Two Case Reports
Author String	K Patel, PJ Kim, D Reich, I Sachmechi Queens Hospital Center/Mount Sinai School of Medicine, Jamaica, NY
Body	**Objective** Report of two cases of acute hepatitis induced by sitagliptin. **Case 1:** A 58 year old male with type 2 diabetes mellitus was admitted to the hospital for further evaluation o chest pain. Liver function tests (LFTs) revealed a 6 to 10 fold in increase in aminotransferases: alanine (ALT) and aspartate (AST) from baseline. The patient had been taking pravastatin and ezetimibe for a few years for control of lipids. Sitagliptin was added to patient's medical regimen for better control of his diabetes. After initiation of sitagliptin patient's ALT and AST increased gradually over 6 months of therapy. Hepatitis B and C serologies were negative. Abdominal sonogram was negative for gallstones. After discontinuation of sitagliptin, pravastatin, and ezetimibe, ALT and AST returned to baseline levels. Resumption of pravastatin and ezetimibe was not associated with elevation of ALT and AST levels. **Case 2:** A 44 year old female with type 2 diabetes and normal LFTs, experienced a more than 10 fold elevation of ALT and AST levels after 6 months of sitagliptin therapy. Further work-up revealed positive hepatitis B-surface antigen with a normal liver songram. The patient's ALT and AST levels returned to norma after discontinuation of sitagliptin, pioglitazone and rosuvastatin. Resumption of pioglitazone and rosuvastati was not associated with elevation of ALT and AST levels. Both patients did not have any history of alcohol abuse, acetaminophen use, or chronic liver disease. **Discussion:** Sitagliptin is primarily excreted by the kidneys and only 16% of it undergoes hepatic metabolism The reported magnitude of liver enzyme derangement with sitagliptin is generally mild and transient (two fold upper limit of normal). We present two unique patients who had marked increases in their aminotransferases (six to ten fold increase above upper normal limit) after starting sitagliptin therapy. These elevations of ALT and AST are enough to meet the criteria for drug induced hepatitis. This acute derangement of ALT and AST in our two patients was due to sitagliptin, since the ALT and AST normalized shortly after sitagliptan was discontinued and remained at baseline after resuming all other medications. **Conclusion:** These are the first reported cases describing sitagliptin-induced acute hepatitis. We recommend periodic LFT monitoring in patients prescribed sitagliptin. Nothing to Disclose: KP, PJK, DR, IS

Pub #	P1-567
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	A Case of Transient Neonatal Diabetes and Congenital Diaphragmatic Hernia
Author String	LA Minarich, DW Kays, RT Zori, CA Williams, MJ Haller University of Florida, Gainesville, FL; University of Florida, Gainesville, FL; University of Florida, Gainesville, FL
Body	Neonatal diabetes mellitus (NDM) is a rare disorder (incidence 1/500,000 live births) that can be transient or permanent. Herein we present an unusual case with the previously unreported combination of transient NDM (TNDM) and congenital diaphragmatic hernia (CDH, incidence 1/4,000 live births). Our patient was the 2401 gram product of a 38 week pregnancy born to a primigravida mother by elective cesarean section. She was prenatally diagnosed with left CDH. Within hours of birth she developed hyperglycemia (493 mg/dl) on total parenteral nutrition (GIR 8 mg/kg/min). On examination she had little subcutaneous tissue and a relatively large tongue. Serum insulin level was undetectable and c-peptide was 0.2 ng/ml (0.9-1.7). Comparative genomic hybridization study revealed a micro duplication in chromosome 6q24.2 which includes the paternally expressed PLAGL1 and HYMAI genes associated with TNDM. Her father, who does not have glucose abnormalities, carries the same duplication. She was treated with a continuous intravenous infusion of regular insulin. She underwent surgical repair of he CDH on day of life 5 and remained on an insulin drip until she transitioned to oral feeds 1 week post-operatively. To prepare for discharge, she was transitioned to a continuous subcutaneous insulin infusion (CSII) using a single basal rate of 0.3 u/hr U-10 lyspro insulin and pre-breast feeding correction boluses (no carbohydrate ratio). Upon discharge insulin requirements waned quickly and CSII therapy was stopped at 7 weeks of age. At 3 months of age she was thriving, with a weight of 4004 grams and an A1c of 4.7%. Transient NDM is caused by overexpression of imprinted and paternally expressed genes on a critical region on chromosome 6q24. Management of NDM is difficult because infants require very small doses of insulin to manage hyperglycemia while avoiding hypoglycemia. We found U-10 lyspro insulin delivered by CSII could be safely and effectively titrated to control blood glucose in this infant. While TNDM resolves spontaneously by one year of age, it carries a 50% risk of diabetes recurrence in adulthood. TNDM has never been reported in association with CDH. However, 40% of CDH patients have other congenital malformations. As TNDM is a rare form of diabetes we questioned whether or not TNDM and CDH are entirely unrelated. Given the incidence of each, the chance of independently observing a child with both TNDM and CDH is approximately 1 in 2,000,000,000. Nothing to Disclose: LAM, DWK, RTZ, CAW, MJH

Pub #	P1-568
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	Importance of Clinician Awareness of Insulin Concentration in the Evaluation of an Insulin Regimen
Author String	S Hegde, H Narula Stony Brook University, Stony Brook, NY
Body	**Introduction**: Insulin concentrated at 100 units per milliliter (mL) (U100) is most commonly prescribed in th United States (US) for the treatment of Diabetes Mellitus (DM). However, although unavailable for human use in the US, insulin concentrated at 40 units of insulin per mL (U40) is prescribed in some countries. We report a case of a patient who presented with hyperglycemia and uncontrolled Type 2 DM suspected to be secondary to the use of U40 insulin purchased in Bangladesh with U100 insulin syringes obtained in the US. The lack of insulin standardization worldwide stresses the importance of clinicians being aware of both the concentration of insulin being used and assuring that the proper type of insulin delivery device is being utilized. **Case**: A 60-year old male with history of Type 2 DM with recent travel from Bangladesh two months prior presented to the emergency room with symptoms of weakness, fatigue, and noted point of care glucose values greater than 350 mg/dL (N=70-110). Laboratory results demonstrated serum glucose of 366 mg/dL and Hemoglobin A1C of 11.9% (N=4.3-6.1). Records from two years prior indicated his overall glycemic control had worsened from 8.6% on a similar insulin regimen. The change in glycemic control and his recent travel from Bangladesh led to further questions about his insulin regimen. He was using insulin *Mixtard*[reg] 30/70 from Bangladesh with U100 standard insulin syringes purchased in the US. The insulin was suspected to be U40, as it was purchased in Bangladesh. He was admitted for further evaluation and restarted on his home insulin regimen using U100 NPH and Lispro insulin. At time of discharge, point of care glucose testing was less than 200 mg/dl. **Conclusions**: Although insulin concentrations are becoming more standardized, current variations in insulin concentrations still exist. This case demonstrates a potential complication with insulin administration that clinicians must be aware of: the use of insulin concentrations with improper insulin delivery devices, which can result in the incorrect amount of insulin being delivered. Clinicians taking care of an increasingly diverse patient population with international travel must recognize the importance of determining the insulin concentration a patient is using and that the proper insulin delivery device is being used. Nothing to Disclose: SH, HN

Pub #	P1-569
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	Case Series -- Hemichorea-Hemiballismus (HC-HB) in Diabetes Mellitus (DM), and a Literature Review
Author String	T Araki, B Alfonso, E Liao Beth Israel Medical Center, New York, NY

Body

Introduction:

We report two cases of HC-HB associated with DM and a literature review

Case presentations:

Case 1:

58 year old female with type 2 DM and gastric banding (GB) presented with 1 week of involuntary movements in the left upper and lower extremity. After GB and 65 pounds weight loss, her glucose levels normalized. Lately she developed hyperglycemia (glucose 400-500 mg/dl). On presentation, glucose was >500 mg/dl and Hb A1c was 14.4 %. Physical exam was positive for HC-HB. Brain MRI showed right basal ganglia hyperintensity on T1 images. HC-HB was diagnosed. Symptoms resolved with hyperglycemia treatment.

Case 2:

38 year old female with type 1 DM, hypertension, stroke with left hemiparesis, and thyroid cancer presented with 1 week of involuntary movements in the right upper and lower extremity. Patient had hyperglycemia (glucose 200-250 mg/dl) a few weeks prior. Hb A1c was 8.9 %. On presentation the glucose was normal; the physical exam was pertinent for HC-HB. Brain MRI revealed increased T1 signal in the left putamen. She wa diagnosed with HC-HB and symptoms did not resolve.

Discussion:

HC-HB is a rare neurological complication related to DM (1). It mostly occurs in uncontrolled type 2 DM. Contralateral striatal hyperintensisty on MRI T1 is the characteristic finding (2). There are very few reports of HC-HB in type 1 DM (3) (4), as it is in case 2. New onset of DM can manifest with HC-HB, therefore, it should be included in differential diagnosis (5).

The mechanism is not well understood. Underlying chronic focal cerebro-vascular disease secondary to DM may accelerate the acute blood-brain barrier dysfunction and synergetic metabolic effects during hyperglycemia (6).

1. Maccario, M., Arch Neurol, 1968. 19(5): p. 525-34.
2. Lai, P.H., et al., Neuroradiology, 2001. 43(7): p. 525-31.
3. Nabatame, H., et al., Intern Med, 1994. 33(8): p. 472-5.
4. Abe, Y., et al., Intern Med, 2009. 48(13): p. 1135-41.
5. El Otmani, H., et al., Funct Neurol, 2009. 24(3): p. 129-32.
6. McCall, A.L., Diabetes, 1992. 41(5): p. 557-70.
7. Oh, S.H., et al., J Neurol Sci, 2002. 200(1-2): p. 57-62.
8. Cadario, F., et al., Minerva Pediatr, 2007. 59(1): p. 49-52

Nothing to Disclose: TA, BA, EL

Pub #	P1-570
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	A Case of Secondary Diabetes Mellitus Associated with Chronic Arsenic Exposure
Author String	J Taverna, D Ciltea, J Moorman, M Dillon, L Guseila Akron General Medical Center, Akron, OH; Akron General Medical Center, Akron, OH; Akron General Medical Center, Akron, OH; Northeastern Ohio Universities College of Pharmacy, Rootstown, OH
Body	**Objective:** To describe a patient with new onset diabetes mellitus secondary to chronic arsenic exposure. **Methods:** We present the clinical, laboratory and pathologic findings of a patient with diabetes mellitus secondary to chronic arsenic exposure. We also review the potential effects of arsenic on glucose homeostasis and discuss the underlying pathophysiologic mechanisms of arsenic-induced diabetes mellitus. **Results:** A 54-year old woman presented with new-onset hyperglycemia while receiving continuous tube feedings and corticosteroid therapy. The patient exhibited an abnormal response to insulin therapy, which prompted a workup for causes of secondary diabetes mellitus. An extensive evaluation was performed, which ultimately led to discovery of a urine arsenic level of 233.5 mcg/gram creatinine (reference range <50 mcg/gram creatinine). The patient was treated with British anti-Lewisite for arsenic toxicity and with insulin therapy for diabetes. **Conclusions:** Chronic exposure to arsenical compounds may lead to secondary diabetes mellitus with varying degrees of insulin resistance. It is therefore important to consider heavy metal poisoning, specifically with arsenic, as a possible cause for new-onset hyperglycemia and insulin resistance when common causes are ruled out. Nothing to Disclose: JT, DC, JM, MD, LG

Pub #	P1-571
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	Delayed Diagnosis of New-Onset Diabetes Mellitus after Renal Transplantation
Author String	A Inankur, L Tannock University of Kentucky, Lexington, KY
Body	

INTRODUCTION

New Onset Diabetes After Transplantation (NODAT) is a common complication after solid organ or bone marrow transplantation. We present a case of NODAT with self-limited hyperglycemia after transplantation which was lost to follow up and represented four years later.

CLINICAL CASE

A 37 year-old African American female presented with symptomatic hyperglycemia, involving polyuria, polydipsia and polyphagia. She had developed bilateral renal failure following pregnancy and received a cadaver donor renal transplant 4 years prior to presentation. She had experienced transient hyperglycemia after transplantation but was not routinely screened for diabetes mellitus. Her post-transplant regimen included tacrolimus and mycophenolate mofetil. She was also taking prednisone, 5 mg daily, for lupus erythematosis. Family history was positive for diabetes mellitus in grandparents.

Ophthalmologic evaluation was negative for retinopathy, and she had no evidence of peripheral diabetic neuropathy. Her A1C was 10.5% with a random blood glucose of 482 mg/dL. Urine albumin to creatinine ratio was elevated at 31 (0-30 mg/g). Plasma lipids in mg/dL: total cholesterol 203, HDL 41, LDL 148, Triglyceride 71. A full metabolic panel was normal other than the presence of elevated creatinine.

The patient was started on NovoLog 70/30, 6 units before breakfast and supper. One month later, she had titrated NovoLog to 10 units twice daily. Prednisone had concurrently decreased from 5 mg to 2.5 mg daily. Six months-post diagnosis, her A1C was 5.9%, and she was only taking prednisone PRN. The patient was given the option to transition off insulin to oral agents. She preferred to continue insulin, however, because of fewer drug interactions.

CLINICAL LESSON

NODAT is a common complication of renal transplantation with an incidence ranging from 2% to 50% in the first post-transplant year.[1] Our patient had several known risk factors for NODAT, including a family history of diabetes mellitus,[2] African American race,[3] deceased donor,[4] and exposure to the calcineurin inhibitor tacrolimus.[3] NODAT negatively influences graft and patient survival after transplantation, and this observation forms the basis of recommending meticulous screening for glucose intolerance before and after transplantation.[3,5] This case illustrates the importance of long-term surveillance for hyperglycemia after kidney transplantation.

(1) Montori V, Basu A, Edwin P, et al. Posttransplant diabetes: a systematic review of the literature. Diabetes Care 2002; 25(3):583-92.
(2) Depczynski B, Daly B, Campbell LV, Chisholm DJ, Keogh A. Predicting the occurrence of diabetes mellitus in recipients of heart transplants. Diabet Med 2000; 17:15-9.
(3) Kasiske B, Snyder J, Gilbertson D, Matas A. Diabetes mellitus after kidney transplantation in the United States. Am J Transplant 2003;3:178-85.
(4) Mazali FC, Lalli CA, Alves-Filho G, Mazzali M. Posttransplant diabetes mellitus: incidence and risk factors. Transplant Proc 2008; 40:764-6.
(5) Wilkinson A, Davidson J, Dotta F, et al. Guidelines for the treatment and management of new-onset diabetes after transplantation. Clin Transplant 2005; 19:291-8.

Nothing to Disclose: AI, LT

Pub #	P1-572
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	Total Daily Insulin Dose (TDD) in a Child with Type 1 Diabetes Mellitus (T1DM) Receiving Standard-Risk Chemotherapy for Acute Lymphoblastic Leukemia (ALL); Mercaptopurine (MP) and Oral Methotrexate (MTX) Appear To Increase Insulin Needs over and above That Caused by Dexamethasone
Author String	GC Dougan, G Hale, DI Shulman University of South Florida, Tampa, FL; All Children's Hospital, St Petersburg, FL
Body	Background: While exogenous glucocorticoids increase insulin resistance in diabetes mellitus, the effects of other antineoplastic chemotherapeutics are less evident. Clinical case: A 9-year old girl with a one year preceeding history of TIDM was diagnosed with standard-risk ALL. Per Children's Oncology Group (COG) protocol AALL0331, she received chemotherapy over a course of 26 months. During the different treatment cycles, the TDD varied from 0.5 units/kg of sc insulin glargine to 5.43 units/kg of insulin lispro delivered via continuous subcutaneous infusion (CSI) *plus* 31.63 units/kg of regular insulin administered sc 3-4 times daily at a concentration of 500 units/mL in attempts to achieve blood glucose (BG) values less than 200 mg/dL. During the maintenance cycles of chemotherapy, due to an apparent progressive increase in insulin needs, meticulous records were kept by the family with regard to mean daily BG and TDD. The TDD increased as expected during scheduled 5-day dexamethasone (DEX) administration. The peak TDD was greater than 30 units/kg with a mean BG generally below 200 mg/dL. However, hyperglycemia and TDD during DEX administration notably reduced following temporary cessation or reduction of MP and MTX due to leucopenia on multiple occasions with a mean TDD of 3 units/kg and mean BG less than 200 mg/dL. Five weeks after discontinuation of cancer treatment, TDD returned to near prediagnosis levels of 0.86 units/kg with the corresponding hemoglobin A_1c (HgA_1c) of 5.5%. Conclusion: In addition to glucocorticoids, MP and MTX appear to antagonize insulin action in a cumulative, dose-dependent fashion. The effects of the alteration in chemotherapy regimen in this child with T1DM were manageable with insulin at high doses and appeared to be reversible with the cessation of all chemotherapy. Nothing to Disclose: GCD, GH, DIS

Pub #	P1-573
Session Information	POSTER SESSION: CLINICAL - Case Reports in Diabetes (1:30 PM-3:30 PM)
Title	Hemiballism-Hemichorea in a Diabetic with Non-Ketotic Hyperglycemia
Author String	A Giestas, M Almeida, S Teixeira, A Maia, T Azevedo, D Vaz, AC Carvalho, M Magalhaes, I Palma Hospital Santo António, Centro Hospitalar do Porto, Oporto, Portugal; Hospital Santo António, Centro Hospitalar do Porto, Oporto, Portugal
Body	Introduction: Chorea or ballism can be caused by a wide variety of degenerative, metabolic, toxic or vascular disorders affecting the basal ganglia. Non-ketotic hyperglycaemia has been reported as a rare cause of these involuntary movements, which resolves with correction of the metabolic disturbance. Case report: We report an 88-year-old woman with long-standing insulin-dependent diabetes mellitus who was admitted with hyperglycaemia (412 mg/dl) and hemiballism-hemichorea in the left arm and left leg over a 15-day period. There was no previous story of headache, seizures, fever or stroke-like episodes. She had a tooth infection on treatment with antibiotic and since then her metabolic control got worse. Examination revealed uncontrolled choreiform movements in the left limbs, occasionally accompanied by rapid uncontrolled flinging ballistic movements of her left leg. Neurological examination was otherwise normal. Urine ketone bodies were negative. Serum osmolality was normal. Her total blood count, liver function, renal function, electrolytes, blood lactate, calcium, thyroid and parathyroid hormone levels were normal. Brain CT revealed abnormal density areas with micro-calcification of the subthalamic nucleus on the right side. Blood glucose levels decreased gradually after initiation of intravenous insulin. Achievement of normoglycaemia with insulin therapy determined the involuntary movements to regress partially within a day. Conclusion: We advise checking blood glucose in patients with hemiballism-hemichorea, particularly in older women, although this type of hyperkinesia is rarely caused by dysfunction of glucose metabolism. Nothing to Disclose: AG, MA, ST, AM, TA, DV, ACC, MM, IP

Pub #	P1-574
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	Allele-Dependent Regulation of Aldosterone Synthase Is Mediated by APEX1: *In Vivo* and *In Vitro* Evidence
Author String	F McManus, W Sands, E Davies, JM Connell University of Glasgow, Glasgow, UK; University of Dundee, Dundee, UK
Body	Aldosterone, the principal regulator of sodium and extracellular fluid status, has been implicated as an important cardiovascular hormone and the aldosterone synthase gene is a likely candidate for blood pressure regulation. A single nucleotide polymorphism (SNP) in the promoter of the aldosterone synthase gene (position -344, rs1799998) has been associated with increased plasma aldosterone levels and hypertension but deletion of this site has no effect on gene transcription in vitro and mechanisms that links genotype with phenotype are unclear. We identified a polymorphism at position -1651 T/C (rs13268025) and show it to be in linkage disequilibrium with the -344 SNP. The genotype/phenotype relationship was explored in a study of 60 normal volunteers, examined under standard salt conditions. Carriers of the C allele at position -1651 had higher 24 hour urinary aldosterone (THAldo) excretion (mean = 59.55 [mu]g/24h 95% CI 46.73-72.37 [mu]g/24h) than subjects carrying the T allele (mean = 36.10 [mu]g/24h 95% CI 24.0-48.21 [mu]g/24h) p=0.008. The mechanism underlying this association was further examined using reporter gene constructs containing contrasting alleles at position -1651, which were transfected into H295R cells. The C allele had (75%) greater promoter activity than the T allele. An electromobility shift assay, with oligonucleotides spanning the polymorphism of interest and nuclear protein from H296R cells, demonstrated a difference in protein/DNA complexes between the T and C alleles. Biotinylated pull down assays of the protein: DNA complex were proteolised and peptides identified by tandem mass spectroscopy. Fragments derived from the transcription factor APEX1, were identified bound to the T allele and not the C allele. Chromatin immunoprecipitation confirmed the association of APEX1 to the CYP11B2 promoter in H295R cells. Inhibition of APEX1 activity with a small molecule inhibitor of APEX1 (E3330, Sigma-Aldrich), and siRNA for APEX1, both increased transcriptional activity. APEX1 has been identified as a novel negative regulator of CYP11B2; a SNP at -1651 demonstrates allele dependant binding, leading to increased transcriptional activity in vitro and increased THAldo excretion in vivo. This offers a plausible mechanism behind the genotype/phenotype association in CYP11B2 and increased aldosterone secretion and hypertension. Sources of Research Support: MRC Clincial Research fellowship awarded to FM. Nothing to Disclose: FM, WS, ED, JMC

Pub #	P1-575
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	O-GlcNAc Modification of Mineralocorticoid Receptor Enhances Its Expression and Transcriptional Activity
Author String	R Jo, H Shibata, I Kurihara, A Murai-Takeda, Y Mitsuishi, T Hayashi, Y Motosugi, T Ohyama, H Itoh School of Medicine, Keio University, Tokyo, Japan
Body	[Background] High plasma aldosterone level or mineralocorticoid receptor (MR) activation plays crucial roles in cardiovascular complications. Post-translational modification of nuclear and cytoplasmic proteins by O-linked-N-acetylglucosamine(O-GlcNAc) has emerged as an essential glucose-sensing mechanism. It is strongly associated with type 2 diabetes and its complications. Indeed, administration of MR antagonist is shown to have beneficial effects to protect renal function in diabetic nephropathy, indicating pathological activation of MR even with normal aldosterone level. We therefore hypothesized that MR is modified by O-GlcNAc to enhance its transcriptional activity in diabetes. [Methods] The MR transcriptional activities were investigated in reporter assays in COS-7 cells and real time RT-PCR in stably MR-expressing 293-MR cells. Levels of expression of MR were examined with western blot analysis. O-GlcNAc modification of MR was investigated with coimmunoprecipitation assays. In each experiment, we analyzed the effect of 25-30mM glucose stimulation. In addition, we used PUGNAc, an O-GlcNAcase inhibitor which raises O-GlcNAc level and 6-diazo-5-oxonorleucine (DON) which reduces O-GlcNAc level by inhibiting glucosamine-fructose-6-phosphate amidotransferase. [Results] In reporter-based transient transfection assays, treatment with high glucose and PUGNAc enhanced aldosterone-mediated MR transactivation by 2-fold. The enhanced MR activities were also observed in endogenous Sgk1 mRNA levels in 293-MR cells. In contrast, DON lowered a reporter activity by 75%. In parallel with MR transcriptional activities, levels of expression of MR protein were significantly increased by treatment with high glucose and PUGNAc, whereas those were decreased by DON, indicating that increased O-GlcNAc levels account for increased MR levels and activities. Coimmunoprecipitation assays showed that MR is in fact modified by O-GlcNAc in the presence of PUGNAc. In addition, the N-terminal (amino acids 170-450), but not the C-terminal ligand-binding domain of MR was markedly modified by O-GlcNAc. [Conclusion] We showed for the first time that MR is a target for O-GlcNAc modification. Treatment with high glucose and PUGNAc resulted in O-GlcNAc modification of MR, which is associated with increased MR levels and its transcriptional activities. The epigenetic modification of MR may account for pathological MR activation in diabetes. Nothing to Disclose: RJ, HS, IK, AM-T, YM, TH, YM, TO, HI

Pub #	P1-576
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	Molecular Dissection of the Transcriptional Control of Intracellular Adhesion Molecule-1 (ICAM-1) Promote in Human Endothelial Cells by Mineralocorticoid Receptor
Author String	V Marzolla, A Armani, A Antelmi, C Mammi, M Calanchini, A Fabbri, GMC Rosano, IZ Jaffe, M Caprio IRCCS San Raffaele Pisana, Rome, Italy; CTO- A Alesini Hospital, University Tor Vergata, Rome, Italy; Tufts Medical Center, Boston, MA
Body	In clinical trials, Mineralocorticoid Receptor (MR) antagonists decrease cardiovascular mortality and ischemi suggesting a beneficial role of MR function inhibition. In animal models aldosterone treatment induces vascular macrophage infiltration and atherosclerosis. These effects are reduced by administration of MR antagonists. We have shown that human coronary and umbilical endothelial cells (HUVEC) express functional MR. In endothelial cells MR activation by aldosterone promoted transcription of ICAM-1 (1.5 fold). This effect was inhibited either by MR antagonist spironolactone or by MR knock down with siRNA. Most importantly cell adhesion assays demonstrated that aldosterone promotes leukocyte adhesion to ECs, an effect that was inhibited by spironolactone and ICAM-1 blocking antibody. Furthermore, MR activation was able to up-regulate VCAM and E-selectin mRNA expression (2 and 3.5 fold respectively), whereas P-selectin was not regulated by MR. In transient transfection experiments performed in HUVEC, we showed that aldosterone was able to activate (2 fold) a 3 Kb promoter region upstream the transcription start site of human ICAM-1 gene. Co-incubation with spironolactone was able to inhibit the effect of aldosterone, confirming the presence of elements responsive to signaling pathway(s) activated by MR. In order to localize and characterize MR responsive cis-element(s) and the corresponding transcription factor(s) binding to this regulatory region, five 5'-deletion constructs of ICAM-1 promoter were subcloned in a vector upstream of luciferase gene. Data of transcriptional activity showed the presence of regulatory element(s) required for ICAM-1 expression via MR in the promoter region between nt-872 and -1141. Bioinformatics analysis of this region revealed the presence of four different potentially involved regulatory elements: three SP1 binding sites, one NF-[kappa]B bingind site, one AP1 binding site and one GRE/MRE. The role of each of these transcription factors in MR-mediated regulation of ICAM-1 expression is being explored, using both dominant negative transcription factors and site specific mutagenesis of the putative binding sites. These studies explore the molecular mechanism for the pro-inflammatory effects of MR activation in the vasculature that may contribute to the protective effects of MR antagonists in clinical trials. Nothing to Disclose: VM, AA, AA, CM, MC, AF, GMCR, IZJ, MC

Pub #	P1-577
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	Dicer-Dependent miRNAs Regulate Adrenal Steroidogenesis
Author String	S Wood, SM Mackenzie, R Fraser, JM Connell, E Davies University of Glasgow, Glasgow, UK; University of Dundee, Dundee, UK
Body	The synthesis of cortisol and aldosterone in the adrenal gland involves several enzymes, the expression of which is tightly-regulated. Alterations in their regulation have been implicated in diseases such as hypertension and adrenal cancers. microRNAs (miRNAs) are small, endogenous RNA molecules which negatively regulate gene expression at the post-transcriptional stage by binding the 3' untranslated region of target mRNA. miRNAs are transcribed as long, polycistronic precursor molecules which are cleaved during miRNA maturation. Dicer1 is responsible for catalysing the final cleavage step and is, therefore, vital to miRNA maturation. Using short interfering RNA (siRNA) molecules to knockdown Dicer1, we investigated the impact of miRNAs on the regulation of steroidogenesis in the adrenocortical cell line, H295R. Two siRNAs targeted against Dicer1 and a control non-targeting siRNA were transfected individually into the H295R cell line. 48 hours post-transfection, RNA was isolated and the mRNA of several key steroidogenic enzymes quantified by qRT-PCR. Steroid production was assessed by liquid chromatography with tandem mass spectrophotometry, normalised to protein concentration. Moderate decreases in Dicer1 expression led to significant increases in CYP11A1, CYP21A1, CYP17A1, CYP11B1 and CYP11B2 mRNA. StAR, 3βHSD2 and HSD11B2 mRNA were unaffected by Dicer knockdown. The aldosterone precursors deoxycorticosterone, corticosterone and 18-OH-corticosterone were all significantly increased in Dicer1 knockdown cells relative to controls and aldosterone itself was increased 1.47 fold ($p<0.01$). Cortisol production was 1.33 fold ($p<0.05$) higher in knockdown cells. Neither the cortisol precursor 11-deoxycortisol nor the cortisol metabolite cortisone was significantly affected by knockdown. In conclusion, loss of Dicer1 - and hence mature miRNAs - led to increased levels of mRNA for several enzymes vital to steroidogenesis. Furthermore, Dicer1 knockdown increased the production several intermediate steroids and of both the major corticosteroid products. These results are consistent with miRNAs exerting negative regulatory effects important to the production of adrenal steroids. Interestingly, of the genes analysed, only those encoding cytochrome P450 enzymes were affected by miRNAs. This study supports a role for miRNAs in steroidogenesis and we believe further investigation of this regulatory mechanism will be important to understanding disorders of the adrenal gland. Nothing to Disclose: SW, SMM, RF, JMC, ED

Pub #	P1-578
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	Systematic Screening for Aberrant Hormone Receptors in Primary Aldosteronism: *In Vivo* and *In Vitro* Studies
Author String	LM Mermejo, S Grunenwald, TL Mazzuco, S Oble, I Bourdeau, A Lacroix Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Montreal, Canada
Body	**Context:** The mechanisms involved in the renin-independent regulation of aldosterone secretion in primary aldosteronism (PA) are poorly understood. In ACTH-independent Cushing's syndrome, cortisol secretion can be regulated by the aberrant expression of G-protein coupled receptors (GPCRs). By analogy, some recent studies identified overexpression of several GPCRs as a potential cause for excess aldosterone production in some aldosterone producing adenomas (APA) and in bilateral idiopathic hyperaldosteronism (IH). **Objective:** To investigate systematically patients with PA for aberrant regulation of aldosterone secretion by GPCRs using *in vivo* testing and *in vitro* studies. **Design:** Twenty-three patients (7F, 16M) with PA (13 APA, 10 IH) were studied. An *in vivo* screening protocol, performed under dexamethasone suppression of endogenous ACTH, included measurements of plasma aldosterone, renin, cortisol and ACTH during upright posture, mixed meal or stimulation with GnRH, TRH, vasopressin and metoclopramide or tegaserod. Aldosterone increase over baseline > 50% was defined a a positive response. Additional tests (desmopressin, isoproterenol, LH, GIP, oral glucose) were performed based on the response to the initial screening tests. Expression of mRNAs extracted from resected adrenals (1(patients) was analysed for 16 GPCRs genes by real-time PCR and compared to that of 8 normal adrenals. **Results:** We found a high frequency of aberrant renin and ACTH-independent aldosterone regulation in patients with APA and IH; 87% of the patients presented at least one positive aberrant response of aldosterone. The most frequent stimuli were upright posture (76.5% of the patients), vasopressin (69%), LH (43%) and GnRH (35%). An mRNA overexpression (> 2 S.D. above mean expression ratio of normal glands) was identified for 12 out of 16 GPCR genes in resected lesions. It was mainly observed in 80% of tumors for the GNRHR at 1.1- to 76.7-fold, followed by GIPR (60%, 2.6- to 16.9-fold) and LHCGR (40%, 2.9- to 32.2-fold). We observed a good correlation between the *in vitro* determinations of GPCR and the *in vivo* responses results; however the overexpression of mRNA of some GPCR in tumors does not always correlate with *in vivo* responses. **Conclusions:** This study confirms that aberrant regulation of aldosterone is frequent ir PA secondary to IH or APA. These findings should modify significantly the investigation and treatment of patients with this frequent etiology of human hypertension. Sources of Research Support: Grant MT-13-189 from the Canadian Institutes of Health Research. LMM was recipient of partial support form Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (Fapesp), Brazil, SC from La Societe Fran[ccedil]aise d'Endocrinologie and CHU de Toulouse, and TLM from CHUM Foundation and Endocrine service. Nothing to Disclose: LMM, SG, TLM, SO, IB, AL

Pub #	P1-579
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	CRH Regulates Cardiac Function in Normal Conditions and Infection
Author String	T Tzanavari, A Varela, S Theocharis, C Pantos, D Cokkinos, K Karalis Biomedical Research Foundation of the Academy of Athens, Athens, Greece; Biomedical Research Foundation of the Academy of Athens, Athens, Greece; University of Athens Medical School, Athens, Greece; University of Athens Medical School, Athens, Greece; Children's Hospital, Boston, MA
Body	The myocardial response to stressful stimuli is influenced by many factors. We investigated the contribution of the stress hormone corticotropin (CRH or CRF) in cardiac function using a model of acute endotoxemia in Crh-null (*Crh-/-*) and wild-type (*Crh+/+*) mice. Echocardiographic analysis using a high frequency system (Vivid 7, GE, 13 MHz linear transducer) was performed in mice injected intraperitoneally with normal saline or LPS (120[micro]g per animal). Two-D targeted M-mode imaging was obtained in anesthetized mice from the short axis view at the level of greatest LV dimension. End diastole was determined at the maximal LV diastolic dimension, and end systole was taken at the peak of posterior wall motion. The Heart Rate (HR) and the percentage of LV fractional shortening (FS) were calculated and results were presented as means ± SEM. We found that LPS administration significantly reduced FS at 6 and 20 hours in *Crh+/+* and *Crh-/-* mice; and TUNEL assay revealed significantly increased number of apoptotic/necrotic cells. In line with that, there was significantly reduced expression of AMPKα in *Crh-/-* after LPS administration and compromised expression of PPARγ. After LPS treatment, histological findings in *Crh-/-* cardiac muscle showed increased levels of infiltration and development of fibers surrounding the hyperplastic vessels and fibrosis-like reactive collagen production. *Crh-/-* mice showed increased reduction and unexpected elevated levels of mortality (90-100% after 16-28h post-LPS treatment), compared to no mortality observed among WT. Corticosterone replacement for a week prior to LPS treatment alone, or accompanied with an acute injection of dexamethasone, slightly ameliorated the effect of LPS in CRH-deficient mice. Interestingly, cardiac function was compromised not only in LPS-treated but also in control *Crh-/-* mice compared to *Crh+/+* mice, as demonstrated by significantly lower FS values and lower heart rate. Furthermore, TUNEL revealed increased levels of apoptosis and H&E staining showed fibroblast-like structures in the proximity of the vessels, increased vasculature and hyperplastic intima, as well as signs of fibrosis, in the *Crh-/-* mice alone. Our results indicate a possible new protective action of the stress hormone CRH in normal cardiac function and HR and in states of systemic inflammatory stress such as in endotoxemia. To our knowledge, this is the first indication of CRH acting directly as a cardioprotective factor. Nothing to Disclose: TT, AV, ST, CP, DC, KK

Pub #	P1-580
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	Differential Effects among ARBs on Aldosterone Synthase (*CYP11B2*) Gene Expression in Human Adrenal H295R Cells
Author String	K Matsuda, A Uruno, M Kudo, F Sato, S Ito, A Sugawara Tohoku University Graduate School of Medicine, Sendai, Japan; Tohoku University Graduate School of Medicine, Sendai, Japan; Tohoku University Graduate School of Medicine, Sendai, Japan
Body	Introduction: Aldosterone synthase gene (*CYP11B2*) is the key enzyme of adrenal aldosterone production, and the elucidation of its regulation is essential for the innovation of novel antihypertensive therapy. Although angiotensin (Ang) II receptor blockers (ARBs) are well known to affect *CYP11B2* expression, functional differences among ARBs are not known. We therefore examined the effects of various ARBs on the expression of *CYP11B2* in human adrenal H295R cells. Methods: Human adrenocortical carcinoma H295R cells were transfected with *CYP11B2* promoter/luciferase chimeric vectors, and their transcription level was measured by luciferase assay. *CYP11B2* mRNA expression level in H295R cells was determined by real-time PCR. Aldosterone secretion from H295R cells to the media was measured by EIA. Regarding ARBs, we used telmisartan, losartan, valsartan, olmesartan, and cacdesartan. Results: In the presence of Ang II, all ARBs suppressed the Ang II-induced *CYP11B2* transcription activation However, telmisartan, but not other ARBs, increased *CYP11B2* transcription level in the absence of Ang II. The telmisartan-mediated increase was also observed in *CYP11B*2 mRNA expression and aldosterone secretion. Experiments using *CYP11B2* promoter deletion mutants and Ad1/Ad5 point mutants indicated that the Ad1/Ad5 elements were important for the telmisartan-mediated increase of *CYP11B2* transcription. Telmisartan also increased the expression of transcriptional factors NURR1 and NGFIB which activates the Ad5 element. Furthermore, the overexpression of NURR1 or NGFIB augmented the telmisartan-mediated increase of *CYP11B2* transcription. However, siRNAs of NURR1 and NGFIB did not affect the telmisartan-mediated increase. We are currently investigating the involvement of other transcription factors including ATF-1, ATF-2, CREB, and CREM. Conclusion: Telmisartan was shown to increase *CYP11B2* transcription via the Ad1/Ad5 elements. Each ARE was thus demonstrated to have different characteristics for *CYP11B2* regulation. Nothing to Disclose: KM, AU, MK, FS, SI, AS

Pub #	P1-581
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	Distinct Susceptibility to Steatohepatitis and Fibrosis in Dietary Models of Non-Alcoholic Fatty Liver Disease in Mice Associates with Alterations in Intra-Hepatic Glucocorticoid Metabolism
Author String	DP Macfarlane, MJ Nyirenda, X Zou, RL Aucott, PJ Raubenheimer, Z Michailidou, R Andrew, JP Iredale, BR Walker University of Edinburgh, Edinburgh, UK; University of Edinburgh, Edinburgh, UK
Body	Glucocorticoids may induce fatty liver and, through anti-inflammatory and anti-fibrotic actions, influence progression from steatosis to steatohepatitis and fibrosis. Intra-hepatic glucocorticoid levels are controlled by local inactivating (5α- and 5β-reductases) or regenerating (11β-HSD1) enzymes, which are regulated by inflammatory and metabolic signals. Here, we studied whether variation in glucocorticoid metabolism is associated with susceptibility to fatty liver, steatohepatitis or fibrosis in dietary models in mice. C57Bl6 mice (male, 14 weeks) were fed a choline deficient diet (CDD), methionine and choline deficient diet (MCDD), or supplemented control diet (CS) for 4 weeks to induce steatosis ± steatohepatitis. In each dietary group, mice were injected twice weekly with carbon tetrachloride [CCl4, 0.3[micro]l/g in olive oil (OO), 1:3 v/v] or vehicle (OO) to induce hepatic fibrosis (n=7/group). Results are mean±SEM for CS, CDD and MCDD respectively. *P<0.05, **P<0.01, ***p<0.001 versus controls by one way ANOVA or paired t-test as appropriate. Liver triglyceride levels were similarly increased (~2-fold) in CDD and MCDD mice and were unaffected by CCl4. MCDD, but not CDD, caused steatohepatitis (GR1-positive neutrophils 1.3±0.2 vs 4.0±1.2 vs **8.9±1.2 per field) and stellate cell activation (aSMA positive cells 5.2±1.6 vs 18.2±5.7 vs *26.6±6.5 per field), without the addition of CCl4. With CCl4, however, hepatic fibrosis was less marked in MCDD than CDD mice (1.2±0.1 vs 1.4±0.2 vs *0.9±0.2% area staining with picosirius red). This susceptibility to steatohepatitis in MCDD mice was accompanied by lower mRNA for both 11β-HSD1 (0.40±0.14 vs 0.27±0.03 vs *0.10±0.05 AU) and 5α-reductase type 1 (0.43±0.08 vs 0.35±0.08 ***0.05±0.02), but no difference in 5β-reductase or 3α-HSD. With CCl4 administration, differences in 11β-HSD1 were abolished (by up-regulation of 11β-HSD1 in fibrosis-resistant MCDD mice) but those in 5α-reductase persisted. Subject to further dissection of causality, we conclude that suppression of hepatic 11β-HSD1, predicted to reduce hepatic glucocorticoid levels, may promote steatohepatitis in MCDD mice, albeit this may be offset by increased glucocorticoid inactivation through 5α-reductase 1, and is associated with a paradoxically reduced fibrotic response to CCl4. These findings could have significant implications for obese humans with non-alcoholic fatty liver disease being treated with 11β-HSD1 or 5α-reductase inhibitors. Sources of Research Support: Wellcome Trust Clinical Research Fellowship awarded to DPM. Disclosures: BRW: Investigator, Wyeth Pharmaceuticals; Ad Hoc Consultant, Boehringer Ingleheim; Astra Zeneca. Nothing to Disclose: DPM, MJN, XZ, RLA, PJR, ZM, RA, JPI

Pub #	P1-582
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	Ontogenic Expression of Angiotensin-Converting Enzyme in the Human Fetal Adrenal Gland: Implication in Angiotensin II-Mediated Aldosterone Synthesis
Author String	K Doguchi, H Ishimoto, T Matsumoto, A Kondo, S Sato, M Miyazawa, K Minegishi, S-i Izumi, M Mikami, RB Jaffe Tokai University School of Medicine, Isehara, Japan; Keio University School of Medicine, Tokyo, Japan; University of California, San Francisco, San Francisco, CA
Body	Angiotensin-converting enzyme (ACE) is a key component of the tissue rennin-angiotensin system (RAS). Although the human adult adrenal expresses all of the components of the RAS, their expression in the human fetal adrenal (HFA) has not been well characterized. Aldosterone synthesis in the outer definitive zone (DZ) of the HFA likely becomes active by late gestation probably due to the eventual expression of CYP11B2 expression (1). In the present study, we investigated the expression profile of ACE and proteins involved in aldosterone synthesis in midgestation HFAs. A sensitive and spatially-accurate combination of real-time quantitative RT-PCR and laser-capture microdissection demonstrated that the levels of transcripts encoding ACE and CYP11B2 in the DZ increased 2.4- and 6.8-fold, respectively, between 18 and 24 wks. ACE and CYP11B2 mRNA in the inner fetal zone (FZ) exhibited 4- and 16-fold lower levels, respectively, than those i the DZ, confirming DZ-specific expression of ACE and CYP11B2. The levels of mRNAs encoding the nuclear receptors NURR1 (NR4A2) and NGFIB (NR4A1), which regulate CYP11B2 gene expression, followed similar zonal and ontogenic expression patterns. We also investigated regulation of ACE using isolated HFA cortical cells. Treatment with angiotensin-II (AGT II) increased ACE mRNA (8 hrs: by 1.7-fold at 100 nM, in DZ cells from a 21-wk adrenal), and such increase was blocked by addition of candesartan (an angiotensin type 1 [AT1] receptor-selective antagonist), but not by PD123319 (an angiotensin type 2 receptor selective antagonist), suggesting involvement of the AT1 receptor subtype in AGT II-stimulated up-regulatior of ACE. In contrast, ACTH markedly decreased ACE mRNA (24 hrs: by 6-fold at 1 nM, in DZ cells from a 20-wk adrenal). Additionally, AGT II up-regulated mRNAs of NURR1, NGFIB and CYP11B2 in isolated DZ cells as expected. Thus, the up-regulation of DZ expression of CYP11B2, NURR1, and NGFIB at late midgestation likely reflects an increasing readiness of this zone to synthesize aldosterone. The fetal adrenal ACE, which shows a similar expression profile, may operate as an amplifier in the machinery for AGT II-mediated aldosterone synthesis because ACE can generate AGT II locally and AGT II increases ACE expression in DZ cells. (1) Ishimoto H and Jaffe RB. Endocrine Reviews 2011; 32 (in press) Sources of Research Support: NIH grant HD08478 awarded to RBJ; Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science #21591423 awarded to HI. Nothing to Disclose: KD, HI, TM, AK, SS, MM, KM, S-II, MM, RBJ

Pub #	P1-583
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	Anti-Tumor Effects of Peptide Analogs Targeting Special Neuropeptide Hormone Receptors in Pheochromocytoma
Author String	CG Ziegler, G Eisenhofer, AV Schally, L Gebauer, M Ehrhart-Bornstein, SR Bornstein University Hospital Carl Gustav Carus, Dresden, Germany; University of Miami Miller School of Medicine, Miami, FL
Body	Pheochromocytoma is a rare but potentially lethal chromaffin cell tumor. The prognosis for malignant pheochromocytoma is particularly poor and there are currently no effective treatments; thus, new therapeutic strategies are urgently needed. The novel targeted therapeutic approach we are pursuing here is based on our previous microarray and RT-PCR analyses, which revealed altered expression of neuropeptide hormone receptors in adrenomedullary tumors and cell lines. Additionally, our work in tumor cell lines of both the adrenal cortex and medulla has shown a significant reduction of cell survival and an increase in apoptosis and necrosis upon incubation of cell lines with several peptide analogues that bind specifically to their expressed receptors. Here we could further demonstrate expression of somatostatin receptor subtype 2 (sst2), growth hormone-releasing hormone (GHRH) receptor and luteinizing hormone-releasing hormone (LHRH) receptor in mouse pheochromocytoma cells (MPC) on mRNA and protein level. Employing various agonists and antagonists for these receptors we could demonstrate a significant reduction of cell proliferation and an increase in programmed cell death upon incubation of MPC cells with peptide analogous. The cytotoxic derivatives of somatostatin AN-162 and to a lesser extent AN-238 significantly reduced cell numbers of MPC cells after 24-72h and significantly increased caspase 3/7 activation in this time interval. Furthermore, we could evidence similar anti-tumor effects also for GHRH antagonist MIA-602 and LHRH antagonist AN-152 on MPC cells. Taking advantage of the same cell line we are now setting up a mouse model of malignant pheochromocytoma, which will than be treated using peptide analogues, selected from *in vitro* studies to establish therapeutic efficacy. In conclusion, this study should help to find the most effective peptide analogues with potential for future targeted treatment of neuroendocrine tumors in humans. Nothing to Disclose: CGZ, GE, AVS, LG, ME-B, SRB

Pub #	P1-584
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	A Potential Role of Calpain in Angiotensin II-Induced Aldosterone Secretion from Adrenal Glomerulosa Cell Models
Author String	MP Seremwe, WB Bollag Medical College of Georgia, Augusta, GA
Body	Aldosterone is a steroid hormone that maintains sodium homeostasis and is important in the regulation of blood volume and pressure. Aberrant synthesis and secretion of aldosterone leads to the development and progression of hypertension and can result in cardiofibrosis and congestive heart failure; therefore it is important to achieve a complete understanding of this process under physiological and pathological conditions. Angiotensin II (AngII) is the primary regulator of aldosterone synthesis and secretion in adrenal glomerulosa cells. AngII promotes these processes by eliciting a signal transduction cascade that involves phosphoinositide turnover and increased intracellular calcium signaling. Calpains are heterodimeric, calcium-dependent cysteine proteases found predominantly in the cytosol and active at neutral pH. In other cell types calpains have been shown to affect the actin cytoskeleton, thought to be essential in steroidogenesis. To test the possible role of calpains in aldosterone secretion we used a selective inhibitor, calpeptin in primary cultures of bovine adrenal glomerulosa cells (AG cells). Our results showed that calpeptin inhibited aldosterone synthesis and secretion basally and after stimulation with secretagogues, namely AngII and potassium (K+). However, in AG cells treated with 22(R)-hydroxycholesterol, a water soluble analogue which bypasses signaling pathways, calpeptin had no effect on aldosterone production, suggesting that inhibition of secretion was not the result of cytotoxicity. In addition, calpeptin exerts its aldosterone secretion inhibitory effect in a dose-dependent manner. To corroborate that the inhibitory effects were a result of calpain inhibition, we used a structurally distinct calpain inhibitor MDL-28170 and observed similar effects. Furthermore, we examined the effect of calpeptin on the actin cytoskeleton using fluorescent phalloidin in AngII-stimulated AG cells; our data suggested that the inhibitor induced cytoskeletal remodeling in our cells. Subsequently, we tested whether calpeptin had similar effects in a human adrenocortical carcinoma cell line (HAC 15 cells from Dr W. Rainey). We found that calpeptin inhibited AngII-induced aldosterone secretion without exhibiting cytotoxicity, suggesting that calpain may play a similar role in HAC15 cells as in AG cells Experiments are in progress to determine whether AngII activates calpain in these two cell models as well as the calpain isoenzyme that is affected. Sources of Research Support: NIH award # HL70046. Nothing to Disclose: MPS, WBB

Pub #	P1-585
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	Luteinizing Hormone Receptor Is Present in Aldosterone-Producing Adrenal Adenoma or Hyperplasia and May Have a Role in Regulation of Steroid Production
Author String	G Nicolini, S Balzan, A Pietrabissa, F Forini, L Sabatino, E Fommei Institute of Clinical Physiology, CNR, Pisa, Italy; University of Pisa, Pisa, Italy; Fondazione Toscana G Monasterio and University of Pisa, Pisa, Italy
Body	Unilateral aldosterone-producing adrenal adenoma or hyperplasia are the most common indications for surgically correctable primary aldosteronism. Mechanisms of aldosterone overproduction in these conditions remain yet poorly clarified and there are evidences about the adrenal expression of G-protein-coupled receptors among which the luteinizing hormone receptor (LH-R) which were described in some adenomas (Saner-Amigh 2006, Ye 2007). Abnormally elevated aldosterone levels have been recently observed in low renin hypertension in women during the LH stimulated phase of their ovarian cycle (Fommei 2009), and may be present after menopause (Olivieri 2008) which is characterized by high plasma LH levels. Aim of this study was to confirm the presence of LHR in human aldosterone producing adenomas or hyperplasia and to assess the possible promoting effects of LH on aldosterone production in vitro. Materials and Methods. We studied tissues obtained from surgically excised adrenal glands of 12 patients with primary aldosteronism and unilaterally functioning adenoma or hyperplasia, who gave their informed consent (7F,5M, 8 with adenoma, 4 with hyperplasia). Western Blot Analysis for LH-Rs and LH stimulation (300ng/ml for 6 hours) in cell cultures were performed in all cases; Real Time PCR for LH-R and cells stimulation with progesterone (100nM for 6 hours) were done in 5 and 4 cases respectively. Results. LH-Rs were present and expressed in all the explored tissues from the 12 patients. In 5 out of 12 cell cultures LH stimulation significanlty increased aldosterone concentration compared with control cultures, with a mean increase by 2-folds ($p<0.05$). Also progesterone induced a significant aldosterone increase by 1.7-folds in all tested cultures ($p<0.05$). Conclusions. Our results 1. confirm observations by others and suggest a high prevalence of adrenal cortex LH-R in both adrenal adenoma ad hyperplasia in primary aldosteronism 3. indicate LH (and/or progesterone) as possible cellular regulator(s) of aldosterone biosynthesis in at least some subsets of primary aldosteronism. By which mechanisms LH itself and/or progesterone may influence aldosterone synthesis at the adrenal cell level should deserve dedicated studies. Nothing to Disclose: GN, SB, AP, FF, LS, EF

Pub #	P1-586
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	YPEL4 Modulates HAC15 Adrenal Cell Proliferation and Aldosterone Production
Author String	K Oki, MW Plonczynski, EP Gomez-Sanchez, CE Gomez-Sanchez G V Montgomery VA Medical Center, Jackson, MS; University of Mississippi, Jackson, MS
Body	Angiotensin II (Ang II) and K regulate aldosterone production in the zona glomerulosa (ZG) of the adrenal. We found that Yippee-like 4 (YPEL4) mRNA is one of the most up-regulated after Ang II or K stimulation in rat ZG cells. YPEL family protein regulates cell proliferation; however the role of YPEL4 in adrenal gland is unknown. The aim of our study was to investigate the effects of YPEL4 on aldosterone production and cell proliferation in the human adrenal cell line, HAC15. YPEL4 over-expression in HAC15 was induced by lentivirus infection. Cells over-expressing YPEL4 had higher basal aldosterone levels than controls (55.4 ± 4.3 pg/ml vs. 38.7 ± 8.7 pg/ml, $P<0.05$), but no significant difference in response to Ang II or K stimulation. There were no differences of mRNA expression levels of StAR, CYP11A1, HSD3B2, CYP21A2, or CYP11B2 between YPEL4 and control cells. The effect of YPEL4 on cell proliferation was measured with the XTT assay. Increased proliferation was detected after 24h (1.31 ± 0.09-fold), (48h 1.33 ± 0.10-fold) and 72h (1.72 ± 0.26-fold). Aldosterone production by YPEL4 over-expressing cells was greater than control HAC15 cells at 48h and 72h, displaying a 1.11 ± 0.04-fold ($P<0.05$) and 1.27 ± 0.04-fold ($P<0.01$) increases, respectively. We determined whether the action of YPEL4 involved the MAPK pathway by incubating cells with the MEK inhibitor U0126. Incubation of both the wt and YPEL4 over-expressing HAC15 cells with 10[mu]M U0126 blocked the effect of YPEL4 on cell proliferation and aldosterone production. In conclusion, YPEL4 stimulated adrenal cell proliferation and basal aldosterone. The effect of YPEL4 in cell proliferation seems to be mediated by MAPK signaling. Sources of Research Support: NIH HL27255-28. Nothing to Disclose: KO, MWP, EPG-S, CEG-S

Pub #	P1-587
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	Association of IGF-I and the IGF-I/IGFBP-3 Ratio with Plasma Aldosterone Levels in the General Populatior
Author String	A Hannemann, H Wallaschofski, R Rettig, H Volzke, S Samietz, M Nauck, M Bidlingmaier, N Friedrich University of Greifswald, Greifswald, Germany; University of Greifswald, Greifswald, Germany; University of Greifswald, Greifswald, Germany; University of Greifswald, Greifswald, Germany; Ludwig-Maximilians-University, Munich, Germany

Body

Background: Previous in vivo studies (1,2) suggested a stimulation of the renin-angiotensin-aldosterone system (RAAS) by the growth hormone (GH) insulin-like growth factor I (IGF-I) axis. While previous clinica studies focussed on small samples of acromegalic (3,4) or GH deficient patients (5), data on the relation of the GH/IGF-1 axis and the RAAS in the general population is sparse. We aimed to analyze the association of serum IGF-I and the IGF-I/IGFBP-3 ratio with plasma aldosterone and the aldosterone/renin ratio in a large, population-based sample including subjects from northeast Germany.
Methods: From the first follow-up of the Study of Health in Pomerania (SHIP-1) 1,504 men and 1,566 women aged 25-88 were selected. Plasma aldosterone and plasma renin concentrations, as well as serum IGF-I and IGFBP-3 levels were determined with immunoassays. Analysis of variance and linear regression analyses were performed.
Results: In women, associations between serum IGF-I or IGFPB-3 and plasma aldosterone (raise per IGF-I SD increase 2.91 ng/l; per IGF-I/IGFBP-3 SD increase 2.17 ng/l) were found. The associations remained significant after exclusion of subjects taking RAAS altering medication and after exclusion of subjects with serum IGF-I levels outside the reference range. Furthermore, a higher serum IGF-I/IGFBP-3 ratio was related to a decreased plasma aldosterone/renin ratio (decrease 0.42 per IGF-I/IGFBP-3 SD increase) in women not taking RAAS altering medication. No relation became apparent in men.
Conclusions: We conclude that not only in acromegaly or GH deficiency, but also in women from the genera population associations of serum IGF-I and IGF-I/IGFBP-3 ratio with plasma aldosterone are present.

(1) Wyse B et al., Am J Physiol 1993; 265:E332
(2) Kamide K et al., J Hypertens 2000; 18:1051
(3) Mulatero P et al., J Clin Endocrinol Metab 2006; 91:5008
(4) Bielohuby M et al., Exp Biol Med 2009; 234:1002
(5) Hanukoglu A et al., J Steroid Biochem Mol Biol 2001; 77:49

Nothing to Disclose: AH, HW, RR, HV, SS, MN, MB, NF

Pub #	P1-588
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	Gender-Specific Effects of Prenatal Betamethesone (Beta) Exposure and Unilateral Nephrectomy on the Intrarenal Renin-Angiotensin System (RAS) in Adult Sheep
Author String	Y Su, J Bi, JP Figueroa, JC Rose Center of Research for Obstetrics & Gynecology, Winston-Salem, NC
Body	**Objective**: Antenatal Beta is used clinically to accelerate fetal lung maturity, but prenatal exposure to elevated levels of glucocorticoids reduces nephron number in animals Reductions in nephron number in early life increase the risk for impaired renal function following a second insult to the kidney. The impaired renal function may be associated with increased angiotensin II (Ang II) actions on the kidney. Therefore we wished to determine if antenatal glucocorticoid exposure in combination with a second insult (unilateral nephrectomy would alter expression of components (Ang II type 1[AT1R], Ang II type 2[AT2R] receptors and renin) of the intrarenal RAS. **Methods**: Pregnant sheep were randomly treated with Beta [2 maternal IM doses (0.17 mg/kg with a maximum of 12 mg) or vehicle 24-h apart] at 80 and 81 d of gestation. The pregnant animals delivered at term and the offspring underwent unilateral nephrectomy at 1-1.5 yr of age. Two to 3 weeks later the second kidney was obtained Kidney cortex was dissected and immediately frozen in liquid nitrogen. AT1R, AT2R and renin proteins were measured by Western blot. Statistical analysis was conducted using two- way ANOVA. **Results**: Beta treatment increased ($F=18.06$, $P<0.0007$) AT1 R expression in males, there was an effect of nephrectomy ($F=7.6$, $P<0.01$), and a Beta, nephrectomy interaction ($F=4.58$, $P<0.05$). Beta treatment ($F=17.29$, $P<0.001$), or nephrectomy ($F=69.65$, $P<0.0001$) decreased AT2 R expression and there was an interaction ($F=5.22$, $P<0.05$) in males. Beta increased renin expression ($F=86.57$, $P<0.0001$). There was an effect of nephrectomy ($F=48.05$, $P<0.001$) and an interaction ($F=24.87$, $P<0.0001$) in males. In contrast, while Beta increased AT1-R ($F=23.8$, $P=0.0002$) expression in females there was no effect of nephrectomy and no effects on AT2 expression were found. Beta increased ($F=13.1$, $P<0.004$) and nephrectomy decreased ($F=9.6$, $P<0.01$) renin expression in females. **Conclusion**: Prenatal Beta and reduction in nephron number have gender specific effects on the expression of Ang receptors and renin in the kidney which may contribute to male- female differences in loss of renal function in adults. Sources of Research Support: NIH grants HD 17644 and HD 47584. Nothing to Disclose: YS, JB, JPF, JCR

Pub #	P1-589
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	Pheochromocytoma and Paraganglioma in Children: Report of Three Cases
Author String	MCP Batista, P Godoy, C Capanema, E Zingler, AEdL Jaculi, BTdA Santos, MF Azevedo, C Stratakis, AA Amato, GB Barra, A Lofrano-Porto University Hospital of Brasilia (HUB/UnB), Brasilia, Brazil; Sabin Institute and Laboratory, Brasilia, Brazil; University of Brasilia (UnB), Brasilia, Brazil; NICHD, National Institutes of Health (NIH), Bethesda, MD
Body	Pheochromocytomas and paragangliomas (PHEO/PGL) are neuroendocrine tumors that arise from neural crest-derived cells. Although uncommon in children, in this age group they are more likely to be multicentric, malignant or familial. Among apparently sporadic cases, up to 25% of patients harbor germline mutations in genes associated with familial syndromes, and genetic testing is considered appropriate for any child with these tumors. We report three cases of PHEO/PGL in children. Patient 1 was a 15-year-old boy who presentec with chronic headache, nocturnal sweating, severe hypertension and progressive weight loss. Urinary dopamine excretion was increased, and CT scan showed two retroperitoneal tumors (6.3 and 5.0 cm), which overlapped with focal areas of increased ^{123}I-MIBG uptake. Patient 2 was a 12-year-old boy who presented at the Emergency Unit with acute abdominal pain. He was normotensive and had a past history of nocturnal sweating since infancy. Malar and hand erythema were noted. CT scan showed a left paraortic tumor (4.3 cm) Plasma norepinephrine levels were greatly increased. ^{123}I-MIBG scan failed to show adrenergic secreting lesions. Both patients were referred to surgical treatment and immunohistochemical analysis indicated PGL. Sequencing analysis of the coding regions of *SDHB* revealed a homozygous synonymous polymorphism in exon 1 (169C>A, rs2746462), and no mutations were found in *SDHD*, *SDHC* and *VHL* genes. After primary treatment, neither patient has shown signs of recurrence. Genetic analysis for large insertions/deletions in *SDH* genes is ongoing. Patient 3 was a 12-year-old girl who presented with paroxysmal episodes of tachycardia and diaphoresis, severe hypertension, syncope and weight loss. Urinary excretion of norepinephrine and dopamine were greatly increased. CT scan of the abdomen revealed a right adrenal tumor (5.2 cm). The patient was referred to surgical treatment, and immunohistochemical and genetic studies are pending. PHEO/PGL are rarely diagnosed in childhood, but are important clinical entities given their greater potential of malignancy as compared to adults. The identification of mutations may influence follow-up of these clinically and genetically heterogeneous neoplasms. Sources of Research Support: FAPDF - Fundacao de Amparo [agrave] Pesquisa do Distrito Deferal; FINATEC - Fundacao de Empreendimentos Científicos e Tecnológicos. Nothing to Disclose: MCPB, PG, CC, EZ, AEdLJ, BTdAS, MFA, CS, AAA, GBB, AL-P

Pub #	P1-590
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	Germline VHL Mutation Q164R Associated with Pheochromocytoma in a Woman with Family History of Severe Hypertension, Adrenal and Pancreatic Tumors
Author String	MCP Batista, P Godoy, AJR Queiroz, BF Luitgards, J Mangussi-Gomes, BTA Santos, AE Jaculi, E Zingler, C Capanema, GB Barra, A Lofrano-Porto University Hospital of Brasilia (HUB/UnB), Brasilia, Brazil; Sabin Institute and Laboratory, Brasilia, Brazil; University of Brasilia (UnB), Brasilia, Brazil
Body	Pheochromocytomas (PHEOs) are rare, catecholamine-secreting tumors arising from adrenal chromaffin cells Von Hippel-Lindau (VHL) disease is characterized by an inherited predisposition for neoplasm development, caused by germline mutations in VHL tumor-supressor gene. VHL-related tumors include retinal and central nervous system hemangioblastomas, renal clear-cell carcinomas, PHEOs, non-secretory pancreatic and endolymphatic sac tumors, and others. Numerous VHL mutations have been described to date and neoplastic transformation occurs when both alleles are inactive. We report a case of a pheochromocytoma in a woman with suspected family history of VHL disease. A 37 year-old woman presented to the Endocrine Unit because of paroxisms of severe hypertension associated with headaches, diaphoresis and, ocasionally, fainting, started one year before. She has had two gestations, 8 and 5 years before, when severe hypertension and eclampsia developed. During her first caesarean section, she also had acute pulmonary edema and a cardiorespiratory arrest that resolved through conventional resuscitation maneuvers. Plasma norepinephrine levels were strongly elevated (10.783 pg/mL; normal < 1700) and CT scan revealed a right adrenal tumor (6.0 cm), showing increased 123I-MIBG uptake. After conventional adrenalectomy, a PHEO was confirmed by immunohistochemical study, and close follow-up has shown no signs of recurrence or evidence of new tumors. Family history was noteworthy for 1 sister that died just after delivery, following a severe hypertensive crisis, and for the 26-year-old niece who presented with a 3 cm left adrenal tumor, an 8 cm pancreatic tumor and disseminated hepatic metastasis, evolving to death within a few months. Immunohistochemical examination of her percutaneous liver biopsy was compatible with a moderately differentiated neuroendocrine carcinoma of uncertain origin. Sequencing analysis of the coding regions of VHL revealed a heterozygous missense mutation in exon 3 (c.491A>G; Q164R) in the index case and her still assymptomatic 5 year-old daughter. Molecular diagnosis was not performed in the affected sister and niece. To our knowledge, the Q164R germline VHL mutation has been described in only 3 cases to date, with variable phenotypes; in 2 cases PHEO occurred at a very young age. The identification of VHL-mutation carriers is essential to provide early diagnosis, treatment, and regular clinical surveillance. Sovinz P, Urban C, Uhrig S, Stepan V, Lackner H, Schwinger W, Benesch M, Moser A, Spuller E, Speicher MR. 2010. Pheochromocytoma in a 2.75-year-old-girl with a germline von Hippel-Lindau mutation Q164R. Am J Med Genet Part A 152A:1752-1755. Sources of Research Support: FAP-DF (Funda[ccedil]ão de Amparo [agrave] Pesquisa do DF), FINATEC, SABIN Institute, Brazil. Nothing to Disclose: MCPB, PG, AJRQ, BFL, JM-G, BTAS, AEJ, EZ, CC, GBB, AL-P

Pub #	P1-591
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	First Description of a Cystic CNS Metastasis in a Patient with Malignant Pheochromocytoma
Author String	DM Schulte, M Faust, M Schmidt, M Ruge, S Grau, M Kocher, T Blau, I Gouni-Berthold, W Krone, M Laudes University of Cologne, Cologne, Germany; University of Cologne, Cologne, Germany; University of Cologne, Cologne, Germany; University of Cologne, Cologne, Germany; University of Cologne, Cologne, Germany; University of Cologne, Cologne, Germany
Body	Approximately 10% of pheochromocytomas (PCC) display a malignant character with high variability in location of metastases and clinical presentation. To our knowledge, cystic CNS metastases have not been reported so far. We present a 73-year old male patient of Caucasian descent who had been diagnosed in 1992 with PCC and was adrenalectomized on the right. In 2002 he complained about dyspnoea and reduced physical fitness. CT scan and I-123-mIBG scintigraphy revealed a local relapse of the PCC and multiple metastases in the lungs and mediastinum. In 2002, 2008 and 2009 three I-131-mIBG therapies achieved a stable disease. During that time, the patient stayed in good physical health. In May 2010 he complained about loss of cognitive function, a homonymous hemianopsia and left sided dysmetria. CNS-MRI displayed a 4x5 cm cystic tumour in the right parietal lobe. I-131-mIBG scintigraphy revealed a doughnut-pattern uptake in the CNS lesion. Since the other metastases in lungs and mediastinum continued to display a stable disease, a combined stereotactic and micro-neurosurgical approach was considered. For the treatment of this cystic metastasis open surgery was performed via a parietal craniotomy. The wall of the cyst was thin but highly vascularised and showed a clear demarcation to surrounding cerebral tissue. The tumour was removed completely. Morphologically and immunohistochemically a PCC was diagnosed. The patient received adjuvant whole brain radiotherapy with 40 Gy at 2Gy fractions. The follow-up MRI showed residual enhancement of scar tissue right parietal. Clinically the patient is without neurological deficite. In summary, these findings demonstrate that CNS metastases in malignant PCC can occur despite recurrent I-131-mIBG therapies. Therefore, in such case a primary surgical therapy should be considered. Nothing to Disclose: DMS, MF, MS, MR, SG, MK, TB, IG-B, WK, ML

Pub #	P1-592
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	Prolonged Suppression of Renin-Angiotensin-Aldosterone System (RAS) after Adrenalectomy for Aldosterone-Producing Adrenal Adenoma (APA)
Author String	O-PR Hamnvik, RG Dluhy Brigham and Women's Hospital and Harvard Medical School, Boston, MA
Body	**Introduction:** Resection is the preferred treatment of aldosterone-producing adenomas (APA). We report a case of prolonged suppression of the RAS after adrenalectomy for APA. **Clinical Case:** A 60-year-old woman had a 30-year history of uncontrolled hypertension (120-160/80-100 mmHg) on hydrochlorothiazide, benazepril and atenolol (doses unknown). Evaluation of an episode of hypertensive urgency revealed hypokalemia of 3.0 mmol/L, and creatinine of 1.6 mg/dL. Her plasma aldosterone level was elevated at 120 ng/dL (normal: [le]21 on normal salt diet) with a suppressed plasma renin activity of <0.6 ng/mL/hr (normal: <0.6-3.0 on normal salt diet). MRI found a 3.1 cm left adrenal lesion with signal loss in the out-of-phase sequences, consistent with a benign lipid-rich cortical adenoma. The contralateral gland appeared normal. The mass was resected and confirmed histologically to be a 3.6 cm adrenal adenoma. She was discharged on metoprolol 50 mg BID. Post-operative renin and aldosterone were Four months after surgery, she still has undetectable renin and aldosterone levels and is requiring low-dose fludrocortisone and supplemental sodium. **Clinical Lessons:** Resection of APA in some patients can be associated with prolonged suppression of the RAS and, as a consequence, of aldosterone production from the contralateral adrenal gland. Risk factors probably include duration and severity of the hyperaldosteronism. Hypoaldosteronism following resection of APA is analogous to the slow recovery of cortisol production after prolonged high-dose glucocorticoid treatment. Clinically, hypoaldosteronism maybe be associated with sodium wasting, orthostasis, hyponatremia and hyperkalemia. Prevention of post-operative hypoaldosteronism can be accomplished by pre-operative treatment with a mineralcorticoid-receptor antagonist. Treatment over weeks/months should activate the suppressed RAS and stimulate aldosterone production from the atrophic contralateral zona glomerulosa. Nothing to Disclose: O-PRH, RGD

Pub #	P1-593
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	Idiopathic Hyperaldosteronism Coexisting with Glucocorticoid and Mineralocorticoid Excess from an Adrenal Adenoma
Author String	HK Ghayee, AS Heyliger, S Woodruff, FE Nwariaku, RJ Auchus UT Southwestern Medical Center, Dallas, TX; UT Southwestern Medical Center, Dallas, TX
Body	Background: Mineralocorticoid excess is characterized clinically by resistant hypertension and hypokalemia, most commonly due to primary aldosteronism. Excessive secretion of other mineralocorticoids, 17-deoxysteroids formed in the zona fasciculata including 11-deoxycorticosterone (DOC) and corticosterone, car lead to similar clinical manifestations. Case: A 71-year old man presented with a history of uncontrolled hypertension and hypokalemia. He had a history of a right adrenal nodule found incidentally on CT scan seven years prior after a motor vehicle accident. The patient also had complaints of skin thinning and easy bruising. The patient was taking six agents for his blood pressure as well as 20 mEq daily of KCl daily. During his workup of his hypertension and hypokalemia, the serum aldosterone was 19 ng/dL with a suppressed renin of <0.4 ng/mL/hr. Repeat abdominal CT scan showed growth of the right adrenal nodule from 4.3 to 4.7 x 3.8 cm. The early morning plasma ACTH was 7.2 pg/mL with a serum cortisol of 13.8 [micro]g/dL and testosterone of 50 ng/dL; the urinary free cortisol was normal. The patient had a 1 mg overnight dexamethasone suppression test in which the serum cortisol fell only to 3.0 mcg/dL, and the serum 11-deoxycortisol and DOC also did not suppress at 46 ng/dL and 6 ng/dL, respectively. Due to the enlarging mass and evidence of autonomous cortisol and cortisol precursor production, the patient underwent a right adrenalectomy. An adrenal adenoma without evidence of malignancy was confirmed histopathologically. One month post operatively, the patient had an aldosterone of 14 ng/dL and suppressed renin. The patient became normotensive 1 month after the procedure but required potassium supplements. Protein and RNA from the tumor tissue were analyzed for steroidogenic enzyme expression. Discussion: This is a case of a glucocorticoid and mineralocorticoid excess from an enlarging adrenal tumor i a patient with primary aldosteronism. Postoperatively, blood pressure control improved, yet the aldosterone/renin ratio remained abnormal with continued need for potassium supplementation. This patient appears to have idiopathic (bilateral) hyperaldosteronism with an adrenal adenoma producing primarily cortisol and precursors with mineralocorticoid activity, contributing to his hypertension. This case underscore the difficulties in evaluating the hormone production of adrenal tumors without comprehensive steroid profiling. Sources of Research Support: Clinical Scientist Award in Translational Research from the Burroughs-Wellcome Fund (to RJA). Nothing to Disclose: HKG, ASH, SW, FEN, RJA

Pub #	P1-594
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Aldosterone, Catecholamines & Cardiovascular Endocrinology (1:30 PM-3:30 PM)
Title	An Unusual Case of Hereditary Pheochromocytoma
Author String	D Gupta, G Lastra-Gonzalez University of Missouri-Columbia, Columbia, MO
Body	Background: Hereditary pheochromocytomas are characterized by presentation at an early age, bilateral and/or recurrent disease and can affect multiple family members. Clinical case: A 29-year-old Caucasian male was diagnosed with right adrenal pheochromocytoma at the age of 11 years during workup for severe hypertension. At that time, he had a right adrenal mass extending into the right kidney and he underwent right adrenalectomy and right nephrectomy followed by radiation therapy. He was symptom free for many years but at the age of 28, presented to his primary care physician with a 2-3 year history of episodes of headaches, palpitations, sweating, pre-syncope and hypertension. As labs were suggestive of recurrent pheochromocytoma, he was referred to Endocrinology for further evaluation. Questioning about family history revealed that his paternal grandfather and father had pheochromocytoma; paternal aunt also had this possible diagnosis. His father was positive for VHL mutation and required three separate surgeries for pheochromocytoma, the first one at age nine. Lab tests were positive for predominantly norepinephrine producing pheochromocytoma - 24-hour urine metanephrines 66 mcmol/mol (normal 0-172), normetanephrines 654 mcmol/mol (0-247), norepinephrine 183 ug/g crt (0-45), plasma metanephrines 0.27 nmol/L (0.00-0.49), normetanephrines 4.52 nmol/L (0.00-0.89). Localization study with CT abdomen and pelvis showed the presence of a left adrenal mass and multiple left renal hyperdense nodules. MIBG scan showed a small focus of increased uptake in the posterior midabdomen just left to the midline corresponding with left adrenal mass. Genetic screening for VHL, RET, SDHB, SDHD was ordered and results are pending. The patient was started on phenoxybenzamine which improved blood pressure control. He successfully underwent laparoscopic left adrenalectomy and was started on glucocorticoid and mineralocorticoid replacement post-operatively. Conclusion: Approximately 15% to 20% of patients with catecholamine-secreting tumors have germline mutations presenting as hereditary pheochromocytomas. Associated syndromes include Multiple Endocrine Neoplasia 1 and 2, von Hippel-Lindau disease, Neurofibromatosis-1 and Familial Paragangliomas. Clinical characteristics like early age of onset (less than 20 years), bilateral or recurrent disease, presence of paraganglioma and/or involvement of multiple family members should prompt genetic screening. Nothing to Disclose: DG, GL-G

Pub #	P1-595
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Developmental Programming: Prenatal Testosterone and Postnatal Obesity Induce Free Fatty Acid Imbalance in Sheep
Author String	A Veiga-Lopez, CF Burant, V Padmanabhan University of Michigan, Ann Arbor, MI; University of Michigan, Ann Arbor, MI; University of Michigan, Ann Arbor, MI
Body	Prenatal testosterone (T)-treated sheep develop similar reproductive and metabolic traits as those seen in women with polycystic ovary syndrome (PCOS) including insulin resistance. Obesity increases the risk of anovulation in women with PCOS and prenatal T-treated sheep. Both prenatal T excess and postnatal obesity induce insulin resistance in sheep. Because free fatty acids (FFA) flux from adipose to non-adipose tissue is implicated in metabolic derangements such as insulin resistance, we determined whether prenatal T treatment causes FFA imbalance with postnatal obesity amplifying it. Pregnant Suffolk sheep were given T propionate (100 mg, i.m. twice weekly) in cottonseed oil from days 30-90 of gestation (term: ~147 d). Control (C) and T females were randomly assigned to either a non-obese (C: n=5; T: n=7) or obese (OB) (COB: n=6; TOB: n=6 groups. Beginning ~14 weeks of age, the OB groups were provided additional ration to increase body weight to 25% above that of C. At 20 months of age, during the follicular phase of a synchronized cycle, blood sample was collected and plasma frozen for determination of FFA index. FFA were quantified by gas chromatography/mass spectrometry. General linear model found a trend for a prenatal T ($P=0.076$) and obesity effects ($P= 0.059$) in the total amount of FFA. Palmitic [16:0], stearic [18:0], oleic [18:1] and, linoleic [18:2] acids were the most highly represented FFA. Prenatal T excess increased the amount of palmitic [16:0] and palmitoleic [16:1] acids ($P<0.05$). Postnatal obesity increased the amount of stearic [18:0] and oleic [18:1 acids ($P<0.05$). In contrast, the concentration of the essential FFA α-linolenic [18:3], and both long chain FFA eicosadienoic [20:2] and arachidonic [20:4] acids were lowered by postnatal obesity ($P<0.05$, $P=0.07$, $P=0.05$ respectively), but not prenatal T treatment. There was no interaction between prenatal and postnatal interventions. In summary, excess prenatal T and postnatal obesity independently impacted FFA metabolism. Considering that FFA have significant influence on both lipid and glucose metabolism, such disruptions may underlie the metabolic perturbations seen in prenatal T females and the exacerbation of the cycle defects by postnatal obesity. Importantly, a FFA imbalance has been suggested (1) as a possible cause in the development of hyperandrogenemia in PCOS women, the reproductive/metabolic attributes of whom the prenatal T-treated females mimic. (1) Mai et al., 2008 JCEM; 93:3900 Sources of Research Support: NIH grant P01 HD44232 awarded to VP. Nothing to Disclose: AV-L, CFB, VP

Pub #	P1-596
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	RNAi Repression of CYP2B Indicates That This Subfamily of P450s Is Involved in Fat Metabolism
Author String	B Damiri, WS Baldwin Clemson University, Clemson, SC
Body	There are few in vivo knockout models available to study the function of the Cyp2 members of the P450 superfamily. Cyp2bs are thought be involved in the metabolism of drugs, environmental toxicants, and endobiotics such as steroid hormones. We produced a Cyp2b-knockdown (KD) mouse for subsequent study o Cyp2b function. There are multiple Cyp2b members in mice (only one, CYP2B6 in humans), therefore we made a quintuple Cyp2b-KD mouse using lentiviral-promoted shRNA homologous to all five Cyp2b subfamily members (Cyp2b10, 2b9, 2b13, 2b19, and 2b23). The Cyp2b-KD mice are viable and fertile. Expression of the three hepatic Cyp2b members, 2b9, 2b10, and 2b13, is significantly repressed as demonstrated by QPCR and Western blotting. Furthermore, the CAR activator and potent Cyp2b inducer was unable to outcompete the shRNA-mediated repression of Cyp2b as Cyp2b induction was 6-11X lower in Cyp2b-KD individuals than WT mice; despite increased CAR expression in TC-treated mice. Cyp2b-KD mic show an increase in liver weight probably due to compensatory mechanisms. In addition, we observed increased abdominal and renal fat in individuals younger than 10-weeks old. This is associated with higher serum cholesterol, HDL, and LDL concentrations. Serum triglycerides are increased in Cyp2b-KD mice treated with TCPOBOP relative to WT mice treated with TCPOBOP. Interestingly, using corn oil as a carrier has some side effects as the mice show induction of CYPs relative to untreated Cyp2b-KD mice and WT corn oil treated mice, indicating perturbations in the normal response to unsaturated fatty acids. A study investigating older mice is currently underway. In summary, we have developed a Cyp2b-knockdown mouse using RNAi and evidence based on this mouse model suggests that Cyp2b isoforms have an endogenous functions that include dietary lipid metabolism. Sources of Research Support: NIH grant ES017321 awarded to WSB. Nothing to Disclose: BD, WSB

Pub #	P1-597
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	ApoE-Deficient Mouse Model Exhibits Significantly Enhanced Mammary Tumor Growth and Pulmonary Metastases
Author String	N Alikhani, R Novosyadlyy, R Ferguson, S Yakar, D LeRoith Endocrinology, New York, NY
Body	Diabetes and obesity have been associated with increased risk for developing cancer, however, the implicated mechanism remains uncovered. To begin to understand how dyslipidemia may affect breast cancer growth and metastases, we used the apolipoprotein E (ApoE) knockout mice ($ApoE^{-/-}$), which show elevated cholesterol levels in plasma when fed with a diet rich in cholesterol. We used two different cancer cell lines; Met-1 (derived from MMTV-PyVmT/FVB-N transgenic mice) and the metastatic Mvt-1 (cells derived from c-myc/vegf tumor explants). Tumor cells were injected into the mammary fat pad of $ApoE^{-/-}$ and wt females and tumor growth was assessed weekly. We found that $ApoE^{-/-}$ mice exhibited increased tumor growth (1.7g vs 0.4g in control mice) five weeks following cell injection. Additionally, the $ApoE^{-/-}$ mice displayed a higher level of spontaneous metastasis to the lungs (11 mets/per mouse vs 5 mets/mouse in controls). Further, retro-orbital injection of Mvt-1 cells led to a greater level of pulmonary metastases in the lungs of ApoE deficient mice (9 mets/per mouse vs 3 mets/mouse in controls). To unravel the molecular mechanism involved in enhanced tumor growth in the $ApoE^{-/-}$ mice we studied the response of Mvt-1 cells to cholesterol, *in vitro*. We found that cholesterol promoted cellular proliferation of Mvt-1 cells and activation of Akt, which could be inhibited by LY-294002 compound. Collectively we suggest that the hypercholestrolemic environment in the $ApoE^{-/-}$ mice is a favorable setting for tumor cell proliferation and metastasis to the lungs and is likely involved in activation of the AKT pathway. Nothing to Disclose: NA, RN, RF, SY, DL

Pub #	P1-598
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Beta-Adrenergic Receptors (β-ARs) and Lipotoxic Effects in Liver and Heart
Author String	Z-J Shu, C-K Yeh, A Kamat University of Texas Health Science Center at San Antonio, San Antonio, TX; University of Texas Health Science Center at San Antonio, San Antonio, TX; University of Texas Health Science Center in San Antonio, San Antonio, TX; Audie L Murphy Veterans Administration, San Antonio, TX
Body	Obesity, age and various stresses increase hepatic fat accumulation (steatosis) and also enhance the release of catecholamines. Numerous biological responses in tissues are modulated by catecholamines acting via beta-adrenergic receptors (β-ARs). We have previously demonstrated that augmentation of β-AR signaling in young mouse hepatocytes that have low levels of endogenous β-ARs increases lipid accumulation while inhibition of β-AR signaling in old rodent hepatocytes reduces fat accumulation. In recent years, increasing clinical evidence suggests a strong association between fatty liver and cardiovascular diseases (CVD). Our long term goal is to determine whether β-ARs play a role in linking fatty liver and lipotoxic effects in the heart. In the present studies we observed that intraperitoneal injection of β-AR agonist isoproterenol (20 [mu] g/g) in young mice induced a 3-fold increase in hepatic fat accumulation. Pharmacological activation of β-AR *in vivo* also increased hepatic expression of lipogenic factor sterol regulatory element binding protein (SREBP)-1 and inflammatory marker C-reactive protein while decreasing expression of peroxisome proliferator-activated receptor alpha (PPARα), a modulator of fatty acid oxidation. These results suggest that both increased lipid synthesis and reduced fatty acid oxidation may play a role in increasing hepatic fat accumulation upon β-AR activation. Interestingly, β_2-AR knockout mouse livers show an increase in PPARα protein levels and a reduction in mRNA levels of lipin-1, a key enzyme in triglyceride synthesis. In further studies we treated 24 month old mice with β-AR antagonist propranolol (0.5g/L drinking water) for 7 weeks. Echocardiogram results of propranolol-treated mice indicated improved left ventricle performance as demonstrated by a number of measurements including increased percent fractional shortening compared to control mice. Expression levels of cytokine IL-6 levels were reduced while no change was observed in PPARα and SREBP-1 mRNA levels in the hearts of propranolol-treated animals compared with controls.Taken together, our studies demonstrate that increased β-AR activation augments hepatic fat accumulation while β-AR inhibition has beneficial effects in the heart. We are currently analyzing the effects of *in vivo* propranolol treatment on hepatic fat accumulation. These studies will lay the foundation for future investigations to study the pathogenetic mechanisms linking fatty liver and CVD. Sources of Research Support: Grant in Aid Award from American Heart Association (AK). Nothing to Disclose: Z-JS, C-KY, AK

Pub #	P1-599
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Up-Regulation of Apolipoprotein A-I Gene Expression in Vitamin D Receptor Knockout Mice
Author String	M Gladysz, E Naem, R Alcalde, S Mesliniene, KR Wehmeier, AD Mooradian, MJ Haas University of Florida-Jacksonville College of Medicine, Jacksonville, FL
Body	Apolipoprotein A-I (apo A-I) is the primary protein component of the anti-atherogenic cholesterol transporter high-density lipoprotein (HDL). Though most epidemiological studies suggest that vitamin D levels are positively associated with plasma HDL levels, it is unclear how vitamin D regulates cholesterol metabolism and reverse-cholesterol transport (RCT). Vitamin D was shown to inhibit apo A-I gene expression in HepG2 cells in vitro. To determine if the vitamin D receptor (VDR) regulates apo A-I gene expression and HDL synthesis in vivo, apo A-I protein levels were measured in two separate lines of VDR knockout mice. In both cases apo A-I protein levels were high in the homozygous knockout animals than in the wild type. Albumin levels were similar for knockout and wild type mice. Likewise, apo A-I mRNA levels were elevated in livers of VDR knockout mice. To determine if other hepatic genes involved in HDL synthesis and RCT are also regulated by the VDR, we measured ATP-binding cassette protein A1 (ABCA1) and scavenger receptor B type 1 (SR-B1) mRNA levels, while albumin and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) mRNA levels were measured as controls. In homozygous VDR knockout mice, apo A-I and SR-B1 mRNA levels were significantly elevated relative to WT mice, while GAPDH, albumin and ABCA1 mRNA levels were similar in each group. These results suggest that the VDR, either directly or indirectly, regulates expression of several genes involved in hepatic HDL synthesis and RCT. Sources of Research Support: Dean's Fund Research Grant from the University of Florida-Jacksonville. Nothing to Disclose: MG, EN, RA, SM, KRW, ADM, MJH

Pub #	P1-600
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Reduced Peak Growth Hormone on Growth Hormone Stimulation Testing Is Independently Associated with an Atherogenic Lipoprotein Particle Size in Obesity
Author String	H Makimura, N Sun, MN Feldpausch, SK Grinspoon Massachusetts General Hospital and Harvard Medical School, Boston, MA
Body	Reduced peak stimulated GH secretion in obesity is associated with increased CVD risk. Lipoprotein particle size, more specifically increased levels of small dense LDL particles, is a known independent risk factor of CVD risk. To assess if reduced peak stimulated GH is associated with increased levels of small dense LDL cholesterol particles, we studied 102 normal weight and obese, men and women without known hypopituitarism (33 normal weight [age: 40.4±1.9 y; BMI: 22.5±0.3 kg/m^2] and 69 obese [age: 42.1±1.1 y; BMI: 38.1±0.8 kg/m^2]). Subjects underwent GH stimulation testing with GHRH-arginine. Lipoprotein concentrations and subspecies distribution was assessed by proton NMR spectroscopy. Univariate analyses demonstrated peak stimulated GH was positively associated with LDL (r=0.50; P<0.0001) and HDL (r=0.57; P<0.0001) but not VLDL (r=-0.19; P=0.06) particle size. Upon examination of lipoprotein subspecies, peak stimulated GH was negatively associated with small LDL (r=-0.48; P<0.0001), medium small LDL (r=-0.50; P<0.0001) and very small LDL (r=-0.47; P<0.0001) particle concentrations and positively associated with large LDL particle concentrations (r=0.27; P=0.007). Peak stimulated GH was negatively associated with small HDL (r=-0.31; P=0.002) and positively associated with large HDL (r=0.49; P<0.0001) but not with medium HDL particle concentrations. Peak stimulated GH was negatively associated with large VLDL/chylomicron (r=-0.30; P=0.003), medium VLDL (r=-0.20; P=0.05) and small VLDL (r=-0.21; P=0.04) particle concentrations. Multivariate regression analysis controlling for age, gender, race, ethnicity, tobacco, lipid lowering medication, BMI and HOMA demonstrated peak stimulated GH remained significantly associated with LDL (β=0.01; P=0.01; R^2=0.42; P<0.0001 for overall model) and HDL particle size (β=0.008 P=0.001; R^2=0.44; P<0.0001 for overall model). However, HOMA, not GH, was the predominant independent variable associated with VLDL particle size (β=1.3; P=0.009; R^2=0.17; P=0.04 for overall model). We demonstrate reduced peak stimulated GH in obesity is associated with smaller mean LDL and HDL particle size such that reduced peak stimulated GH is associated with increased concentrations of very small LDL, medium small LDL and small LDL as well as lower levels of large LDL particles. These results suggest for the first time that reduced peak stimulated GH, is independently associated with a more atherogenic lipoprotein profile in obesity. Sources of Research Support: NIH K23DK087857 (to HM); NIH R01HL085268 (to SKG); NIH K24DK064545 (to SKG). Nothing to Disclose: HM, NS, MNF, SKG

Pub #	P1-601
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Acute Sex Steroid Withdrawal Increases HDL-Associated Clusterin in Young, Healthy Men
Author String	KB Rubinow, AN Hoofnagle, CN Snyder, JK Amory, JW Heinecke, ST Page University of Washington, Seattle, WA

Body

Introduction: There is an inverse relationship between high-density lipoprotein cholesterol (HDL-C) and the risk of cardiovascular disease (CVD) and mortality. Since exogenous testosterone (T) can decrease HDL-C, it has been speculated that the HDL-lowering effects of T contribute to CVD risk in men. However, recent epidemiologic data demonstrate elevated risk of dyslipidemia, insulin resistance, and CVD among hypogonadal men. Emergent data suggest heterogeneity in HDL-associated proteins may modulate its atheroprotective effects to a greater extent than absolute HDL-C quantity.(1) Further, HDL-associated clusterin has been associated with insulin resistance and dyslipidemia.(2) Accordingly, we studied the effects of short-term sex steroid withdrawal in men on the HDL proteome.

Methods: We enrolled 8 healthy men, ages 18-55, with normal baseline T levels. All subjects received the GnRH antagonist acyline (300 mcg/kg/2 weeks x 2) to confer medical castration for 28 days. Subjects were followed for 4 weeks after acyline discontinuation. At baseline, D28, and D56, sex steroids were quantified using liquid-chromatography-tandem mass spectrometry, fasting lipids were measured, and the HDL proteome was determined using mass spectrometry.

Results: Subjects were rendered medically castrate (D28 T=0.8±0.8 nmol/L) with acyline treatment. Serum estradiol (E_2) also decreased substantially (D28 E_2=31±11 pmol/L). HDL increased though not significantly during sex steroid withdrawal (p=0.2). Forty-six proteins were identified in the HDL proteome. Both HDL-clusterin and HDL-vitronectin increased with sex steroid withdrawal (p=0.002, p=0.036, respectively, for D0 v. D28). HDL-clusterin remained elevated (D0 v. D56, p=0.03), whereas vitronectin normalized after sex steroid re-exposure.

Conclusion: Acute sex steroid withdrawal in young, healthy men elevates HDL-associated clusterin and vitronectin, suggesting that sex steroids may modulate HDL protein composition. Moreover, the sustained increase in clusterin indicates that transient changes in sex steroids might confer more enduring effects on HDL composition. Whether androgens or E_2 mediates the observed effects on HDL protein composition remains unclear. Notably, low levels of HDL clusterin have been associated with obesity, insulin resistance, and dyslipidemia. Further investigation of the effects of sex steroids on HDL composition and function may help resolve the apparently conflicting data regarding T, HDL, and CVD risk.

(1) Vaisar T et al., J Clin Invest 2007; 117(3):746-56
(2) Hoofnagle AN et al., Arterioscler Thromb Vasc Biol 2010; 30(12):2528-34

Nothing to Disclose: KBR, ANH, CNS, JKA, JWH, STP

Pub #	P1-602
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Circulating Clusterin/Apolipoprotein J Does Not Display Any Day/Night Variability Pattern and Is Positively Associated with Total and LDL-C Cholesterol in Healthy Young Males
Author String	KN Aronis, MT Vamvini, JP Chamberland, CS Mantzoros Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; Harvard School of Public Health, Boston, MA; Boston VA Healthcare System, Boston, MA

Body

Introduction: Clusterin / Apolipoprotein J is a molecule considered to have anti-inflammatory actions, through inhibition of complement lysis activity and is associated with reverse cholesterol transfer. The physiology of clusterin has not been studied in detail in humans. Little is known about any potential association(s) between clusterin and metabolic syndrome and any associations between clusterin and metabolic or anthropometric parameters in have never been studied in healthy humans. The purpose of this study is to examine whether circulating clusterin levels display a day night variation pattern and to study whether clusterin is associated with anthropometric and metabolic parameters in young healthy individuals.
Methods: Study A: Serum samples initially collected every 15 minutes and subsequently pooled every hour, from 6 healthy male individuals (22.3±3.1 years old) studied in the fed state, were used to evaluate the existence of any day/night variation pattern in circulating clusterin levels. Study B.: Data and samples from 186 apparently healthy males (18.4 ± 0.14 years old) were used to evaluate cross-sectional associations between clusterin levels and anthropometric, metabolic and cardiovascular parameters. 91 of these subjects were studied again 2 years later and clusterin's association with the change of metabolic parameters was then investigated prospectively. Circulating clusterin levels were measured using a commercially available ELISA.
Results: A. Both spectral domain analysis and cosinor non-linear OLS regression analysis, failed to reveal any consistent pattern of day/night variation. B. Spearman's correlation analysis revealed a significantly positive correlation with total and LDL cholesterol ([rho]=0.23 p=0.002 and [rho]=0.20 p=0.005) and a negative association with neutrophil count ([rho]=-0.18 p=0.015). Baseline Clusterin did not predict any other anthropometric, biochemical or metabolic parameters.
Conclusions: We report for the first time that circulating clusterin does not have a day night variation pattern and is associated with total and LDL cholesterol crossectionally, but not prospectively, in healthy adult males. Clusterin is not associated with any other anthropometric, metabolic and cardiovascular parameter.

Sources of Research Support: Grant Number M01-RR-01032-328840 to the Harvard Clinical and Translational Science Center, from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health. Funding was also received from the National Institute of Diabetes and Digestive and Kidney Diseases (grants DK058785, DK079929 and DK081913), and the National Institute on Aging (grant AG032030).

Nothing to Disclose: KNA, MTV, JPC, CSM

Pub #	P1-603
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	High HDL-Cholesterol Is Common in Older Adults with Type 1 Diabetes (T1DM)
Author String	T Alessa, A Szeto, A Mendez, R Goldberg Diabetes Research Institute, University of Miami, Miami, FL
Body	Although HDL-cholesterol (HDL-C) values are usually stated to be normal in T1DM, there have been reports of elevated levels in some studies, though this has not been consistently noted. Since HDL-C is an important negative risk factor for cardiovascular disease (CVD), the issue of whether HDL-C may be elevated or not in T1DM has an obvious clinical significance. In order to examine this question we assessed the prevalence and clinical features of high HDL-C (hHDL-C) in a large bi-ethnic group of subjects with T1DM (men/women 83/111, Hispanic[H]/non-Hispanic White[W] 85/109). Mean ($\pm$SEM) age was 40$\pm$1.1 years, HbA1c 7.9$\pm$0.1%, duration of diabetes 21.5$\pm$1.0 years and BMI 26.1$\pm$0.4, Cutpoints for hHDL-C were arbitrarily set at >60mg/dL for men and >70mg/dL for women, both corresponding to the 85th percentiles of the National Health and Nutrition Examination Survey III report on population distribution of HDL-C. As expected, HDL-C was higher in women (68mg/dL$\pm$2mg/dL) than in men (57mg/dL$\pm$2mg/dL; $p<0.001$) even though the prevalence of hHDL-C was similar (38% vs 36%, respectively). W men (n=37) had a higher HDL-C than H men (n=41), (62mg/dL$\pm$3mg/dL vs 52mg/dL$\pm$2mg/dL; $p<0.05$) with a higher prevalence (49% vs 24% $p<0.05$), but there was no ethnic difference among women. Triglyceride (TG) was lower in men and women with vs without hHDL-C and HDL-C was negatively associated with TG in both men and women ($r= -0.246$, $p<0.05$; $r= -0.350$, $p<0.001$, respectively) and with BMI in women only ($r= -0.246$, $p<0.01$) but not with HbA1c. HDL-C correlated positively with age in men and women ($r=0.262$, $p<0.05$; $r=0.377$, $p<0.001$, respectively) which represented an increase in HDL-C of 0.47mg/dL per year in women ($R^2=0.142$) and 0.30mg/dl in men ($R^2=0.069$) in a linear regression model. There was a higher prevalence of hHDL-C among women $\geq$40 vs <40 years (56% vs 20%, $p<0.001$) and men (43% vs 28%, $p>0.05$). These data indicate that there is an important influence of age on the HDL-C level in T1DM suggesting a unique interaction between aging and the underlying mechanism causing hHDL-C in these subjects. hHDL-C could thus escape notice in studies of subjects with T1DM <40 years of age. The finding of a high prevalence of elevated HDL-C level in older subjects with T1DM raises the question as to its functionality, given their excess risk of CVD. Detailed studies of HDL structure and function in T1DM are therefore needed to evaluate the prognostic significance of these findings. Disclosures: RG: Speaker, Merck & Co., GlaxoSmithKline, Daiichi-Sankyo; Investigator, Abbott Laboratories, Roche Pharmaceuticals, GlaxoSmithKline; Advisory Group Member, GlaxoSmithKline, Pfizer, Inc. Nothing to Disclose: TA, AS, AM

Pub #	P1-604
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Gender Dimorphism in Augmentation of HDL Cholesterol Levels in Healthy Persons with Premicroalbuminuria
Author String	C Edeoga, E Chapp-Jumbo, E Nyenwe, J Wan, S Dagogo-Jack University of Tennessee Health Science Center, Memphis, TN

Body

Background: Microalbuminuria (> 30 mg/g creatinine) signals nephropathy and cardiometabolic risk, but the significance of variations in microalbumin (MA) excretion within the normal range is unclear.
Objective: We tested the hypothesis that variations in MA excretion within the normal range reflect differences in cardiometabolic risk.
Subjects and Methods: We studied 352 healthy persons (109 men, 243 women; 187 Black, 165 White), whose mean (±SD) age and BMI were 43.1±11.4y and 29.6 ± 6.99 kg/m^2. Persons with a history of DM, kidney disease, proteinuria or acute illness were excluded. Among the subjects, 275 had parental type 2 diabetes (T2DM) history and 77 had no family history of T2DM. Subjects made outpatient visits after an overnight fast. Spot urine samples were obtained and blood pressure (BP), height, weight and girth were recorded. A 75-gm OGTT was performed, and blood was obtained for chemical analyses. The spot urine MA and creatinine levels were measured, and the ratio (MCR) was calculated.
Results: Blood glucose and creatinine levels were all normal. The mean MCR was 6.33±0.28 mg/g (range 1-29 mg/g). The 50^{th}, 75^{th}, and 90^{th} percentiles for MCR were 5 mg/g, 9 mg/g and 12.5 mg/g, respectively. ANOVA showed interaction of MCR with HDL levels ($F=21.4$, $P<0.0001$) that was seen in Blacks ($P=0.0001$), Whites ($P=0.0083$), persons with ($P=0.001$) or without ($P=0.04$) parental T2DM, and in women ($P=0.0008$) but not men ($P=0.43$). In multiple regression analyses, HDL ($r=0.25$, $P=0.0009$) was the only significant predictor of MCR in models that included age, BMI, girth, BP, FPG, 2h-PG, fasting insulin, LDL and triglyceride levels. The interaction with HDL was limited to subjects with MCR >50^{th} percentile, whose mean HDL level was 49.8 ±0.96 mg/dl compared to 55.4 ± 1.18 mg/dl ($P=0.0005$) for those at ≥ 50^{th} percentile, 58.0 ± 2.23 mg/dl ($P=0.001$) for ≥75^{th} percentile, and 63.0 ± 4.5 mg/dl ($P=0.001$) for those at ≥ 90^{th} percentile.
Conclusions: Excretion of urinary MA levels above the 50^{th} percentile (> 5mg/g creatinine), for which we propose the term [ldquo]premicroalbuminuria[rdquo], is associated with a greater augmentation of HDL cholesterol levels in women than men. Premicroalbuminuria (PMA) probably signals nascent endothelial stress; the augmentation of HDL (an antioxidant, antiinflammatory, and antiatherogenic agent) may be a defensive response to such stress. The mechanisms linking PMA, endothelial stress and gender dimorphism in HDL response are unclear.

Sources of Research Support: NIH (Grants R01 DK067269 and MO1 RR00211) and the American Diabetes Association.

Nothing to Disclose: CE, EC-J, EN, JW, SD-J

Pub #	P1-605
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Lipoprotein(a) Correlations among Diabetic Patients
Author String	MA Ferreira, T Azevedo, A Giestas, AC Carvalho, J Vilaverde, IM Palma Hospital de Santo António - Centro Hospitalar do Porto, Porto, Portugal
Body	Introduction. Lipoprotein(a) [Lp(a)] is a well recognized cardiovascular risk factor. Many studies have been tried to assess if it correlates with anthropometric and metabolic parameters. The results showed that it is an independent risk factor that doesn't correlate with any other parameters. Despite this, the authors tried to correlate Lp(a) with specific clinical parameters such as body mass index (BMI), HbA1c, LDL, and HDL and total cholesterol, triglycerides and some other lipoproteins in type 2 diabetic patients. Methods: Correlation study between Lp(a) levels and BMI, total cholesterol, HDL and LDL cholesterol, triglycerides, ApoA1, ApoB, ApoCII and ApoCIII of a group of diabetic patients who attended the outpatient endocrine clinic during educational sessions in 2009. Correlation was evaluated with the Pearson correlation coefficient using SPSS 17.0. Preliminary analyses were performed to ensure no violation of the assumptions of normality and linearity. Results: 103 patients (61±10 years old) have attended all the sessions which compose the educational plan performed at the clinic: 59% were men; 81% with type 2 diabetes. Their BMI varied between 19.0 and 46.4 kg/m2 (average ± standard deviation=28.2±4.8 kg/m2). They had 11±9 years of diagnosed diabetes and a low rate of complications. They had 8.5±2.1% of HbA1c, 34.76±40.33 mg/dL of Lp(a), 184.1±44.98 mg/dL of total cholesterol, 158±82 mg/dL of triglycerides, 47±13 mg/dL of HDL, 105.5±38.9 mg/dL of LDL cholesterol, 148.16±27.35 mg/dL of ApoA1, 84.32±24.95 mg/dL of ApoB, 6.5±3 mg/dL of ApoCII and 16.24±6.45 mg/dL of ApoCIII. There was no correlation between Lp(a) and total cholesterol (r=0.042; p=0.672), triglycerides (r=0.006; p=0.95), HDL cholesterol (r=-0.001; p=0.995), LDL cholesterol (r=0.01; p=0.921), ApoA1 (r=-0.004; p=0.972), ApoB (r=-0.053; p=0.604); ApoCII (r=0.066; p=0.51), ApoCIII (r=0.124; p=0.21), BMI (r=-0.093; p=0.351) and HbA1c (r=-0.047; p=0.638). Conclusions. These results are similar to the published data. Once again it was showed that there is no direct correlation between Lp(a) and the major analytic cardiovascular risk factors, HbA1c and BMI. Lp(a) seems to have an independent behavior as a cardiovascular risk factor. Nothing to Disclose: MAF, TA, AG, ACC, JV, IMP

Pub #	P1-606
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Postprandial Peaking and Plateauing of Triglyceride and Very Low Density Lipoprotein (VLDL) Levels Were Observed in Patients with Cardiovascular Disease Even on Normal Fat Diet and Maintained on Statin Treatment
Author String	CE Samson, LM Asis University of Santo Tomas, Manila, Philippines

Body

CONTEXT

Dyslipidemia is associated with increased cardiovascular morbidity and mortality. Recently, abnormal postprandial lipid levels (triglyceride and VLDL) have been shown to be significantly correlating with arterial stiffness and elevated endothelial inflammatory markers leading to increased atherosclerosis.

OBJECTIVE

To demonstrate the occurrence of postprandial lipemia among patients with cardiovascular disease (i.e.coronary artery disease/cerebrovascular disease) despite normal fat diet, normal fasting lipid levels and statin treatment.

METHODOLOGY

This is a prospective descriptive study of patients >18 years of age diagnosed with cardiovascular disease for > 6 months, on statin for [ge] 6 months and normal fasting lipid levels. Qualified patients were given standard diet of 30 kcal/kg composed of 60% carbohydrate,25% fat,and 15% protein, divided into 3 meals and 2 snacks given at time 0800h,1000h,1200h,1600h,and 1800h. Total cholesterol, triglyceride, High Density Lipoprotein (HDL), Low Density Lipoprotein(LDL) and Very Low Density Lipoprotein(VLDL) were taken at baseline (fasting), before each meal; 2 hours and 4 hours after each meal. ANOVA and post- hoc Wilcoxon Signed-Rank tests were used for data analysis.

RESULTS

A total of 9 patients were studied. Triglyceride levels showed a sharp rise from fasting level to 2 hours after breakfast with a mean difference of 22.48 mg/dL(p=0.028), then plateaued and further increased in the next 2 hours with a mean difference from baseline level of 37.66 mg/dL(p=0.08). Likewise, VLDL levels increased significantly from fasting level to 2 hours after breakfast with a mean difference of 4.15 mg/dL (p=0.015), then plateaued and further increased in the next 2 hours with a mean difference from the baseline level of 11.5 mg/dL(p=0.008)**.**The mean levels of both the triglyceride and VLDL were significantly higher than fasting level ($p<0.05$) and did not return to their baseline values in the 10- hour observation period. In contrast, the levels of total cholesterol, LDL, and HDL did not show similar pattern.

CONCLUSION

Triglyceride and VLDL peaking and plateauing were observed in patients diagnosed with cardiovascular disease despite normal fat diet and statin treatment. Overall, these findings must lead to a paradigm shift in the diagnosis, management and monitoring of dyslipidemia.

Nothing to Disclose: CES, LMA

Pub #	P1-607
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Higher Blood Levels of Dihomo-γ-Linolenic Acid May Progress Non-Alcoholic Fatty Liver Disease in Type 2 Diabetes Patients
Author String	K Yamashita, T Ichijo, A Yoshifuji, E Yoshida, R Iga, H Ouchi, M Higa Saiseikai Yokohamashi Tobu Hospital, Yokohama, Japan; Toho University School of Medicine, Ota-ku, Japan

Body

AIM

Our aim in this study was to evaluate the correlation between the non-alcoholic fatty liver disease (NAFLD) and the serum levels of polyunsaturated fatty acid (PUFA) fractions in patients with type 2 diabetes.

METHODS

A total of 60 patients with type 2 diabetes and without history of alcohol drinking and viral hepatitis were included in this study and divided into two groups, the fatty liver (FL) group and non-FL (NFL) group, determined by the abdominal ultrasonography. The ultrasonography was always performed by the same readiologist. We measured the plasma glucose, the serum alanine transaminase (ALT) as a hepatic aminotransferasem, the serum triglyceride (TG), ferritin, leptin and four types of serum fatty acid fractions; dihomo-γ-linolenic acid (DGLA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), arachidonic acid (AA) in the both group.

RESULTS

According to the abdominal ultrasonographic reports, 28 and 32 patients were classified into the FL and NFL groups, respectively. There were no significant differences in age, gender, history of smoking, blood pressure and blood glucose level between the two groups. The mean body mass index (BMI) and abdominal circumference (AC) were 25.9±2.8 vs. 22.3±3.0 and 89.4±6.8 vs. 82.6±7.6 cm in the FL and NFL groups, respectively, and both BMI and AC were significantly higher in the FL group ($p<0.001$). The mean serum ALT and TG level were significantly higher in the FL group than those in NFL group, 24.2±10.6 vs. 16.3±6.2 U/L ($p<0.001$) and 145.2±79.0 vs. 97.2±45.6 mg/dL ($p<0.01$). In contrast, although the 3 fatty acid fractions showed no significant differences between the two groups in AA, EPA and DHA levels as well as the EPA/AA ratio, the mean DGLA levels were significantly higher in the FL group than one in the NFL group, 37.5±12.2 vs. 28.6±9.2 [mu]g/mL, respectively ($p<0.01$). The mean serum ferritin and leptin level did not show significant difference between the two groups. The multiple regression analysis demonstrated a significant positive association of DGLA with ALT ($r=0.38$, $p<0.01$). In addition, DGLA also showed significant correlation with the AC ($r=0.30$, $p<0.05$), BMI ($r=0.39$, $p<0.01$), serum TG ($r=0.58$, $p<0.0001$) and leptin ($r=0.68$, $p<0.001$).

conclusionsIt was suggested that the high serum levels of DGLA reflected overeating, but our study indicated it also have a role in the progression of NAFLD by impairing the conversion to long-chain fatty acids and promoting the differentiation of adipocytes.

Nothing to Disclose: KY, TI, AY, EY, RI, HO, MH

Pub #	P1-608
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Addition of Omega 3 Fatty Acid to Statin Improved Triglyceride and LDL Size in Diabetic Dyslipidemia
Author String	D Kim, KJ Kim, DY Shin, SW Park, YD Song, CW Ahn, EJ Lee Yonsei University College of Medicine, Seoul, Republic of Korea; CHA University College of Medicine, Seongnam, Republic of Korea; National Health Insurance Cooperation Ilsan Hospital, Ilsan, Republic of Korea; Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea
Body	**Background:** A substantial proportion of diabetic patients with dyslipidemia does not achieve goals of triglyceride (TG) level while using statins. **Objectives:** To evaluate the effects and the safety of combined treatment with statin and omega-3 fatty acids in dyslipidemic patients with type 2 diabetes **Design:** This is a randomized, open-label, multi-center, parallel group phase IV study that was conducted across 4 sites in Korea. **Intervention/Participants:** Patients with persistent hypertriglyceridemia (TG[ge]200 mg/dl) while taking statin for at least 6 weeks were eligible. 66 patients were randomized to receive either Omacor 4 g, Omacor 2 g, or no drug for 8 weeks while continuing with statin therapy. The main goal was to evaluate the percentage change in TG level and low-density lipoprotein (LDL) particle size from baseline to week 8. **Results:** After 8 weeks of treatment, the mean percent change from baseline TG level was significantly greater in patients who took 4 g of Omacor, reaching an mean change of -41% compared to -24.2% in patients who maintained statin therapy alone (P=0.049). The mean percent change of LDL particle size was greater in patients prescribed with 4 g of Omacor with statin than in patients receiving statin monotherapy (P=0.024). Adverse events observed in patients receiving 4 g of Omacor included diarrhea, dyspepsia, and flushing (5.9%, 5.9%, and 5.9%, respectively). **Conclusions:** Coadministration of omega-3 with statin decreases TG and increases LDL particle size in dyslipidemic patients with type 2 diabetes. Combination therapy of 4 g of Omacor and statin was well tolerated without significant adverse effects. Sources of Research Support: Grant from the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (No. A085136). Nothing to Disclose: DK, KJK, DYS, SWP, YDS, CWA, EJL

Pub #	P1-609
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Treating Hypercholesterolemia with a Combination of Ezetimibe and Simvastatin (Vytorin[trade]) in Patients with Statin-Induced Muscle-Related Symptoms
Author String	TD Hoang, VQ Mai, PW Clyde, MKM Shakir National Naval Medical Center, Bethesda, MD
Body	Patients (pts) who develop statin-related myalgia may be managed by alternate, albeit less optimal treatment. Herein we report 3 pts with statin-induced myalgia responding to Vytorin[trade]. Case 1: A 62 y/o M with coronary heart disease and hypercholesterolemia (total cholesterol (C) 242 mg/dL, triglyceride (TG) 120 mg/dL, LDL-C 190 mg/dL, HDL-C 28 mg/dL, VLDL-C 24 mg/dL) was placed on atorvastatin 40 mg qd. Two weeks later, pt developed severe myalgia; creatinine kinase (CK) was 190 (nl 21-232 U/L). Atorvastatin dose was reduced but the symptoms persisted. Treatments with rosuvastatin, pravastatin, niacin, and red yeast rice were unsuccessful. Pt was then placed on Vytorin[trade] (10 mg ezetimibe + 40 mg simvastatin), and 3 months later his lipids were LDL-C 102 mg/dL, HDL-C 33 mg/dL , VLDL-C 20mg/dL. Liver enzymes and CK levels remained normal and pt had no myalgia for the next 3 years. Case 2: A 60 y/o M s/p CABG with hypercholesterolemia (total-C 243 mg/dL, TG 60 mg/dL, LDL-C 210 mg/dL, HDL-C 24 mg/dL, VLDL-C 12 mg/dL) was placed on atorvastatin 80 mg daily Three weeks later pt developed thigh muscle weakness, myalgia and elevated CK levels (832 U/L). Pt tried reduced doses of atorvastatin as well as other drugs without any success. Three months later pt was placed on Vytorin[trade] (10 mg ezetimibe + simvastatin 40 mg), and lipid levels improved (LDL-C 101mg/dL, HDL-C 26 mg/dL, VLDL-C 10 mg/dL). Pt continued on Vytorin[trade] for the next 2 years. Case 3: A 48 y/o M s/p coronary angioplasty presented with serum LDL level of 238 mg/dL (total C 289 mg/dL, TG 142 mg/dL, HDL-C 23 mg/dL, VLDL-C 28 mg/dL). During atorvastatin therapy pt developed myalgia and serum CK was 967 U/L, and pt was subsequently treated with Vytorin[trade] (10 mg ezetimibe + 20 mg simvastatin) every other day for 4 weeks and then increased to 10 mg ezetimibe + 40 mg simvastatin daily over a period of 3 months. On this regimen, lipid levels were LDL-C 118 mg/dL, HDL-C 26 mg/dL and VLDL-C 23 mg/dL. Serum CK levels remained normal, and pt tolerated Vytorin[trade] without any symptoms for the next 4 years. Discussion & Conclusion In the 3 cases reported here, other treatments (weekly therapy and other hypolipidemic drugs) were tried without any success. Although Vytorin[trade] contains statin, this drug was still able to successfully lower LDL levels without causing untoward side-effects. The exact mechanism by which simvastatin when given in combination with ezetimibe does not cause myalgia and CK elevation remains unclear. Nothing to Disclose: TDH, VQM, PWC, MKMS

Pub #	P1-610
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Randomized Clinical Trial Comparing Soybean Oil-Based and Olive Oil-Based Lipid Emulsion in Medical-Surgical Patients Requiring Parenteral Nutrition
Author String	G Umpierrez, D Smiley, R Spiegelman, V Zhao, I Pinzon, T Morris, L Peng, C Thomas, H Garcia, D Griffith, T Ziegler EUSOM/Grady Memorial Hospital, Atlanta, GA; Emory University Hospital, Atlanta, GA; Emory Rollins School of Public Health, Atlanta, GA; Emory University, Atlanta, GA
Body	The administration of parenteral nutrition (PN) has been associated with increased risk of complications in critically-ill patients. The underlying mechanism for the higher risk of complications is unknown but may be related to the use of soybean oil-based lipid emulsions (Intralipid[reg]), the only FDA-approved lipid formulation for PN. We have shown that **soybean oil-based** PN results in higher blood pressure and endothelial dysfunction compared to **olive oil-based PN** (ClinOleic[reg]) in healthy subjects; however, it is not clear whether **olive oil-PN** improves clinical outcome over traditional formulations. Thus, this double-blinded, randomized trial determined differences in nosocomial infections (wound, respiratory, blood stream, urinary), non-infectious complications, mortality, glycemic control, inflammatory markers (CRP, TNF-α), oxidative stress markers (cystine, cysteine, glutathione, glutathione disulfide), and immune function (phagocytic and oxidative burst activity of monocytes and granulocytes) in medico-surgical, ICU patients treated with s**oybean oil**- and olive oil-based PN. A total of 100 patients received **soybean oil-PN** (n=49) and **olive oil-based PN** (n=51) for up to 28 days. Patients received **soybean oil-PN** (51±15 yrs, BMI:27±6 kg/m^2, APACHE:15) or **olive oil-based PN** (46±19 yrs, BMI:27±8 kg/m^2, APACHE:15) for a mean duration of 12.9±8 days. For the entire cohort, the hospital LOS was 43.7±42 days and total hospital mortality was 13% without significant differences between groups (LOS: 47±47 vs. 41±36 days, p=0.49; mortality 16% vs. 10%, p=0.38, respectively). Patients treated with s**oybean oil** and **olive oil-based PN** had similar rates of nosocomial infections (43% vs. 57%, p=0.16), pneumonia (10% vs. 14%, p=0.76), bacteremia (22% vs. 22%, p=0.92), UTI (14% vs. 14%, p>0.99), acute renal failure (27% vs. 18%, p=0.33), length of ICU stay (15±14 vs. 17±18 days, p=0.77) and mortality (10% vs. 8%, p=0.74). The mean BG concentration during PN was 125±9 mg/dl without differences between groups. In addition, we observed no differences in inflammatory and oxidative stress markers or in immune function between groups. In summary, our results indicate that the administration of PN containing soybean oil-based (Intralipid[reg]) and olive oil-based (ClinOleic[reg]) lipid emulsion result in similar rates of infectious and non-infectious complications as well as in no significant differences in metabolic, inflammatory or oxidative stress markers in ICU patients. Sources of Research Support: Baxter Pharmaceuticals. Disclosures: GU: Principal Investigator, Baxter Pharmaceuticals. Nothing to Disclose: DS, RS, VZ, IP, TM, LP, CT, HG, DG, TZ

Pub #	P1-611
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	The Sex-Specific Association of the Hepatic Secretory Protein Fetuin with All-Cause and Cardiovascular Mortality among Older Adults: The Rancho Bernardo Study
Author String	GA Laughlin, E Barrett-Connor, CL Wassel, K Cummins, JH Ix University of California San Diego, La Jolla, CA; University of California San Diego, La Jolla, CA

Body

Background: Fetuin-A, a member of the cystatin family, is an important circulating inhibitor of calcium deposition in the vasculature and of insulin action in muscle and fat. Elevated levels of fetuin-A have been associated with insulin-resistance and diabetes, and fetuin-A may also be involved in the pathogenesis of cardiovascular disease (CVD).

Methods: This is a prospective study nested within the Rancho Bernardo Study, an ongoing study of healthy aging. Participants were 663 men and 1024 women age 50+ (median=73.1 yrs) who had CVD risk factors evaluated and blood samples collected in 1992-96 and who were followed for vital status through 2010 (95% follow-up). Fetuin-A levels were measured on archived plasma samples by ELISA kit (Epitope Diagnostics) in 2010.

Results: Plasma fetuin-A (g/L ± SD) was highest in women using oral estrogen (E) (0.55 ± 0.12), intermediate for women not using oral E (0.51 ± 0.10), and lowest for men (0.50 ± 0.10), all $p<.001$. Lower concentrations of fetuin-A were associated with older age in both sexes ($p<.001$), but with lower levels of several other CVD risk factors including adiposity, blood pressure and lipids (p's <.01); fetuin-A was higher ($p<.001$) in women with diabetes. Mortality: During the median 12.1 yr follow-up, there were 636 deaths overall (344 women, 292 men); 273 of these were attributed to CVD (153 women, 120 men). In Cox models adjusting for age, lifestyle, CVD risk factors and diabetes, the hazards ratio (HR) (95% confidence interval) for the lowest quartile (Q1) of fetuin-A vs the highest (Q4) was 1.56 (1.14-2.12) for all cause and 1.91 (1.22-3.02) for CVD mortality in women; HRs for Q2 and Q3 were not significantly different from Q4. In men, low fetuin-A was not significantly related to all cause or CVD mortality [Q1 vs Q4 HR = 1.00 (0.71-1.42) and 0.93 (0.55-1.56), respectively]. The association of low fetuin-A with all cause mortality was markedly stronger ($p=.005$) for women with hypertriglyceridemia (>150 mg/dl) compared to those without [HR=3.85 (2.05-7.20) and 1.32 (1.03-1.70), respectively.]

Conclusions: Despite being favorably associated with several CVD risk factors, low levels of fetuin-A predicted earlier CVD and all cause mortality in women, particularly those with hypertriglyceridemia. Fetuin-A was not related to mortality in men. These associations, and their absence in men, may be related to some maladaptive response to metabolic challenge specific to women.

Sources of Research Support: NHLBI Grant 1R01HL09851 awarded to JHI; AHA SDG Grant #0930073N awarded to GAL.

Nothing to Disclose: GAL, EB-C, CLW, KC, JHI

Pub #	P1-612
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Total Adiponectin Levels in Dyslipidemic Individuals: Relationship to Metabolic Parameters and Intima-Media Thickness
Author String	D David Karasek, H Vaverkova, M Halenka, D Jackuljakova, Z Frysak, D Novotny University Hospital, Olomouc, Czech Republic; University Hospital, Olomouc, Czech Republic
Body	*Introduction:* Adiponectin is adipocytokin with anti-inflammatory and anti-atherogenic effects. However, studies examining the relationship between adiponectin and cardiovascular diseases have had inconsistent results. *Aims:* The aim of this study was to evaluate the plasma levels of adiponectin in clinically asymptomatic subjects with various dyslipidemic phenotypes. The associations between adiponectin and risk factors for atherosclerosis, markers of insulin resistance, and the intima-media thickness of the common carotid artery (IMT) were also evaluated. *Methods:* 234 asymptomatic subjects were divided into four dyslipidemic phenotypes (DLP) according to apolipoprotein B (apoB) and triglycerides (TG): DLP1 (n=58, apoB<1.2 g/l and TG<1.5 mmol/l), DLP2 (n=47, apoB<1.2 g/l and TG[ge]1.5 mmol/l), DLP3 (n=31, apoB[ge]1.2 g/l and TG<1.5 mmol/l) and DLP4 (n=98, apoB[ge]1.2 g/l and TG[ge]1.5 mmol/l). DLP1 (normo-apoB /normo-TG) served as a control group. *Results:* Significant differences in adiponectin levels between normolipidemic phenotype - DLP1 (16.1 [10.3-20.8] mg/l) and hypertriglyceridemic phenotypes - DLP2 (9.5[6.8-13.0] mg/l, p<0.01) and DLP4 (10.1 [7.4-16.8] mg/l, p<0.01) after adjustment for age, sex and body mass index were found. Adiponectin correlated positively with high-density lipoprotein cholesterol and apolipoprotein A1 (apoA1), negatively with triglycerides, apoB/apoA1, high-sensitivity C-reactive protein, insulin, homeostasis model assessment and waist circumference. ApoA1 and insulin were detected as independent predictors for adiponectin levels in multivariate regression analysis. Adiponectin did not correlate with IMT. *Conclusions:* Individuals with hypertriglyceridemic phenotypes showed decreased adiponectin levels in comparison with normolipidemic subjects. Adiponectin was associated with lipid parameters, markers of insulin resistance, chronic inflammation and visceral obesity. But no association between adiponectin and IMT was found. Sources of Research Support: Grant source IGA MZCR NS/10284-3. Nothing to Disclose: DDK, HV, MH, DJ, ZF, DN

Pub #	P1-613
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Trends in Cardiovascular Risk Factors in Tehranian Children and Adolescents between Years 2000 and 2006
Author String	S Moradi, F Azizi Endocrine Research Center of Firouzgar, Tehran, Islamic Republic of Iran; Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Islamic Republic of Iran
Body	**Objective**: The aim of this study is to investigate the prevalence of overweight and its associated risk factors between 2000 and 2006 in Iran, as a country experiencing a nutritional transition phase in the recent decades. **Materials and Methods**: The participants in the study were boys and girls 3 to 17 years of age in Tehran city as a sample of the study target population in Tehran Lipid and Glucose Study (TLGS) who attended in three separate time periods in 2000, 2003 and 2006(1). The subjects including both boys and girls were categorized into three age groups 3-6, 7-12, and 13-17 years of age. Weight, height, waist circumference, systolic and diastolic blood pressure, fasting blood sugar, total cholesterol, high density lipoprotein(HDL), and triglycerides were measures and low density lipoprotein(LDL) was calculated. The prevalence of dyslipidemia based on the Franklin criteria(2), waist circumference above 75% percentile based on Caspian study, overweight and obesity based on more than 80% and 90% international percentile were determined and compared between different periods of time(3). **Results**: Mean BMI in both genders in age groups 7-12 and 13-17 years showed a significant increase from 2000 to 2006. Waist and waist to hip circumference ratio in boys in all age groups had a significant increase from 2000 to 2006, but this change in girls was significant only in the age group of 7-12 years of age. The blood pressure in all ages group and in both genders had a significant decrease from 2000 to 2006. Total cholesterol and LDL in some age groups of boys and girls had a significant decrease from 2000 to 2006. Serum triglyceride showed a significant decrease in age groups of 13-17 years of age in girls and 7-12 years in boys from 2000 to 2006. **Conclusions**: The results show increase in the rate of obesity but decrease in blood pressure and serum lipid concentrations in Tehranian children and adolescents. (1)Azizi F et al. Tehran Lipid and Glucose Study: Rationale and Design. CVD prevention 2000;3:242-47. (2)28-Franklin Fa et al. Evaluation and management of dyslipidemia in children. Endocrinol Metab Clin North Am 1998; 27:641-54. (3) Cole TJ et al. Establishing a standard definition for child overweight and obesity worldwide: International survey. BMJ 2000;320:1240-43. Nothing to Disclose: SM, FA

Pub #	P1-614
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Dietary Fructose and Risk of Metabolic Syndrome in Adults: Tehran Lipid and Glucose Study
Author String	P Mirmiran, F Hosseini-Esfahani, Z Bahadoran, F Azizi Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Islamic Republic of Iran; Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Islamic Republic of Iran
Body	**Background:** Studies have shown that excessive fructose intake may induce adverse metabolic effects. There is no direct evidence from epidemiological studies to clarify the association between usual amounts of fructose intake and metabolic syndrome. **Objective:** The aim this study was to determine the association of fructose intake and prevalence of metabolic syndrome (MetS) and its components in Tehranian adults. **Methods:** This cross-sectional population based study was conducted on 2537 subjects (45% men and 55% women) aged 19-70 y, participants of the Tehran Lipid and Glucose Study in 2006-2008. Dietary data were collected using a validated 168 items semi-quantitative food frequency questionnaire. Dietary fructose intake was calculated by sum of natural fructose (NF) in fruits and vegetables and added fructose (AF) in commercial foods. MetS was defined according to the modified NCEP ATP III for Iranian adults. **Results:** The mean age of men and women were 40.5±13.6 and 38.6±12.8 years, respectively. Mean total dietary fructose intakes were 46.5±24.5 (NF: 19.6±10.7 and AF: 26.9±13.9) and 37.3±24.2 (NF: 18.6±10.5 and AF: 18.7±13.6) gr/day in men and women, respectively. After adjustment for age, energy intake, physical activity and smoking status, mean of waist circumference across quartiles of total fructose intakes was 93.1, 93.3, 95.1 and 96.4 cm (P trend = 0.03) in men, and 83.4, 84.7, 85.9 and 86.7 cm (P trend = 0.007) in women. Total dietary fructose intake in men were inversely associated with systolic (P trend = 0.02) and diastolic blood pressure (P trend = 0.013); in women this association was observed for systolic blood pressure (P = 0.04). There were no significant differences in mean of serum fasting glucose, triglycerides and HDL-C across dietary fructose categories in men and women. The odds ratio (95% CI) of the MetS in the fourth quartile of fructose intake as compared with the first quartile was 1.33 (CI=1.15-1.47, P trend=0.002) in men, and 1.20 (CI=1.09-1.27, *P*=0.006) after adjustment for potential confounding variables. **Conclusion:** The higher intake of total dietary fructose is associated with the higher risk of MetS, waist circumference and systolic blood pressure.

Nothing to Disclose: PM, FH-E, ZB, FA

Pub #	P1-615
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Prevalence of Metabolic Syndrome and Associated CVD Risk Factors among Women of South Asian Ethnic Subgroups
Author String	MA Banerji, G Mahajan, N Potter, A Kansara SUNY Downstate Medical Center, Brooklyn, NY
Body	**Background**: The South Asian population is amongst the fastest growing ethnic group within the US. The size and diversity of the NYC population allows us to reliably estimate disease risk among South Asian subgroups. CVD associated mortality is 2-3 times higher in South Asians than Caucasians. We estimated traditional and non-traditional CVD risk factors in women representing two predominant ethnic subgroups, Gujarati and Sikh. **Method**: Our data represents participants in faith based organization in Metropolitan New York, who were screened for CVD risk factor. The convenience sample was compromised of 69 Gujarati and 70 Sikh women, mean age 43 and 48.5 years. All were instructed to fast before coming. **Result**: Traditional CVD risk factors were similar and common to both groups. Prevalence of obesity and overweight were 43 and 72% in Gujarati, 53 and 74% in Sikh, by Asian criteria of a BMI>23 and 25 respectively. Using (IDF) modified NCEP ATP III criteria the prevalence of age adjusted metabolic syndrome (MetS) was (30 vs 56%, p <0.002). An analysis of components of MetS showed Sikh women more frequently had higher waist circumference > 80 cm (91 vs 71%, p<0.005), fasting plasma glucose > 100 mg/dl (30 vs 9%, p<0.003) and Triglycerides >150 mg/dl (41 vs 12%, p<0.0001) as observed in Sikh and Gujarati respectively. Systolic BP >130 mm Hg was twice as prevalent in Sikh(40vs 20%, p<0.06) although not reaching statistical significance. Hdl<50, Diastolic BP > 85 mm Hg were similar (50 vs 38%, 16 vs 17%). The rate of diabetes based on A1c criteria >6.5 % was (11 vs 7%) and pre-diabetes based on A1c (5.7 - 6.5%) was (25 vs 35%) in Sikh and Gujarati women respectively and not different. The MetS rate correlated with age in both subgroups, (r= 0.446, p= 0.0001) consistent with data from a national study (1). **Conclusion**: These data suggest that there may be significant ethnic heterogeneity with higher rates of non-traditional CVD risk factors, especially fasting triglycerides, waist circumference and fasting plasma glucose among Sikh women. Studies should target differences in gene-environment interactions, culture and pathophysiology of CVD risk factors in ethnic subgroups. Programs aimed to decrease CVD risk should targe groups differently, including older women, beyond traditional risk factors to decrease CVD mortality and associated health care burden. (1) Misra R et al. J Diabetes Complications 2010;24:145-53 Nothing to Disclose: MAB, GM, NP, AK

Pub #	P1-616
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Differences in Prevalence of Metabolic Syndrome in South Asian Male Subgroups Living in New York
Author String	A Kansara, MA Banerji SUNY Downstate Medical Center, Brooklyn, NY; Wykoff Hospital, Brooklyn, NY
Body	Background: South Asians have the highest ethnic-specific rates of CVD in the US, with mortality two to three times higher than Caucasians. Traditional CV risk factors do not explaine this. Since the South Asian population is highly heterogeneous, we sought to determine differences in non-traditional CVD risk factors between 2 groups of South Asian men in New York. Methods We report CVD health fair data from participants in faith based organizations on 155 Sikh & 66 Gujerati (G) men, mean (SD) age 47(12) years. Results: The Sikhs were taller with a higher BMI (27.01 vs 25.6, p=0.012) than the G men. Overweight/obesity was similar and common: 80-90% were overweight/obese and 60- 70% were obese by the Asian criteria of a BMI > 23 and 25 respectively. Using International Diabetes Federation (IDF) modification of NCEP ATP III criteria, metabolic syndrome (MetS) was1.66 fold more common among Sikh than G men (63 %vs 38 %, p<0. 004 BMI adjusted). Analysi of the components of the MetS showed that Sikh compared with Gujerati men had high waist circumference > 90 cm (88% vs 64%, p<0.0001), fasting plasma triglyceride > 150 mg/dl (52 vs 35%, p<0.02), diastolic BP $\geq$ 85 mm Hg (46% vs 26%, p<0.004) and fasting plasma glucose > 100 mg/dl (23% vs 15%, p=0.003). The commonest phenotype was increased waist circumference & fasting plasma triglyceride levels, followed by blood pressure, HDL & fasting glucose. Compared with a national study of South Asians in the US (1), which did not distinguish subgroups, MetS rat of 38% was similar to NY Gujerati but lower than Sikh men (63%). Rates of MetS for both South Asian subgroups were higher than White, Black or Hispanic men (NCEP III criteria 25%, 23% & 40% respectively) (2). In both sub-groups, MetS correlated highly with BMI (r=0.3, p=0.0001), A1C (r=0.186, p=0.012) but not age or LDL cholesterol. Using A1c criteria of > 6.5%, diabetes was present in ~14% and pre-diabetes in an additional one-third, while elevated systolic blood pressure > 140 mm Hg and LDL-cholesterol > 140 mg/dl were each present in 25% of the men. 9% had an A1C $\geq$ 7.0% . Conclusions: There is significant ethnic heterogeneity and high rates of obesity and non-traditional CVD risk factors among South Asian, especially Sikh men. A lack of relationship to age suggests early onset of risk factors. Multi-level interventions are essential to understand mechanism, avoid significant morbidity and mortality and plan appropriate local health care resource allocation. (1) Misra R et al. J Diabetes Complications 2010;24:145-53 (2) Ford ES et al. JAMA 2002, 287:356-359 Sources of Research Support: NY State ECRIP fellowship awarded to MAB for AK. Nothing to Disclose: AK, MAB

Pub #	P1-617
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Decreased Cardiovascular Mortality in Patients with Early Gastric Cancer May Be Associated with Amelioration of Dyslipidemia and Atherosclerosis by Marked Reduction of Visceral Fat after Gastrectomy
Author String	Y-h Lee, SJ Han, HC Kim, WJ Hyung, HJ Lee, SH Lee, ES Kang, CW Ahn, BS Cha, EJ Lee, HC Lee Yonsei University College of Medicine, Seoul, Korea; Ajou University School of Medicine, Suwon, Korea; Yonsei University College of Medicine, Seoul, Korea; Yonsei University College of Medicine, Seoul, Korea
Body	**Background:** Bariatric surgery including gastric banding and gastric bypass are effective to induce weight loss and resolve obesity-related comorbidities in obese patients. This study investigated the cardiovascular mortality among patients undergone subtotal gastrectomy due to early gastric cancer (EGC) and analyzed the changes of metabolic parameters in those patients after surgery. **Methods:** Total 2,477 patients with gastrectomy for EGC were enrolled between 1995 and 2004, then followed up for mortality through 2007. To compare cardiovascular mortality of EGC patients to that of the general population, standardized mortality ratio (SMR) were calculated using the sex and age-specific cardiovascular mortality of the total Korean population in 2005. Effects of gastrectomy on changes of body weight and metabolic parameters were investigated in 51 EGC patients who were enrolled between 2004 and 2007. Their blood metabolic parameters, abdominal fat areas based on computed tomography, and carotid artery intima-media thickness were repeatedly measured before and after gastrectomy. **Results:** During the 15096.4 person-years of follow-up for the 2,477 patients, 244 deaths were observed. The all-cause mortality was not significantly higher than expected: SMR (95% CI) = 1.01 (0.88-1.14). However, cardiovascular mortality was significantly lower than expected: SMR (95% CI) = 0.35 (0.21-0.54). Metabolic investigations for the 51 patients observed that total/subtotal gastrectomy significantly lowered body weight, body mass index, and visceral fat areas regardless of obesity status before surgery. Triglycerides, LDL-cholesterol, and plasminogen activator inhibitor-1 levels were significantly decreased, while HDL-cholesterol and adiponectin levels were increased after surgery. Carotid intima-media thickness was significantly decreased only in non-obese patients. **Conclusions:** EGC patients experienced a lower cardiovascular mortality when compared with the general population. Significant reduction of body weight and visceral fat after surgery may improve lipid metabolism and prevent from atherosclerotic changes. Nothing to Disclose: Y-HL, SJH, HCK, WJH, HJL, SHL, ESK, CWA, BSC, EJL, HCL

Pub #	P1-618
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Lipids, Lipoproteins & Cardiovascular Disease (1:30 PM-3:30 PM)
Title	Serum Levels of Adipocyte Fatty Acid Binding Protein Relate to Sarcopenia and Sarcopenic Obesity
Author String	JC Won, TN Kim, KW Ko, N Kim, J Han, BD Rhee Inje University, Seoul, Republic of Korea; Inje University, Busan, Republic of Korea
Body	**Background:** Reductions in muscle mass and physical activity (Sarcopenia) results in accumulation of visceral fat muscle mass (Sarcopenic obesity, SO) and is known to be associated with increased risk of metabolic syndrome (MetS). Adipocyte fatty acid-binding protein (AFABP) has been identified as a marker o MetS. We examined the association of sarcopenia/or SO with AFABP in healthy Korean subjects. **Methods:** Two hundred ninety eight (male: 119, female: 179) adults aged 20 ~ 70 years were included and examined using dual X-ray absorptiometry. Sarcopenia was defined as the appendicular skeletal muscle mass index (ASMI) divided by weight (%) of < 1 SD below the mean values of young adults in both sex. Obesity was defined as visceral fat area [ge] 100 cm^2 using abdominal computed tomography. Serum AFABP levels were measured using an enzyme-linked immunosorbent assay. **Results:** Levels of AFABP were significantly increased in subjects with SO/or sarcopenia compared to non-sarcopenic and lean subjects, and negatively correlated with ASMI in both sex. Increased levels of AFABP was independently associated with ASMI and presence of SO even after adjustment of age, sex, body mass index, and smoking status **Conclusions:** Serum levels of AFABP are elevated in subjects with sarcopenia and SO and may contribute to pathologic link to MetS in subjects with SO. Sources of Research Support: Priority Research Centers Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education, Science and Technology(2010-0020224). Nothing to Disclose: JCW, TNK, KSK, NK, JH, BDR

Pub #	P1-619
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Adrenocortical Carcinoma (1:30 PM-3:30 PM)
Title	Etoposide, Doxorubicin, Cisplatin, and Mitotane *vs.* Streptozotocin and Mitotane in Adrenocortical Carcinoma -- (Preliminary) Results of the FIRM-ACT Trial
Author String	M Fassnacht, M Terzolo, B Allolio, E Baudin, H Haak, A Berruti, K Balthasar, H-H Mueller, B Skogseid University Hospital, Würzburg, Germany; University of Turin, Orbassano, Italy; Institute Gustave Roussy, Villejuif, France; Máxima Medical Centre, Eindhoven, Netherlands; University of Marburg, Marburg, Germany; University of Munich, Munich, Germany; University Hospital of Uppsala, Uppsala, Sweden
Body	Abstract Embargoed.

Sources of Research Support: German Ministry of Research (BMBF) grant # 01KG0501.

Nothing to Disclose: MF, MT, BA, EB, HH, AB, KB, H-HM, BS

Pub #	P1-620
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Adrenocortical Carcinoma (1:30 PM-3:30 PM)
Title	Steroid Profiling in Adrenocortical Carcinoma Reveals Mitotane as a Strong Inducer of CYP3A4 and Inhibitor of 5α-Reductase with Major Implications for Cortisol and Androgen Metabolism
Author String	V Chortis, P Schneider, AE Taylor, JW Tomlinson, BA Hughes, DJ Smith, R Libe, B Allolio, X Bertagna, J Bertherat, F Beuschlein, M Fassnacht, M Mannelli, F Mantero, G Opocher, E Porfiri, M Quinkler, M Terzolo, CHL Shackleton, PM Stewart, S Hahner, W Arlt University of Birmingham, Birmingham, UK; University of Würzburg, Würzburg, Germany; René Descartes University, Paris, France; University Hospital Innenstadt, Munich, Germany; Charité University Medicine, Berlin, Germany; University of Turin, Turin, Italy; Section of Endocrinology, Department of Clinical Physiopathology, Florence, Italy; University of Padua, Padua, Italy
Body	Mitotane (o,p'DDD) is the first-line treatment for metastatic adrenocortical carcinoma (ACC) and is also regularly used in the adjuvant setting, in particular in patients deemed to have a high risk of recurrence after surgical removal of the primary tumor. In the past the use of mitotane has been limited due to severe gastrointestinal side effects. However, these symptoms were actually due to mitotane-induced hypocortisolisn and it is now generally accepted that mitotane-treated patients need to receive a higher than usual glucocorticoid replacement dose, to avoid adrenal crisis otherwise precipitated by mitotane-induced increases in cortisol-binding globulin and subsequent drop in free cortisol. Mitotane is considered an adrenolytic substance but no information is available on any distinct effects on steroidogenesis. Here we carried out urinary steroid profiling by gas chromatography/mass spectrometry in 141 patients with ACC, 118 patients with tumor and 23 patients without tumor at time of 24-h urine collection. Results revealed a strong induction of CYP3A4 by mitotane, as reflected by a significantly increased 6b-hydroxycortisol/cortisol ratio comparing steroid profiles before and after mitotane (all $p<0.001$). In mitotane-treated patients >90% of hydrocortisone was inactivated to 6b-OH-cortisol. Mitotane also exerted a strong inhibition of 5a-reductase as reflected by a highly significant decrease in the ratios 5a-tetrahydrocortisol/cortisol and androsterone/etiocholanolone (all $p<0.001$). The inhibitory effect on 5a-reductase as reflected by both these ratios was similar to the established 5a-reductase inhibitor finasteride documented by 24-h urine analysis in five finasteride-treated patients (finasteride vs. mitotane, n.s.). In addition to these two distinct effects on two enzymes involved in androgen and glucocorticoid metabolism, CYP3A4 and 5a-reductase, mitotane significantly downregulated overall steroid output. The latter effect might be mediated by mechanisms impacting on cholesterol import or other early steps of steroidogenesis. Taken together, mitotane has significant effects on steroid metabolism, highly likely to impair both glucocorticoid and androgen action. Induction of CYP3A4 by mitotane will impact on drug metabolism in ACC patients. The therapeutic potential of mitotane as a 5a-reductase inhibitor e.g, in the context of testicular leydig cell carcinoma or prostate cancer deserves further exploration. Nothing to Disclose: VC, PS, AET, JWT, BAH, DJS, RL, BA, XB, JB, FB, MF, MM, FM, GO , EP, MQ, MT, CHLS, PMS, SH

Pub #	P1-621
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Adrenocortical Carcinoma (1:30 PM-3:30 PM)
Title	Mutational Analyses of EGFR and Downstream Pathways in Adrenocortical Carcinoma: Correlation with Mitotane Response and Survival
Author String	IGC Hermsen, HR Haak, R de Krijger, TMA Kerkhofs, RA Feelders, W de Herder, JW Wilmink, JWA Smit, AJ Gelderblom, NFCC Miranda, R v Eijck, T Wezel, H Morreau MaXima Medical Centre, Eindhoven, Netherlands; Erasmus Medical Centre, Rotterdam, Netherlands; Erasmus Medical Centre, Rotterdam, Netherlands; Amsterdam Medical Centre, Amsterdam, Netherlands; Leiden University Medical Centre, Leiden, Netherlands; Leiden University Medical Centre, Leiden, Netherlands; Leiden University Medical Centre, Leiden, Netherlands
Body	Adrenocortical carcinoma (ACC) is a rare disease with a very poor prognosis in which therapeutic options are limited. Mitotane is considered the most active drug, nonetheless, a number of limitations exist, making progress in the use of this drug highly important. As only 30% of the patients show objective tumour response, defining patients who will respond to treatment would be of clinical importance. The Epidermal Growth factor Receptor (EGFR) is known to be involved in the regulation of many cell processes and its role has been implicated in the development of multiple solid tumours. EGFR pathway related proteins in ACC have been investigated, however without available clinical data and relation to survival. In the present study mutational status of EGFR and downstream signalling pathways was evaluated in 47 ACC patients on mitotane using direct sequencing, a TaqMan allele-specific assay and immunohistochemistry. Objective tumour response was observed in 37.8 % and was significantly associated with mitotane serum levels [ge] 14 mg/l (p=0.00) and longer survival (p=0.03). One *BRAF*, three (possible) activating *EGFR*, 11 *TP53*, but no *PIK3CA* and *KRAS* mutations were found. No relation was found between mutational status, immunostaining and mitotane response or survival. Remarkable patterns of pERK staining were observed. Our data suggest that in a very low percentage of ACC patients, EGFR TKI inhibitors might give benefit, whereas in other ACC patients EGFR monoclonal therapy could be discussed, due to the almost absent mutations in downstream effectors of EGFR. Nothing to Disclose: IGCH, HRH, RdK, TMAK, RAF, WdH, JWW, JWAS, AJG, NFCCM, RvE, TW, HM

Pub #	P1-622
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Adrenocortical Carcinoma (1:30 PM-3:30 PM)
Title	A Computerized, Operator-Independent Morphometric Model for the Histological Assessment of Adrenal Neoplasia: Preliminary Data
Author String	P Dalino Ciaramella, M Vertemati, D Petrella, E Grossrubatscher, E Giovannini, ME Randazzo, M Gambacorta, M Ripamonti, L Vizzotto, P Loli AO Niguarda Ca' Granda, Milano, Italy; AO Niguarda Ca' Granda, Milano, Italy; Universit[agrave] Statale - UNIMI, Milano, Italy; Consiglio Nazionale delle Ricerche - CNR, Milano, Italy
Body	Specific biomarkers for diagnosis and prognosis of adrenocortical carcinomas (ACCs) are still lacking.(1) The proliferation marker Ki-67 is under evaluation for validation as a reliable IHC marker of malignancy for ACCs.(2) Although a well defined diagnostic cutoff has not been demonstrated yet, Ki-67 staining between 5-7% is thought to be a feature of AT malignant behavior.(3) As for the morphological markers currently used in the determination of AT malignancy, also for Ki-67 an interobserver variability is frequently reported. The aim of this pilot study was to determine, with an operator independent and repeatable technique, the proliferative activity and cell morphology of AT histological samples; a preliminary comparison with visual perception was also attempted. **METHODS** IHC study of 14 ACCs and 7 adrenal adenomas (AAs) samples was done (Rabbit anti-human Ki-67 monoclonal Ab, Clone SP6, 1:400); two pathologists performed a blinded examination with a consensus determination and according to Weiss score. We generated a computerized morphometric model on AT slices to evaluate the volume fractions occupied by nuclei (nVF) and cytoplasm (cVF) of both Ki-67(+)/Ki-67(-) neoplastic cells, and by inflammatory infiltrate. All morphometric variables were obtained by a computerized image analyzer (Kontron-Zeiss KS400) with a color camera attached to a light microscope (40x objective) for microscopic fields examination. More than 200 fields were systematically selected and examined by an automatically controlled procedure to assure unbiased sampling. The method of point counting by using a counting frame was used to determine the relative volume proportion of the investigated structures. Statistics: t-test. **RESULTS** nVF ($p<0.0001$), cVF ($p<0.0001$), nuclear/cytoplasmic ratio ($p<0.0001$) were the most discriminatory features between ACCs/AAs. Moreover nVF of Ki-67(+) cells was more prominent in ACCs than in AAs ($p<0.0001$) but in some cases an overlapping was still evident. When compared with computerized analysis, Ki-67 pathologist's evaluation in ACCs showed a marked overestimation and a wide range (average of 9,9% vs 1,4%, range 1-25 % and 0,2-4,2% respectively; p=0,00036). **CONCLUSIONS** Based on these preliminary data our computerized morphometric method could be considered in the future as an helpful tool for pathologists in the histological assessment of ACCs/AAs, particularly minimizing subjectivity and possibly integrating the actual morphologic Weiss criteria. (1) Volante M, Buttigliero C, Greco E, Berruti A, Papotti M. Pathological and molecular features of adrenocortical carcinoma: an update. J Clin Pathol. 2008 Jul;61(7):787-93 (2) Stojadinovic A, Brennan MF, Hoos A, Omeroglu A, Leung DH, Dudas ME, Nissan A, Cordon-Cardo C, Ghossein RA. Adrenocortical adenoma and carcinoma: histopathological and molecular comparative analysis Mod Pathol. 2003 Aug;16(8):742-51 (3) Morimoto R, Satoh F, Murakami O, Suzuki T, Abe T, Tanemoto M, Abe M, Uruno A, Ishidoya S, Arai Y, Takahashi K, Sasano H, Ito S. Immunohistochemistry of a proliferation marker Ki67/MIB1 in adrenocortical carcinomas: Ki67/MIB1 labeling index is a predictor for recurrence of adrenocortical carcinomas. Endocr J. 2008 Mar;55(1):49-55 Nothing to Disclose: PDC, MV, DP, EG, EG, MER, MG, MR, LV, PL

Pub #	P1-623
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Adrenocortical Carcinoma (1:30 PM-3:30 PM)
Title	SNP Array Profiling of Childhood Adrenocortical Tumors Highlights Focal Peak Regions of Alteration and Demonstrates a Founder Effect for the R337H TP53 Mutation in Southern Brazil
Author String	E Letouze, R Rosati, H Komechen, M Doghman, L Marisa, GP Zambetti, BC Figueiredo, MAD Pianovski, E Lalli Ligue Nationale Contre Le Cancer, Paris, France; Hospital Pequeno Principe, Curitiba, Brazil; CNRS - UNS, Valbonne, France; St Jude Children's Research Hospital, Memphis, TN; UFPR, Curitiba, Brazil
Body	Childhood adrenocortical tumors (ACT) are rare malignancies (0.3-0.4 annual cases per million children under the age of 15), except in southern Brazil, where a higher incidence rate (3.4-4.2 cases per million) is associated to a high frequency of the R337H TP53 mutation. In order to identify the genetic aberrations in this disease, we analyzed DNA from 13 childhood ACT from southern Brazil and 6 matched peripheral blood samples from the same patients using Illumina 370K SNP arrays. We found 18 significantly recurrent chromosome aberrations (q-value < 0.1), 3 of which were present in all the Brazilian tumors: loss of heterozygosity (LOH) of the whole chromosome 17, LOH at 11p15, gain at 9q34. Peak regions were delimited within each significant region of alteration, highlighting potential genes involved in ACT pathogenesis and/or progression. In addition, we describe a new statistical method for detecting founder mutations from SNP array data. Using this method, we identified a cluster of SNPs with significantly conserved genotypes around the TP53 gene in Brazilian ACT patients. By comparing the genotypes of these SNPs in matched tumor and blood samples, we characterized a 500 Kb haplotype around TP53 common to all mutant alleles, demonstrating that all R337H TP53 mutations in that geographic area derive from a single founder. These data have an important impact on our understanding of the pathogenesis of childhood ACT. Sources of Research Support: La Ligue contre le Cancer (Cartes d'Identité des Tumeurs program); Institut National du Cancer; and CNRS (LIA NEOGENEX) to E.L.; CNPq; and CAPES to B.C.F. Nothing to Disclose: EL, RR, HK, MD, LM, GPZ, BCF, MADP, EL

Pub #	P1-624
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Adrenocortical Carcinoma (1:30 PM-3:30 PM)
Title	SHRNA-Mediated Gene Silencing of β-Catenin Inhibits Growth of Human Adrenocortical Cancer Cell H295R *In Vivo*
Author String	M Keramidas, C Maisin, A Salomon, J-J Feige, M Thomas U823, Grenoble, France; U1036/Biology of Cancer and Infection, Grenoble, France
Body	Adrenal carcinoma (ACC) is a rare endocrine neoplasm in humans, notorious for its aggressive behavior, metastatic potential and poor outcome. Standard therapy is ineffective partly because ACC is intrinsically resistant to conventional chemotherapy. Progress into the elucidation of the genes and pathways involved in the pathogenesis of ACC has helped to identify new putative targets for the development of selective treatment. Mutations of b-catenin gene(CTNNB1) have been found in a third of ACC. Here we studied the effects of its inhibition on the *in vitro* and *in vivo* growth of the human ACC cell line H295R, which harbors the CTNNB1 Ser45 mutation. This mutation leads to a stabilized protein that is constitutively active. The cells were infected with lentiviral particles expressing short hairpin RNA (shRNA)-mediated silencing b-catenin. Two of three shRNAs used, induced specific and substantial down-regulation of b-catenin protein levels. *In vitro* cell proliferation assays showed that the expression of these shRNAs decreased cell growth as compared to cells expressing a non-targeting shRNA vector. We then assessed the *in vivo* effects of targeting b-catenin in H295R cells by transplanting them beneath the kidney capsule of Icr-Scid mice. The animals were sacrificed 28 days following transplantation. Tumor growth suppression was achieved by the two shRNAs showing *in vitro* efficacy. Xenografts were then processed for histological and immunohistological analyses. Despite a decrease in b-catenin protein expression, neither cyclin D1 nor c-myc downstream gene targets was down-regulated in silenced tumors. Proliferation measured with Ki-67, a marker expressed on all proliferating cells during late G1, S, M and G2 phases of the cell cycle was not significantly reduced in silenced tumors compared to the control ones. In contrast, p57, a cyclin-dependent kinase inhibitor, was found expressed at high level in silenced tumors which might modulate cell cycle progression. Finally, TUNEL assay, assessing DNA fragmentation, revealed no significant differences between the number of apoptotic cells in control and shRNA-mediated silencing b-catenin. Taken together, these findings indicate that the blockade of b-catenin can inhibit tumor growth of b-catenin-activated tumor cells *in vivo* and might represent a therapeutic strategy for ACC. Sources of Research Support: Association pour la Recherche contre le Cancer. Nothing to Disclose: MK, CM, AS, J-JF, MT

Pub #	P1-625
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Adrenocortical Carcinoma (1:30 PM-3:30 PM)
Title	Analysis of Potential Mechanisms Implicated in *IGF1R* Overexpression in Pediatric and Adult Sporadic Adrenocortical Tumors
Author String	TC Ribeiro, AA Jorge, MQ Almeida, BM Mariani, MCB Fragoso, BB Mendonca, AC Latronico Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil
Body	**Background:** Sporadic adrenocortical tumors are frequently diagnosed as incidentalomas in adult individuals but these tumors are rare in children. However, a remarkably high prevalence of adrenocortical tumors has been reported in children from Southern Brazil. Overexpression of *IGF2* and/or *IGF1R* may trigger a cascade of molecular events that can ultimately lead to adrenocortical malignancy. We previously demonstrated that IGF1 receptor gene (*IGF1R)* overexpression was a biomarker of pediatric adrenocortical carcinomas (1). The molecular mechanism implicated in its upregulation remains unknown. Recently, *IGF1R* gene amplification was demonstrated in only one adrenocortical carcinoma which had *IGF1R* overexpression. However, it was absent in the great majority of adrenocortical tumors studied (2). **Objective:** To evaluate if *IGF1R* overexpression observed in human sporadic adrenocortical tumor is associated with allelic variants in *IGF1R* gene. **Patients and methods:** Thirty-eight adrenocortical tumors (24 adenomas and 14 carcinomas) diagnosed in 19 children (14 girls and 5 boys) and 17 adults (14 women) were evaluated. Endocrine syndromes were demonstrated in 89% of these patients: isolated Cushing's syndrome (30%), 14 virilization (41%), 9 mixed syndrome (26%) and feminization (3%). *IGF1R* overexpression was previously demonstrated in 20 (15 children and 5 adults) out of 38 tumors by quantitative real time PCR. Genomic DNA was extracted from adrenocortical tumors tissues. The entire coding region of *IGF1R* (21 exons) was amplified and directly sequenced. **Results:** No new *IGF1R* variant was identified in the entire cohort of adrenocortical tumors. Three distinct previously described exonic polymorphisms were detected in *IGF1R* (exon 11 rs_3743262; exon 16 rs_2229765, exon 21 rs_17847203). The frequency of these polymorphisms was 10.5%, 65.8% and 7.9% respectively. No correlation between the *IGF1R* variants was demonstrated in the adrenocortical tumors whicl had or not *IGF1R* overexpression. In addition, other 6 already known polymorphisms were identified in intronic regions (rs_ 7174918, rs_2272037, rs_951715, rs_ 1464430, rs_ 4486868, rs_2593053). **Conclusion:** *IGF1R* polymorphisms were not associated with *IGF1R* overexpression in adrenocortical tumors. Other molecular mechanisms such as microRNA abnormalities and/or epigenetic alterations might be involved in the *IGF1R* upregulation in malignant adrenal tumors. (1) Almeida et al. 2008; J Clin Endocrinol Metab. 93:3524-31 (2) Ribeiro et al. 2010; Growth Hormone & IGF Research, submitted Nothing to Disclose: TCR, AAJ, MQA, BMM, MCBF, BBM, ACL

Pub #	P1-626
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Adrenocortical Carcinoma (1:30 PM-3:30 PM)
Title	Low Expression of LIN28, an RNA-Binding Protein, Is Highly Associated with Poor Prognosis in Pediatric and Adult Adrenocortical Carcinomas
Author String	AM Faria, IC Soares, TC Ribeiro, AM Lerario, BMP Mariani, A Wakamatsu, R Ressio, VAF Alves, BB Mendonca, MCBV Fragoso, AC Latronico, MQ Almeida Hospital das Clinicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Hospital das Clinicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil
Body	**Background:** LIN28, a highly conserved RNA-binding protein, has emerged as a modulator of the processing of let-7 miRNA family. LIN28 may control cell reprogramming and pluripotency through both miRNA-dependent and independent pathways. Overexpression of LIN28 has been demonstrated in many different human tumors with an overall frequency of 15% (1). **Patients and Methods:** In this study, we evaluated LIN28 expression in a large cohort of pediatric and adult adrenocortical adenomas (ACA) and carcinomas (ACC). LIN28 expression was assessed in a tissue microarray including 52 ACA and 49 ACC (37 children and 67 adults). The staining intensity and the percentage of positive tumor cells were calculated for each specimen in triplicate to obtain a final semiquantitative H score from 0 to 3 (a score [ge] 1.0 was chosen a priori as the cutoff point for separating tumors with LIN28 strong staining from those with weak staining). **Results:** LIN28 expression was significantly lower in metastatic ACC when compared to ACA and non-metastatic ACC (p= 0.0001). Among the carcinomas, a strong LIN28 staining was evidenced in 82% of the non-metastatic ACC and in only 25% of the metastatic ACC (X^2= 16.8, $p < 0.0001$). The specificity of a weak LIN28 staining for the diagnosis of metastatic ACC was 94% and 75% in children and adults, respectively. LIN28 expression was inversely correlated with tumor weight (r= -0.4, p= 0.004), but not with Weiss score in both children and adults. In pediatric patients, LIN28 expression was lower in mixed (Cushing syndrome plus virilization) ACC than in isolated virilizing ACC (p= 0.05). In univariate analysis, a weak LIN28 staining was significantly associated with metastasis and reduced overall survival in patients with ACC (p= 0.0001 and p= 0.027, respectively). The median disease-free survival time was 86 months in patients with ACC and a strong LIN28 staining and 31 months in those with a weak LIN28 staining. In multivariate analysis, LIN28 expression remained as a stage-independent predictor of metastasis in ACC patients (hazard ratio 0.42, confidence interval 0.18-0.96, p= 0.04). **Conclusions:** A strong LIN28 expression was found in the majority of ACA and non-metastatic ACC, but low expression of LIN28 was a hallmark of metastatic ACC in both children and adults. Therefore, LIN28 expression might be clinically useful to select ACC patients for mitotane adjuvant therapy, mainly in the pediatric group where prognostic factors are not well established. (1) Viswanathan SR, Daley GQ. Lin28: A microRNA regulator with a macro role. Cell. 2010 19;140 (4):445-9. Nothing to Disclose: AMF, ICS, TCR, AML, BMPM, AW, RR, VAFA, BBM, MCBVF, ACL, MQA

Pub #	P1-627
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Adrenocortical Carcinoma (1:30 PM-3:30 PM)
Title	Analysis of *CTNNB1* in a Cohort of Pediatric and Adult Adrenocortical Tumors
Author String	LO Lima, IC Soares, MQ Almeida, MCBV Fragoso, AM Lerario, TC Ribeiro, BMP Mariani, BB Mendonca, AC Latronico Hospital das Clínicas - FMUSP, São Paulo, Brazil
Body	**Introduction**: Hyperactivation of the Wnt signaling pathway has frequently been identified in cancers, particularly in digestive tract tumors. β-catenin is a key signaling molecule in this activation. Somatic activating mutations of *CTNNB1*, the gene encoding β-catenin, were identified in adrenocortical adenomas and carcinomas in adults. **Objective**: The aim of this study was to investigate the β-catenin expression and the frequency of *CTNNB1* mutations in a large cohort of adrenocortical tumors from children and adults. **Patients and methods**: A cohort of 104 Brazilian patients with adrenocortical tumors (64 adults and 40 children) was studied by immunohistochemistry in a tissue microarray manner for β-catenin and p53. Exon 3 of the *CTNNB1* was sequenced in 79 adrenal tumors using automatic direct sequencing. Seventy eight patients (39 adults and 39 children) exhibited p53 nuclear overexpression in TMA and 24 (of 57 patients studied) carried the known germinative p.R337H. **Results:** The majority of adrenocortical tumors (89.4%) exhibited the usual distribution of beta-catenin at the plasma membrane. In the adult group, 10 adenomas (27.0%) and 5 carcinomas (18.5%) showed abnormal cytoplasmic and/or nuclear β-catenin immunohistochemical staining. In the pediatric group, 5 adenomas (15.7%) and 1 carcinomas (14.2%) showed abnormal immunohistochemical β-catenin accumulation, indicating Wnt/β-catenin pathway dysregulation. We observed in the adult group that adrenocortical tumors with great intensity of β-catenin immunostaining in the nucleus compartment are associated with a dismal prognosis ($p = 0.02$). However, we cannot confirm the same hypothesis in the pediatric group maybe due to the size of the sample. We do not found any significant statistically relationship between anomalous β-catenin and p53 immunostaining. Seven distinct *CTNNB1* mutations (Ser45Pro, Ser45Phe, Asp32Asn, Pro44Ala Ser45Pro, His36Gln Ser37Lyn, p.E9GfsX14) were identified in 9 adrenocortical tumors; all of them showed abnormal immunohistochemical β-catenin accumulation, except one novel frameshift due to a single nucleotide insertion. **Conclusions:** Abnormal expression and activating mutations of the β-catenin was demonstrated in both adult and pediatric adrenocortical tumors (15% and 23.4% respectively), indicating participation of the Wnt pathway in the adrenal tumorigenesis. A greater nuclear intensity of β-catenin in adults was associated with a dismal outcome of adrenocortical carcinomas. Nothing to Disclose: LOL, ICS, MQA, MCBVF, AML, TCR, BMPM, BBM, ACL

Pub #	P1-628
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Adrenocortical Carcinoma (1:30 PM-3:30 PM)
Title	Sf-1/NR5A1 and Pod-1/TCF21 Expression in Different Human Adrenocortical Tumor Cell Cultures
Author String	MM Franca, MG Santos, CFP Lotfi University of São Paulo, São Paulo, Brazil; University of São Paulo, São Paulo, Brazil
Body	Steroidogenic factor 1 (SF-1/Ad4BP/NR5A1) is an important transcription factor to the development and function of the adrenal cortex. SF-1 is amplified and overexpressed in pediatric adrenocortical tumors in comparison to adult tumors (1, 2). Pod1/Capsulin/TCF21, member of bHLH transcription factor family, has been shown to inhibit the expression of Sf-1 by antagonizing the activity of USF on the proximal E-box (3). Moreover, Pod-1 deficient-Leydig cells present an increase expression of Sf-1 and Cyp11a1. In a previous work of expression data set of human ACC tissue samples, Pod-1 is markedly down-regulated in adrenocortical carcinoma (ACC) in comparison to adrenocortical adenoma (ACA) and normal adrenal tissue (4). In this study we evaluate the expression of Sf-1 and Pod-1 in two different culture cells from ACCs, a NCI-H295A cell line and a secondary cell culture from adult carcinoma (T53 cells); also in two culture cells from ACA, a functional pediatric adrenocortical adenoma (T7 cells) and a secondary cell culture from adult adenoma (T54 cells), by using quantitative PCR. In order to evaluate the effect of overexpression of Pod-1 in the Sf-1 expression, we transfected transiently T7 cells with Pod-1 pcDNA3Pod1 [T7pcDNA3Pod1 and T7pcDNA3 (empty vector)]. Our results showed that T7 adenoma pediatric cell culture and T53 adult carcinoma cell culture express Sf-1, respectively, 25-fold and 3.5 fold increase, when compared with NCI-H295A cell line. The T54 adult adenoma cell culture showed similar Sf-1 expression in relation to NCI-H295A cell line. The Pod-1 expression was 8.6-fold and 4.2 fold increase in, respectively, T7 adenoma pediatric cell culture and T53 adult carcinoma cell culture. Preliminary results of T7pcDNA3Pod-1 cells, that expressed 6-fold Pod-1 increase, have shown an increase of Sf-1 expression when compared with T7pcDNA3 cells. In summary, our results of Sf-1 and Pod-1 gene expression in adrenocortical adenomas and carcinomas are consistent with the results described in the literature. However, the results of increase of Sf-1 expression in the pediatric adenoma cell culture that overexpress Pod-1 are unexpected, and contrast with the results that suggest a inhibitory effect of Pod-1 in the proximal E-box of Sf-1 promoter. (1) Figueiredo et al., J Clin Endocrinol Metab. 2005; 90:615-619 (2) Almeida et al., J Clin Endocrinol Metab. 2010; 95: 1458-1462 (3) Cui et al., Development 2004; 131:4095-4105 (4) Giordano, TJ, et.al., Clinical Cancer Research 2009 15(2):668-676 Sources of Research Support: FAPESP, CAPES, CNPq and PrP-USP. Nothing to Disclose: MMF, MGS, CFPL

Pub #	P1-629
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Adrenocortical Carcinoma (1:30 PM-3:30 PM)
Title	Familial Adrenocortical Carcinoma Associated with HNPCC
Author String	N Kandasamy, S Nik-Zainal, AK Annamalai, L Happerfield, J Paterson, M Arends, M Gurnell Addenbrooke's Hospital, Cambridge, UK; Addenbrooke's Hospital, Cambridge, UK; Addenbrooke's Hospital, Cambridge, UK
Body	Introduction: We report the first case of *familial* adrenocortical carcinoma (ACC) in association with hereditary non-polyposis colorectal cancer (HNPCC) in a family with a *MSH2* germline mutation. HNPCC, an autosomal dominant disorder caused by mutations in one of the DNA mismatch repair (MMR) genes, is the commonest cause of hereditary colon carcinoma, and is associated with an increased risk of certain non-colonic cancers (eg. endometrial, ovarian, urinary tract, biliary tract). Currently, ACC is not a recognised feature of the HNPCC syndrome, and there are only two previous reports of ACC arising in the context of HNPCC, both of which were *non-familial* tumours. The Amsterdam II/Revised Bethesda criteria are used to select patients, based on family history of colorectal cancer or other HNPCC-associated tumours, for immunohistochemical (IHC) analysis to detect loss of MMR protein expression, or for microsatellite instability (MSI) in the tumour DNA. Those with loss of MMR expression or MSI are offered mutation analysis of the four MMR genes that are associated with HNPCC - *MLH1, MSH2, MSH6 and PMS2*. Clinicalc case: The proband presented aged 54y with generalised abdominal pain. Her past medical history included ovarian cancer (age 44y) and a malignant colonic polyp (age 47y). Investigation showed an extensive right adrenal mass, which was surgically resected. Histology confirmed an ACC with capsular, vascular and lymphatic invasion. Review of the family history revealed several individuals with colorectal cancer and other HNPCC-associated tumours. In addition, her mother had died of metastatic ACC. IHC analysis demonstrated loss of MSH2 protein expression in the ACC from both the proband and her mother. Mutation analysis confirmed a germline MSH2 mutation (deletion of exons 1-3) in the proband and several other family members. Conclusion: The familial occurrence of a rare tumour in this HNPCC family, strongly argues for a causal link with the underlying MMR defect. Nothing to Disclose: NK, SN-Z, AKA, LH, JP, MA, MG

Pub #	P1-630
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Adrenocortical Carcinoma (1:30 PM-3:30 PM)
Title	Virilizing Adrenocortical Carcinoma Associated with High LH Levels in a 16-Year-Old Girl with Previous Gonadotropin-Dependent Precocious Puberty
Author String	AM Lerario, MCBV Fragoso, MQ Almeida, AC Latronico, BB Mendonca Hospital das Clinicas da Universidade de São Paulo, São Paulo, Brazil
Body	We report a case of a 16 year-old girl with a six-month history of virilization signs, amenorrhea and a 18 cm right-sided adrenocortical tumor. Noteworthy, unexpectedly high basal serum LH levels were observed, in spite of high serum testosterone levels (35 U/L and 800 ng/dL respectively). Interestingly, the patient had a previous history of gonadotropin-dependent precocious puberty (GDDP) successfully treated with leuprolide acetate until the age of 10 yr. Six months after discontinuing leuprolide acetate, she had menarche and regular menses. In order to understand the mechanism underlying the lack of negative feedback of testosterone on LH secretion in this patient, we performed a GnRH stimulation test. A huge increase in LH level was observed, suggesting that LH secretion by the pituitary was chronically stimulated (35 U/L and 92 U/L for basal and post-GnRH LH levels, respectively). The patient underwent a right adrenalectomy, achieving normalization o testosterone level in two days and a progressive fall in LH levels, reaching normal values in two weeks. The GnRH stimulation test was repeated 15 days after the surgery and LH hyper-responsive pattern was again observed (10 U/L and 54 U/L, for basal and post-GnRH LH levels, respectively) Unfortunately, the patient had a relapse and died from progressive disease 18 months after the surgery. We hypothesized that an unidentified factor produced by the tumor had a stimulatory effect on pituitary LH release and might also had been a causative factor of GDDP in this girl. In an attempt to identify such factor, we studied by real-time PCR the expression levels of three genes which products are known stimulators of LH release: KISS1, TAC3 and GDF11. However, neither gene was overexpressed in comparison to other adrenocortical tumor tissues. In conclusion, we described an unusual LH secretion in a girl with a virilizing adrenocortical carcinoma but we were not able to define the underlying mechanism of LH secretion in this patient. Nothing to Disclose: AML, MCBVF, MQA, ACL, BBM

Pub #	P1-631
Session Information	POSTER SESSION: CLINICAL - Hypothalamic-Pituitary-Adrenal Axis (1:30 PM-3:30 PM)
Title	Intramuscular Glucagon Stimulation Test for Assessing Adrenal Function in Short Children
Author String	L de Vries, A Tenenbaum, M Phillip Schneider Children's Medical Center of Israel, Petah Tiqva, Israel; Tel Aviv University, Tel Aviv, Israel

Body

Background: The glucagon stimulation test (GST) has been shown to be effective in evaluating growth hormone (GH) secretion in children but there are few data on its use in evaluating the hypothalamic-pituitary axis (HPA).
Objective: To investigate the diagnostic value of the glucagon test in evaluating the adrenocortical response in short children.
Patients and Methods: Intramuscular glucagon was used to assess the HPA axis in addition to GH in children evaluated for short stature. A total of 194 children aged 7.7±4.4 years were evaluated (158 healthy children; 36 with various disorders). Adrenal function was considered normal if peak cortisol was >550 nmol/l and/or absolute increase of cortisol was >250nmol/l. A 250-[mu]g ACTH test was performed in 31 children with inadequate response to GST.
Results: Abnormal adrenal response to GST was found in 25.7% of the cohort. Inadequate cortisol response was significantly more common among males than among females (28.7% vs, 16.4%, $p<0.04$) and among children [ge] 6 years than among younger children (32.7% vs. 18.4%, $p<0.02$). Both mean basal and mean peak cortisol levels were significantly higher in the females than in the males: 381±165 vs. 319±151 nmol/l ($p=0.003$) and 741±102 vs. 595±208 nmol/l ($p<0.001$), respectively. By 180 minutes peak cortisol was achieved in 98% of the patients, with the highest proportion (44%) of patients showing peak cortisol response at 180 minutes. In only 4 of the 31 patients undergoing an ACTH stimulation test was peak cortisol <550 but higher than 500 nmol/l. The response to ACTH stimulation was significantly more robust than that to GST: peak cortisol 677±173 vs. 403±95 nmol/l, $p<0.001$. There were no significant differences in proportions of patients with abnormal cortisol response, mean basal or mean peak cortisol level, based on GH secretory status. Analyses including only healthy children yielded the same results.
Conclusions: GST may serve as a useful screening tool for adrenal function in both healthy and "abnormal" children with suspected hypopituitarism, especially in children <6 years old and in female girls. The adrenal response to GST is age and gender related. Larger studies are needed for establishing the best cut-off level for adequate cortisol response to the GST.

Nothing to Disclose: LdV, AT, MP

Pub #	P1-632
Session Information	POSTER SESSION: CLINICAL - Hypothalamic-Pituitary-Adrenal Axis (1:30 PM-3:30 PM)
Title	Clinical Indicators for Cushing Syndrome, as Derived from the ERCUSYN (European Registry on Cushing Syndrome) Database
Author String	E Valassi, A Santos, M Yaneva, M Toth, CJ Strasburger, P Chanson, JAH Wass, O Chabre, M Pfeifer, RA Feelders, S Tsagarakis, PJ Trainer, H Franz, K Zopf, S Zacharieva, SWJ Lamberts, SM Webb Hospital Sant Pau, UAB, Barcelona, Spain; Medical University of Sofia, Sofia, Bulgaria; Semmelweis Egyetem, II, Budapest, Hungary; Charité- Universit[auml]tsmedizin, Berlin, Germany; H[ocirc]pitaux de Paris, Paris, France; Churchill Hospital, Oxford, UK; H[ocirc]pitaux de Grenoble, Grenoble, France; Hospital de Ljubljana, Ljubljana, Slovenia; Erasmus Medical Center, Rotterdam, Netherlands; Evangelisnos Hospital, Athens, Greece; Christie Hospital, Manchester, UK; Lohmann & Birkner, Berlin, Germany
Body	The European Registry on Cushing's Syndrome (ERCUSYN) is designed to collect prospective and follow-up data at EU level on patients with Cushing's syndrome (CS). Baseline data on 481patients (new patients since 2008 and retrospective cases since 2005) from 36 centers in 23 countries have been included**.** Mean age (±SD) is 44±14 yrs (390 females, 91 males). Patients were divided into 4 major etiologic groups: pituitary-dependent CS (PIT-CS) (66%), adrenal-dependent CS (ADR-CS) (27%), CS from an ectopic source (ECT-CS) (5%) and CS from other etiologies (OTH-CS) (2%). Mean delay between onset of symptoms and diagnosis was 2.9 ± 3.7 yrs. The ECT-CS was the only group with a male prevalence ($p<0.05$).The ADR-CS group was older than the PIT-CS group (46.9±13.6 vs. 42.7±13.5, $p<0.05$). ECT-CS patients had a higher prevalence of hirsutism compared to global values (92% vs. 60%) and higher diabetes (74% vs. 38%) than the other groups ($p<0.05$ and $p<0.01$). Skin alterations (78%), menstrual irregularities (63%) and hirsutism (63%) were more prevalent in PIT-CS than in ADR-CS ($p<0.01$). Reduced libido was more prevalent in men (60% vs. 40%; $p<0.01$), as well as vertebral osteoporosis (40% vs. 20%; $p<0.05$), and vertebral (52% vs. 18%; $p<0.001$) and rib fractures (34% vs. 23%; $p<0.05$). Diabetes was more prevalent in ECT-CS than the others (74% in ECT-CS vs. 33% in PIT-CS, 34% in ADR-CS, and 20% in OTH-CS; $p<0.01$ for all comparisons), and the former had consulted a diabetologist prior to correct diagnosis of CS more frequently than those with ADR-CS ($p<0.05$); gynecologists were consulted more by women with PIT-CS or ADR-CS than with ECT-CS group ($p<0.05$). Weight gain resulted significantly more common in women than men ($p<0.01$). Quality of Life (QoL) evaluated by a generic (EuroQoL) and a disease-generated (CushingQoL) questionnaires were available in 27% of the patients; mean score of Visual Analogue Score (VAS) of EuroQoL was 54 ± 19 (reference values from France 83 ± 15 and Spain 71 ± 18), while mean score of the CushingQoL score was 39, lower than previously reported in active -46- and [ldquo]cured[rdquo] -56- CS patients. EuroQoL-VAS score in PIT-CS was significantly better than in ADR-CS ($p<0.05$). We observed a long delay to diagnosis, with a high number of specialists consulted prior to a correct diagnosis, high morbidity at diagnosis, low bone mass, and impaired QoL. Altogether this determined that less than half the cohort was actively working (mean age 44 yrs). Sources of Research Support: PHP grant 800200 of the EU.; ESE is an associated partner of ERCUSYN. Nothing to Disclose: EV, AS, MY, MT, CJS, PC, JAHW, OC, MP, RAF, ST, PJT, HF, KZ, SZ , SWJL, SMW

Pub #	P1-633
Session Information	POSTER SESSION: CLINICAL - Hypothalamic-Pituitary-Adrenal Axis (1:30 PM-3:30 PM)
Title	Contribution of Clinical Evaluation in Estimation of Pre-Test Probability in the Diagnosis of Cushing Syndrome under a Bayesian Perspective
Author String	D Cipoli, EZ Martinez, M Castro, AC Moreira School of Medicine of Medicine of Ribeirão Preto - University of São Paulo, Ribeirão Preto, Brazil; School o Medicine of Ribeirão Preto - University of São Paulo, Ribeirão Preto, Brazil
Body	The diagnosis of Cushing's syndrome (CS) is difficult. UFC, dexamethasone test and salivary midnight cortisol had shown similar accuracy. Elamin et al (2008) summarized that the Likelihood Ratio results of thes tests in a Fagan nomogram can estimate the post-test probability of CS. There are no descriptions on pre-test probability in the diagnosis of CS. The difficulties in estimating pre-test probability of CS are derived of rarity of CS and from the overlap in clinical findings between CS and other diseases. The Bayesian theory combines the objective test results with clinical suspicion to calculate the probability that a patient has a disease. In this study, we applied the Bayes theorem to estimate the pre-test probability of CS diagnosis. **Methods:** A questionnaire was submitted to physicians on 7 endocrine meetings: Brazil (cities: Salvador, Sao Luiz, Franca Belo Horizonte); Colombia (Bucaramanga), Italy (Naples); USA (San Diego), from 2008 to 2010 asking the questions: [ldquo]Based on your personal expertise, after obtaining clinical history and physical examination, without using laboratorial tests, what is your probability (expressed in percentage) to diagnose Cushing's Syndrome?[rdquo]; [ldquo]For how long have you been practicing Endocrinology?[rdquo], [ldquo]Where do you work: Primary and Secondary Healthcare Units; Private Office; Public, Private or University Hospital [rdquo]. **Statistical analysis:** a Bayesian beta regression (Brascum et al, 2007) investigated the relationship between the average probability of diagnosing CS and the independent variables: duration and place of endocrine practice and city of the meeting. The software WinBugs based on Markov chain Monte Carlo methods was used. **Results**: 294 questionnaires were obtained. The mean percentage and 95%CI of probability to diagnosis CS were: Salvador 0.47 (0.42-0.52); S.Luiz 0.63 (0.51-0.73); Franca 0.61 (0.49-0.71) B.Horizonte 0.49 (0.40-0.58); Bucaramanga 0.37 (0.31 - 0.43); Naples 0.62 (0.55-0.68); S. Diego 0.59 (0.53-0.65). The overall mean was 0.51 (0.48-0.54). There was a direct relationship between the probability o clinical diagnosis and years of practicing. There were no differences among places of endocrine practice. **Conclusions:** The mean pre-test probability of CS diagnosis using clinical evaluation is 51%. These results are influenced by previous endocrine experience. Although, the degree of subjectivity cannot be avoided, Bayesian logic may integrate the results of the clinical picture with laboratory tests. Sources of Research Support: FAPESP;CNPq-Brazil. Nothing to Disclose: DC, EZM, MC, ACM

Pub #	P1-634
Session Information	POSTER SESSION: CLINICAL - Hypothalamic-Pituitary-Adrenal Axis (1:30 PM-3:30 PM)
Title	Initial Management and Complications of Cushing Disease (CD): A French Multicenter Study of 437 Patients
Author String	C Baudry, M Martinie, M Fathallah-Sahnoun, B Gatta-Cherifi, R Bou Khalil, L Guignat, E Gay, G Raverot, T Brue, A Tabarin, O Chabre, J Bertherat Université Paris Decartes et H[ocirc]pital Cochin, Assistance Publique H[ocirc]pitaux de Paris, Paris, France; Centre Hospitalier Universitaire H[ocirc]pital Albert Michallon, Grenoble, France; H[ocirc]pital de la Timone, Assistance Publique H[ocirc]pitaux de Marseille, Université de la Méditerranée, Marseille, France; Centre Hospitalo-Universitaire de Bordeaux, Haut Lév[ecirc]que, Bordeaux, France; H[ocirc]pital Cochin, Assistance Publique H[ocirc]pitaux de Paris, Paris, France; Groupement Hospitalier Est, Hospices Civils de Lyon, Université Lyon 1, Lyon, France
Body	**Introduction:** CD is a rare disease. Our aim was to describe demographic and clinical characteristics together with initial management of patients with CD. **Patients and methods:** a retrospective analysis of 437 CD patients diagnosed between 1996 and 2009, followed in 5 tertiary centers (Bordeaux, Grenoble, Lyon, Marseille, Paris) was performed in the frame of the national program for rare diseases. **Results:** 347 (80%) were women. Mean age at diagnosis was 40.5 ± 15.8 years, and mean time between first symptoms and diagnosis was 34.4 ± 38.4 months. A bilateral inferior petrosal sinus sampling was performed in pretreatment evaluation in 32 % of patients.Pretreatment pituitary magnetic resonance (MR) imaging was normal in 20% of patients, allowed adenoma visualisation in 66.7 % and was inconclusive in 13.3%. Initial clinical and biological evaluation before treatment showed: body mass index above 25 kg/m[sup2] and 30 kg/m[sup2] in respectively 31 % and 35 %, hypertension (53%), elevated fasting or post-prandial glycemia (26%), psychiatric disorder (32%), clinical hypogonadism (38%), and fractures (21%). Pretreatment bone mineral density was only available in 110 patients: lombar T-score and femoral T-score were under 2.5 DS in respectively 16 % and 8 % of these patients. 338 patients (77%) had transsphenoidal surgery as first line treatment, leading to positive histology of corticotroph adenoma in 81 %. In the 299 patients with immediate postoperative data available, remission was obtained in 225 (75%) cases. Remission rate was higher in patient with positive pre-treatment MR (89% versus 71%, p=0.004). **Conclusion:** this large multicenter cohort of patients with CD allowed to characterize initial demographics and clinical manifestations of the disease, together with immediate pituitary surgery outcomes. The longitudinal study of this cohort should enable to determine long term evolution of CD manifestation after treatment. Disclosures: JB: Investigator, HRA Pharma. Nothing to Disclose: CB, MM, MF-S, BG-C, RBK, LG, EG, GR TB, AT, OC

Pub #	P1-635
Session Information	POSTER SESSION: CLINICAL - Hypothalamic-Pituitary-Adrenal Axis (1:30 PM-3:30 PM)
Title	Clinical Usefulness of Growth Hormone-Releasing Hormone-2 Test in Evaluation of the Hypothalamic-Pituitary-Adrenal Axis
Author String	S Nishigaki, Y Mizuno, K Waki, M Noda, Y Naiki, R Horikawa National Center for Child Health and Development, Tokyo, Japan
Body	**Context:** Growth hormone-releasing hormone-2 (GHRP-2), which exerts stimulatory effects on GH secretion is also known to activate hypothalamic-pituitary-adrenal (HPA) axis. Recent studies suggested GHRP-2 test is useful for assessing HPA axis function. **Objective:** To investigate the clinical usefulness of GHRP-2 test in evaluation of HPA axis by comparing with corticotrophin-releasing factor (CRF) test. **Subjects and Methods:** We retrospectively analyzed the result of GHRP-2 and CRF tests performed in 89 subjects (42 male and 47 female; 7 months-16 years 4 months); 33 with short stature (SS), 33 with intracerebral tumor (ICT) and 23 with those suspected of having HPA disorders. Blood samples were obtained at 0, 15, 30, 45 and 60 minutes after 2 mcg/kg of GHRP-2 loading, and at -30, 0, 30, 60, 90 and 120 minutes after 1.5 mcg/kg of CRF loading. **Results:** There were no serious complications in GHRP-2 tests. There were significant positive correlation of peak ACTH and cortisol levels between CRF and GHRP-2 test (P=0.0259 and 0.0391, respectively). Among 89 subjects, 5 patients with ICT showed discordant response of ACTH to CRF and GHRP-2. Peak cortisol levels were more than 17 mcg/dl in all SS in response to CRF and GHRP-2, except 2 subjects (one small for gestational age-SS and one GHD). Both peak ACTH and cortisol levels showed age-dependent decline (P=0.2530 and 0.0185, respectively). **Discussions:** Previous studies suggested that GHRP activates ACTH release via CRH. Additionally, according to recent reports, GHRP-2 has the direct effect on the pituitary gland to stimulate ACTH release. The present data suggest that GHRP-2 acts via the different way from CRF. Peak ACTH and cortisol showed significant correlation in CRF and GHRP-2 test. The younger children showed higher response to GHRP-2 in both ACTH and cortisol, suggesting the higher sensitivity to GHRP-2 in younger age group or stressed condition after overnight fast. **In conclusion,** GHRP-2 test is safe and simple and comparable to CRF test in assessing the HPA axis. Nothing to Disclose: SN, YM, KW, MN, YN, RH

Pub #	P1-636
Session Information	POSTER SESSION: CLINICAL - Hypothalamic-Pituitary-Adrenal Axis (1:30 PM-3:30 PM)
Title	The Value of 25 [mu]g Cortrosyn Stimulation Test in Evaluation of the Hypothalamic-Pituitary-Adrenal Axis
Author String	SV Peechakara, A Gupta, L Kennedy, C Faiman, BA Hatipoglu, RJ Weil, AH Hamrahian Cleveland Clinic Foundation, Cleveland, OH; Cleveland Clinic Foundation, Cleveland, OH; Cleveland Clinic Foundation, Cleveland, OH

Body

Introduction:
The ideal test to evaluate secondary adrenal insufficiency (AI) has been a matter of debate. The 250 [micro]g and 1 [micro]g cortrosyn stimulation test (CST) have been criticized as having low sensitivity and specificity to detect partial AI, respectively. We have used a 25 [micro]g CST since 2004 based on the literature that 20 [micro]g and 30 [micro]g cortrosyn dose, results in peak ACTH of 200 and 350 pg/mL respectively, approximating levels during ITT and metyrapone test.

Methods:
3 patient groups were extracted from a pituitary database: **G1 (n=53)** had both ITT and 25 [micro]g CST; 34 had macroadenoma. **G2 (n=50)** had microadenoma or hyperprolactinemia with a normal MRI. None had evidence of pituitary hormone deficiency except hypogonadism in those with hyperprolactinemia. **G3 (n=100** had cortisol [ge]18 [micro]g/dL, 30 or 60 minute (min) after 25 [micro]g cortrosyn, and underwent pituitary surgery without glucocorticoid coverage. Cortrosyn administered (IM) using 0.1 ml of 250 [micro]g cortrosyn vial.

Results:
The median (range) age, %female and tumor size (mm) were as follows: G1: 44 (19-64), 64%, 15 (4-52); G2: 36 (20-59), 78%, 3 (0-9); G3: 53 (18-84), 44% and 25 (10-44). Peak cortisol during CST occurred at 30 and 60 min in 55 % and 45% of patients, respectively.
In G1, the interval between CST and ITT was 32 days (1-79). Peak cortisol during CST and ITT were 22.7 [micro]g/dL (2.3-43.6) and 22.0 [micro]g/dL(1.8-34.6) respectively with Pearson coefficient correlation, r=0.65 (P<0.0001). Among 36 who passed the CST, 7(19.4%) failed the ITT, among 17 who failed CST, 8 (47%) passed the ITT, with sensitivity and specificity of 56% and 78%, respectively.
Peak cortisol in G2 and G3 were 25.8 [micro]g /dL (17.6-40) and 25.6 [micro]g/dL (18.5-43.8) respectively. One patient in G2 failed the CST but did not require glucocorticoid therapy. All patients in G3 had uneventful pituitary surgery with mean arterial pressure 88.8mmHg ±11.4 (SD).

Discussion:
The finding that all patients who passed 25 [micro]g CST preoperatively had uneventful pituitary surgery and only one patient in G2 had a slightly subnormal response, represents excellent performance of the test in real clinical practice. Peak cortisol levels during ITT and 25 [micro]g CST in G1 correlated significantly, eventhough the test performed suboptimally compared to ITT, as has been reported with 1 and 250 [micro]g CST.

Conclusion:
The 25 [micro]g CST is clinically useful for evaluation of AI. Whether or not it will prove superior to 1 or 250 [micro]g CST warrants prospective head to head studies.

Nothing to Disclose: SVP, AG, LK, CF, BAH, RJW, AHH

Pub #	P1-637
Session Information	POSTER SESSION: CLINICAL - Hypothalamic-Pituitary-Adrenal Axis (1:30 PM-3:30 PM)
Title	Comparison of Calcified and Non-Calcified Plaque from Cardiac CT in Cushing Syndrome with Age- and BMI-Matched Controls
Author String	NM Neary, OJ Booker, BS Abel, A Gharib, LK Nieman Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD; National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD; National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD

Body

Patients with Cushing's syndrome (CS) have an increased risk of cardiovascular disease (CVD), even after successful surgical cure (1). To investigate the etiology, we performed cardiac CT scans in CS patients and controls (n=15 per group) and quantified calcified plaque (using standard Agatston score) and non-calcified coronary plaque volumes. Calcified plaque is thought to represent old, relatively stable lesions whereas non-calcified plaque may be more prone to rupture.

CS was diagnosed by clinical and standard biochemical assessment. 24-h urinary free cortisols (UFC) at first presentation were elevated, ranging from 73-12392 [mu]g/24h, median 1534 (nl <50). Diagnostic testing and imaging studies showed that one patient had pituitary dependent Cushing's and 14 had an ectopic source of ACTH (7 occult). Cardiac CT was performed 0.3-13 years, median 5, from the onset of first Cushing's features and before any successful surgical resection. Controls had at least one risk factor for cardiac disease and were selected to match the patients for age (range: CS 32-61 years, median 52; controls 35-62, median 56), sex (10 males and 5 females in each group) and BMI (range: CS 23-46 kg/m2, median 32; controls 23-37 median 30).

Before cardiac CT 15/15 CS patients and 7/15 controls were hypertensive (14 and 7 were on anti-hypertensives respectively). Seven CS patients and 3 controls had a history of diabetes (5 and 1 on diabetes medication). One CS patient and 3 controls were current smokers. Four CS patients and 5 controls were on statin therapy. Two CS patients and 3 controls had known CVD. At the time of the CT scan, 5/15 CS patients were eucortisolemic; 4 on ketoconazole and one with cyclic Cushing's. Blood pressure was 150±6/89±4 mmHg in CS patients and 131±3/71±2 in controls ($P<0.05$ for systolic and diastolic comparisons).

CS patients had higher calcified plaque scores than controls, although this did not reach statistical significance (Agatston score: CS patients 236±99, controls 81±61; P=0.2). CS patients had significantly greater non-calcified plaque volumes (CS patients 64±12mm^3, controls 21±6; P=0.004).

These data suggest that excess glucocorticoids associated with CS may increase non-calcified plaque formation explaining, at least in part, the elevated risk of CVD seen in CS as well as the risk of events after cure. Further work is needed to investigate whether this effect is independent of changes in blood pressure and lipids associated with Cushing Syndrome.

(1) Colao A et al., J Clin Endocrinol Metab 1999; 84(8):2664-72

Sources of Research Support: The intramural programs of the National Institute of Child Health and Human Development.

Nothing to Disclose: NMN, OJB, BSA, AG, LKN

Pub #	P1-638
Session Information	POSTER SESSION: CLINICAL - Hypothalamic-Pituitary-Adrenal Axis (1:30 PM-3:30 PM)
Title	Coronary Microvascular Function in Cushing Syndrome: A Study Performed with Transthoracic Doppler Echocardiography
Author String	F Fallo, D Capizzi, N Sonino, A Paoletta, E Osto, S Iliceto, F Tona University of Padova, Padova, Italy; University of Padova, Padova, Italy; Cittadella City Hospital, Cittadella, Italy; University of Padova, Padova, Italy
Body	**Background**: Current evidence indicate a strong association between Cushing's syndrome, characterized by a cluster of systemic complications, and increased cardiovascular morbidity and mortality. **Objective:** To evaluate coronary flow reserve (CFR) by transthoracic Doppler echocardiography, an index of coronary microvascular function, in patients with Cushing's syndrome **Methods**: 12 newly diagnosed patients with Cushing's syndrome (11 females, aged 41.6±9.6 years), selected for having no clinical evidence of ischemic heart disease, were studied. There were 10 cases of pituitary-dependent Cushing's disease 2 of adrenal adenoma. Twelve subjects matched for age, sex, and major cardiovascular risk factors (diabetes, dyslipidemia hypertension, obesity, smoking habit) were used as controls. Coronary flow velocity in the left anterior descending coronary artery was detected by transthoracic Doppler echocardiography at rest and during adenosine infusion. CFR was obtained as the ratio of hyperaemic diastolic flow velocity (DFV) to resting DFV. **Results**: Mean CFR was similar in patients with Cushing's syndrome and in controls (2.9±0.4 versus 3.0±1.3, *P* ns). A reduced coronary reserve (i.e., [le]2.5) was found in 3/12 (25%) of Cushing's syndrome patients and in 4/12 (30%) of controls. CFR was inversely related to urinary cortisol levels in patients with endogenous hypercortisolism (Spearman's rho = -0.59, *P*=0.04). **Conclusions**: Coronary microvascular function, as assessed by CFR, is impaired in a high proportion of patients with Cushing's syndrome without clinical evidence of ischemic heart disease and in controls with matched risk factors. The relationship betweer CFR and cortisol may contribute to explain the increased risk of cardiovascular mortality in Cushing's syndrome. The potential pathogenic role on coronary dysfunction of hypercortisolism *per se* versus the associated cardiovascular risk factors requires further studies. Nothing to Disclose: FF, DC, NS, AP, EO, SI, FT

Pub #	P1-639
Session Information	POSTER SESSION: CLINICAL - Hypothalamic-Pituitary-Adrenal Axis (1:30 PM-3:30 PM)
Title	Verbal and Visual Memory Performance and Hippocampal Volumes, as Determined by 3Tesla MRI, in Patients with Cushing Syndrome
Author String	E Resmini, A Santos, B Gomez-Anson, Y Vives, P Pires, I Crespo, M de Juan-Delago, MJ Portella, SM Webb Hospital Sant Pau,Autonomous University of Barcelona (UAB), Barcelona, Spain; Hospital Sant Pau, UAB, Barcelona, Spain; UAB, Barcelona, Spain; Hospital Sant Pau, UAB, Barcelona, Spain
Body	Cushing's syndrome (CS) is associated with deficits in cognitive function, in particular concentration and memory. Hippocampus, a brain structure critical for learning and memory, is rich in glucocorticoid (GC) receptors, thus being particularly vulnerable to GC excess. **Objective:** The aim of our study was to evaluate both verbal and visual memory performance and hippocampal volumes (HV) on 3Tesla Magnetic resonance Imaging (MRI) in patients with CS. **Patients and methods:** 33 right-handed patients with CS and 34 healthy controls, matched for age, sex and years of education, were evaluated with Rey Auditory Verbal Learning Test (RAVLT) and Rey-Osterrieth Complex Figure (ROCF) memory tests. HV were calculated on dedicated 3T MRI of the brain, using Freesurfer image analyses software. **Results:** CS patients had lower performances in verbal (REY5 $p<0.001$, RETENTION INDEX $p<0.001$, TOTAL RECALL SCORE $p<0.001$, RECOGNITION-A $p=0.01$, RECOGNITION-B $p<0.05$), and delayed visual memory (FIG-REY-DELAYED $p<0.05$) than controls. There were no differences in hippocampal volumes either left or right between all CS patients and controls. Using normative data, 16 CS patients had pathological memory performances (6 in verbal memory, 10 in visual memory, and 4 in both). Significant decreased hippocampal volumes were observed in this subgroup. **Conclusion:** In CS patients with pathological memory performances, HV were reduced and correlated with memory deficits, the left with verbal memory and the right with visual memory, indicating that endogenous GC excess has deletereous effect on hippocampal volume. Moreover RAVLT and ROCF are usefull tools in clinical daily practise for evaluating verbal and visual memory. Sources of Research Support: FIS080302 and ERCUSYN PHP800200 and FIS 07/770. Nothing to Disclose: ER, AS, BG-A, YV, PP, IC, Md-J-D, MJP, SMW

Pub #	P1-640
Session Information	POSTER SESSION: CLINICAL - Hypothalamic-Pituitary-Adrenal Axis (1:30 PM-3:30 PM)
Title	Comparison of Quality of Life between Patients with Cushing Syndrome and Obese Subjects without Cushing Syndrome
Author String	BS Abel, SB Abraham, D Rubino, T Nansel, S Ramsey, LK Nieman The Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD; Washington Center for Weight Management and Research, Arlington, VA; The George Washington University, Washington, DC; The Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD
Body	Introduction: Patients with untreated Cushing's syndrome (CS) and subjects with obesity (OB) have impaired health-related quality of life (HRQL) compared with healthy individuals. Past studies have not directly compared HRQL between obese subjects with and without CS despite several common associated co-morbidities such as diabetes, hypertension, cardiovascular disease and depression, which can decrease HRQL We hypothesized that patients with CS would have lower HRQL compared to obese subjects as a result of hypercortisolemia. To test this, we compared the SF-36 responses of the two groups Methods: We evaluated HRQL in 56 patients with untreated CS (age, 39.9±1.7 yr; 79% White; 78.6% F; etiology: 79% pituitary, 21% ectopic) and in 319 non-CS OB patients presenting to a weight management clinic (age, 48.5±0.66 yr; 74% White; 72.7% F). CS was excluded in all obese subjects. HRQL was assessed with the SF-36, a 36-item HRQL survey measure with 8 subscales (physical functioning, role physical, bodily pain, general health, vitality, social functioning, role emotional and mental health) that comprise physical and mental component summary scores. Scores range from 0 to 100, with higher scores indicating better HRQL. Group differences in HRQL were examined using analysis of covariance controlling for gender, age, BMI and diabetes diagnosis. Data are shown as mean ± SEM adjusted for the above covariates. Results: Patients with CS had lower physical component summary scores compared to OB subjects (32.9±1.3 vs. 45.7±0.54, $p<0.001$). They also had significantly worse domain scores for physical functioning, role physical, bodily pain, and general health perceptions compared to OB subjects ($p<0.01$ for all). The mental component summary scores of CS patients and OB subjects were similar (44.0±1.7 vs. 43.0 ±0.67, $p=0.56$). CS patients had significantly worse domain scores for social functioning and role emotional ($p<0.05$ for both) However, domain scores for vitality and mental health were not statistically different between the groups. Diagnosis (CS vs. OB), gender, age, and BMI all had a significant effect on the physical component summary score but only age had a significant effect on the mental component summary score. Diabetes diagnosis was not associated with any HRQL score. Conclusion: After controlling for the effect of gender, age, BMI and diabetes, the presence of hypercortisolism significantly worsens HRQL especially in the physical domains. Sources of Research Support: In part by the intramural program of The Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health. Nothing to Disclose: BSA, SBA, DR, TN, SR, LKN

Pub #	P1-641
Session Information	POSTER SESSION: CLINICAL - Hypothalamic-Pituitary-Adrenal Axis (1:30 PM-3:30 PM)
Title	Health Care Costs among Patients with Cushing Disease Receiving Pituitary Surgery -- A Two-Year Retrospective Longitudinal Study
Author String	B Swearingen, N Wu, S-Y Chen, S Pulgar, BMK Biller Massachusetts General Hospital, Boston, MA; United BioSource Corporation, Lexington, MA; Novartis Pharmaceuticals, Florham Park, NJ
Body	Background: Surgery to remove pituitary adenoma is the first-line therapy for Cushing's disease (CD) and successful remission from hypercortisolemia is critical for decreasing morbidity and mortality. The impact of successful surgery on subsequent healthcare costs vs. costs associated with persistent disease is unknown. Methods: Medical and pharmacy claims during 2004-2008 from a large population with commercial or Medicare supplemental insurance were analyzed using a retrospective cohort design. Patients were included if they had claims of Cushing's syndrome (ICD-9-CM: 255.0) and hypophysectomy (07.6x). The date of first surgery was denoted as the index date and patients with at least one year of continuous enrollment before and after the index surgery were included. Cases were defined as being in remission when there were no claims fo bilateral adrenalectomy, radiotherapy, second transsphenoidal procedure, or pharmacy claims for ketoconazole, mitotane, metyrapone, bromocriptine, aminoglutethimine, or cabergoline during the 12 months after the index surgery. Total healthcare costs and comorbidity-related costs during the 12 months before and after the index surgery were compared between patients in remission vs. those who were not in remission. Non-parametric Wilcoxon tests were used to compare costs; $p<0.05$ was considered statistically significant. Results: 100 CD patients (mean age=43; 77% female) who received pituitary surgery were identified, 53 of whom were in remission after surgery. Annual healthcare costs increased significantly among patients not in remission from $15,829 in the 12 months prior to surgery to $36,795 in the 12 months after surgery ($p=0.01$), and decreased among patients in remission from $28,501 to $19,896, although this was not statistically significant ($p=0.21$). Costs associated with outpatient care significantly decreased among patients in remissior ($16,983 vs. $12,465, $p<0.001$). When comparing the two groups, there was a statistically significant difference in the changes in total annual healthcare costs incurred in pre and post surgery periods (patients in remission: -$8,605 vs. patients not in remission: +$20,967, $p=0.006$). Conclusions: The findings suggest that successful surgical treatment for CD patients leads to a decrease in healthcare costs but patients not in remission incur increased costs. The decrease in outpatient care after successful surgery may be partly attributable to reduced costs in managing comorbidities. Disclosures: SP: Employee, Novartis Pharmaceuticals. BMKB: Principal Investigator, Novartis Pharmaceuticals, Corcept Therapeutics; Consultant, Novartis Pharmaceuticals. Nothing to Disclose: BS, NW, S-YC

Pub #	P1-642
Session Information	POSTER SESSION: CLINICAL - Hypothalamic-Pituitary-Adrenal Axis (1:30 PM-3:30 PM)
Title	Hypercortisolism Is Necessary to Ensure Appropriate CRH and IPSS Responses in Cushing Syndrome
Author String	O Suer, EH Oldfield, LK Nieman The Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD; National Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, MD; University of Virginia, Charlottesville, VA
Body	Background: An absent ACTH response to CRH stimulation in Cushing's syndrome (CS) patients (pts) characterizes ectopic ACTH secretion (EAS) and primary adrenal disorders (AD). This result reflects cortisol inhibition of corticotrope secretion. Reduced negative feedback after adrenalectomy (ADX) or steroidogenesis inhibitors may result in a false positive response to CRH and a central-to-peripheral ACTH gradient during inferior petrosal sinus sampling (IPSS) suggesting Cushing's disease (CD). We present 9 adult CS pts who illustrate this problem. Cases: Short-term eucortisolism effects A pt with AD converted a flat CRH response to one suggesting CD after 3-weeks treatment with the glucocorticoid antagonist mifepristone. Another pt had 10-fold normal urine free cortisol (UFC) 5 weeks earlier but normal UFC on admission; IPSS indicated CD. CRH and dexamethasone (DEX) suppression tests suggested EAS. She was cured after resection of a pulmonary carcinoid (PulmCoid). Use of DEX in pts with unknown recent UFC A man admitted with high UFC had a CD IPSS response but an EAS response to CRH and DEX suppression. After DEX 2mg/d for 2 days, IPSS indicated EAS. He had gastrinoma. A woman had IPSS during septic shock; it suggested CD. Subsequent IPSSs suggested EAS: one was done 4 weeks after stopping steroidogenesis inhibitors, and one after DEX 4 mg/d for 4 days. She was cured after resection of a PulmCoid Evaluation after ADX Three of 4 ADX pts had CD. One (CD) had nearly 3-fold elevated UFC, probably from adrenal rest tissue, the others had low to normal UFC. One (PulmCoid) received replacement hydrocortisone. All had IPSS results suggesting CD. A subsequent ADX patient (CD) had a positive IPSS after taking DEX 2 mg/d for 3 weeks. Summary: Short-term eucortisolism (3-5 weeks) and severe stress (hypotension) can evoke an ACTH response to CRH in EAS pts by reversing/overcoming inhibition of the normal corticotrope. DEX at doses of 2-3 mg/d for 2-4 days in EAS converts IPSS responses from CD to EAS. ADX patients require supranormal glucocorticoid to suppress the normal corticotrope, as one ADX EAS pt on replacement hydrocortisone had a CD response to IPSS. Conclusions: Hypercortisolism should be confirmed in the weeks before IPSS, and not just within 1-2 days. Patients with cyclic CS may achieve correct diagnostic results if pre-treated with DEX. The optimal approach to ADX is not clear. Sources of Research Support: In part by the intramural NIH research programs of NICHD and NINDS. Nothing to Disclose: OS, EHO, LKN

Pub #	P1-643
Session Information	POSTER SESSION: CLINICAL - Hypothalamic-Pituitary-Adrenal Axis (1:30 PM-3:30 PM)
Title	ACTH-Secreting Pituitary Macroadenomas: Outcomes of Multimodal Therapy
Author String	R Ness-Abramof, Y Greenman, Y Toledano, S Ilan Meir Hospita, Sackler School of Medicine, Tel Aviv University, Kfar Saba, Israel; Tel Aviv- Sourasky Medical Center and Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; Maccabi Health Services, Hadera, Israel; Rabin Medical Center, Beilinson Hospital, Petach Tikva, Israel
Body	**Introduction:** Cushing's disease is rare, with an incidence of 2-3 /1,000,000. It is commonly associated with a pituitary microadenoma; however, 4-10% of patients with an ACTH-secreting pituitary tumor have a macroadenoma (1). Comparison of patients with microadenomas *vs*. macroadenomas demonstrated that the latter had higher plasma ACTH levels, reduced suppression on high dose dexamethasone and lower surgical cure rates, necessitating radiation and medical therapy to control hormone secretion (2,3). **Aim of the study**: To evaluate the biochemical and anatomical presentation of patients with ACTH-secreting pituitary macroadenomas, the need of different therapeutic options and the long- term cure/remission rates. **Methods:** Multicenter retrospective study. Clinical, biochemical, radiological and therapeutic data were retrieved from charts. **Results:** Twelve patients (5 male and 7 female) with ACTH-secreting pituitary macroadenomas (mean size 27.7 ± 10.3 mm) were included. Mean age at diagnosis was 41.4 ± 11.7 yr and mean follow up was 6.5 ± 4.2 yr (range 2-15 yr). At diagnosis, mean plasma ACTH and mean urinary free cortisol were 21.6 pmol/L ± 15.6 (nl:2-10.1) and 1185 ± 1616 nmol/day (nl:20-208 nmol/day) respectively. Three patients had evidence of visual field defects, and 5/12 patients had partial hypopituitarism. Transsphenoidal surgery was the primary therapy in 11/12 patients and medical treatment with pasireotide in one. Postoperative remission rate was 36% (4/11 patients). Reoperation was performed in one patient due to relapse and in another patient due to a rapid regrowth of the tumor with neurologic symptoms. Six patients were referred to pituitary radiotherapy, two had conventional radiotherapy and 4 had fractionated stereotatic radiotherapy. In the subgroup that had radiation therapy, 4/6 (67%) patients are in remission while one is still on medical therapy, and one was lost to follow up. Mean adenoma size of patients cured by surgery was smaller than those with a surgical failure, but the difference was not statistically significant (21.5 mm ± 6.6 *vs.* 31.5 ± 10.6 mm, respectively, *P*=0.12). **Conclusions:** Transsphenoidal surgery alone frequently fails to cure Cushing's disease caused by ACTH-secreting pituitary macroadenomas, therefore, postoperative pituitary radiation and/or medical therapy are often necessary and effective. 1. Lynnette K. Nieman,et al. An Endocrine Society Clinical Practice Guideline. J. Clin. Endocrinol. Metab., May 2008; 93: 1526 - 1540. 2. Woo YS,et Clinical and biochemical characteristics of adrenocorticotropin-secreting macroadenomas. J Clin Endocrinl Metab; 2005: 4963-9. 3. B. M. K. Biller, et al. Treatment of Adrenocorticotropin-Dependent Cushing's Syndrome: A Consensus Statement. J. Clin. Endocrinol. Metab., Jul 2008; 93: 2454 - 2462. Nothing to Disclose: RN-A, YG, YT, SI

Pub #	P1-644
Session Information	POSTER SESSION: CLINICAL - Hypothalamic-Pituitary-Adrenal Axis (1:30 PM-3:30 PM)
Title	Night Shift Work Affects Circadian Rhythm of Cortisol in Women Only
Author String	F Miyauchi Ehime Rosai Hospital, Niihama, Japan
Body	Introduction The previous study showed that circadian rhythm of cortisol was observed in plasma and saliva simultaneously and that it was maintained in nurses working on the daytime shift and the evening shift. As these results were obtained from the study of female nurses, presence (or absence) of sex differences in the circadian rhythm of cortisol and in the influence of shift works were investigated in this study. Materials and Methods Twenty-seven female nurses and twenty-four male nurses who enrolled voluntarily in this study were healthy, and all female nurses had regular menstrual cycles. To examine the circadian rhythm, blood and saliva were obtained every 2 hours from 8:00 for 24 hours and at 17:00 from six off-duty nurses in both sexes. The correlation between the concentrations of cortisol in saliva and those in plasma were examined. The nurses worked on three different shifts from 8:00 to 17:00 (daytime shift, N=13), from 17:00 to 24:00 (evening shift, N=12), and from 24:00 to 8:00 (night shift, N=14). Blood and saliva were obtained from each nurse at the start and the end of her or his shift. Concentrations of cortisol were determined by LC-MS/MS. The statistical difference was calculated using the student t test. Results Circadian rhythm of cortisol was observed in plasma and in saliva simultaneously in both sexes. Cortisol concentrations in saliva were well correlated with those in plasma, respectively. The nadir of cortisol concentrations was observed at 0:00 and the peak was at 8:00. Cortisol concentrations at the end of the daytime shift did not differ from those of the off-duty group in both sexes. Cortisol concentrations in the evening shift did not differ from those of the off-duty group in both sexes. Despite finding no differences of the cotisol concentrations at the end of the first day of the night shift, those at the beginning of the second day of the night shift were significantly elevated in female. Cortisol concentrations in male did not differ from those of the off-duty group at the first day and the second day of the night shift. Conclusions Circadian rhythms of cortisol in plasma and saliva were same in both sexes, respectively. Circadian rhythm of cortisol was maintained during the daytime shift and the evening shift in both sexes, but affected by the night shift work in female only. These results might suggest male be able to adapt more easily rather than female in night shift work. Nothing to Disclose: FM

Pub #	P1-645
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	NCoR Action in the Pituitary Establishes the Set Point of the Hypothalamic-Pituitary-Thyroid Axis
Author String	RH Costa-e-Sousa, I Astapova, KR Vella, FD Ye, FE Wondisford, AN Hollenberg Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; Ministry of Education of Brazil, Brasília, Brazil; Johns Hopkins University School of Medicine, Baltimore, MD
Body	The development of mouse models that can assess nuclear corepressor action have determined that NCoR and SMRT play a role in both ligand-independent repression of positive targets in hypothyroidism and T3-sensitivity of these same targets in the euthyroid state. However, their role in negative regulation has not been determined. To explore this further we developed mice that globally express a NCoR allele (NCoR[Delta]ID) that cannot interact with the TR. Remarkably, despite low T4 and T3 levels, these mice are hyperthyroid in peripheral target tissues and have normal TSH levels. Indeed, the central axis, including TRH neurons in the hypothalamus and pituitary thyrotrophs appears to be reset and recognizes the low T4 and T3 levels as normal (1). To understand how disruption of the NCoR-TR interaction in global NCoR[Delta]ID mice alters the set point of the HPT axis, we have now developed mice that express NCoR[Delta]ID selectively in the pituitary (P-NCoR[Delta]ID) by mating mice that express the cre recombinase under control of the common α-glycoprotein subunit promoter (α-GSU-Cre) with mice possessing the same NCoR conditional allele (NCoR lox/lox) used to develop the global model described above. To ensure that the cre recombinase in α-GSU-Cre mice was expressed in thyrotrophs we used a GFP-reporter strain and could demonstrate colocalization of TSHβ and GFP in the pituitary, confirming the specificity of our approach. P-NCoR[Delta]ID mice developed normally with similar body weight and length to controls. However, in adult P-NCoR[Delta]ID female mice T4 levels were reduced by 29% ($P=0.03$), T3 levels by 25% ($P<0.001$) and serum TSH levels by 79% ($P<0.01$) when compared to the control group. Accordingly, mRNA expression of both TSH subunits was decreased to 60% of control levels in the pituitaries of female P-NCoR[Delta]ID mice ($P=0.01$ for α[minus]GSU; $P=0.06$ for TSHβ). Thus, the conditional expression of NCoR[Delta]ID in the pituitary allows for the set point of the HPT axis to be altered, replicating the central phenotype seen in mice that express NCoR[Delta]ID globally. Because NCoR[Delta]ID cannot interact with the TR, the phenotype observed suggests that NCoR plays a direct role in regulating α[minus]GSU and TSHβ gene expression in the thyrotroph and establishes NCoR as a critical modulator of negative regulation by the thyroid hormone receptor. (1) Astapova I et al., Mol Endo in press Sources of Research Support: NIH Grant DK-056123 awarded to ANH. Nothing to Disclose: RHC-e-S, IA, KRV, FDY, FEW, ANH

Pub #	P1-646
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	Thyroid Hormone-Induced Changes in H3K4 Methylation and H3K9 Acetylation in the TSHB Promoter Region Suggest a Role for Epigenetic Modifications in TSHB Gene Regulation
Author String	AR Sidhaye, S Matsumoto, R Huang, S Barnett, PA Cole, FE Wondisford Johns Hopkins University School of Medicine, Baltimore, MD; Johns Hopkins University School of Medicine, Baltimore, MD
Body	Negative regulation of the hypothalamic-pituitary-thyroid axis is important for maintaining normal thyroid hormone levels. This is accomplished at the level of the pituitary by the action of thyroid hormone (T3) such that increasing levels of thyroid hormone cause decreased levels of circulating TSH as well as decreased Tshb mRNA levels. Precise molecular mechanisms for down-regulation of Tshb are lacking. The TαT1.1 cell line is a mouse thyrotroph derived cell line which displays down-regulation of Tshb and Trhr after treatment with T3 suggesting that this may be a good in vitro model system in which to carry out further investigations into the mechanism of T3 mediated down-regulation of Tshb. Gene activation is typically associated with acetylation of core histone tails. In addition, the methylation status of H3K4 has been associated with regions being defined as either promoters or enhancers. As a result we hypothesized acetylation or methylation of core histone tails may be important in regulation of Tshb. We utilized the ChIP assay to scan a 10kb region surrounding the Tshb transcriptional start site (TSS) to determine the enrichment of H3K4 di-methyl (H3K4me2), H3K9 acetylation (H3K9ac), and RNA polymerase 2 (RNAP2) after treatment with T3 10nM or vehicle. We found that T3 caused a time dependent increase in H3K4me2 enrichment across this region but most prominently in the 2kb region (>4 fold) proximal to the TSS. In contrast it appears that H3K9ac is decreased by upto 8-fold in a similar region. Thus we find reciprocal changes in H3K9ac and H3K4me2 marks in the promoter region. In addition, RNAP2 enrichment was decreased around the TSS after T3 treatment. To determine if altering the level of histone acetylation or methylation in and of itself could alter Tshb mRNA levels, we utilized the following pharmacologic inhibitors: trichostatin A (TSA), an inhibitor of histone deacetylases, or LSD1 inhibitors (PEH and PPH). We found dose dependent increases in Tshb mRNA with TSA (~ 3 fold), PEH (~ 4 fold) and PPH (~ 10 fold) following 14 hours of treatment, as measured by RT qPCR. Based on these data we suggest that both histone acetylation and histone methylation play important roles in regulating Tshb mRNA levels. Further work is needed to determine how these epigenetic marks are made and whether they are involved in the dynamic regulation of Tshb by physiologic modulators such as T3 and TRH. Nothing to Disclose: ARS, SM, RH, SB, PAC, FEW

Pub #	P1-647
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	Thyroid Hormone Levels Regulate Thyrotrope Development in the Zebrafish Pituitary
Author String	KN Tonyushkina, RO Karlstrom Baystate Medical Center/Tufts University School of Medicine, Springfield, MA; UMASS Amherst, Amherst, MA
Body	Central hypothyroidism in humans is caused by both genetic and environmental factors that alter normal cell proliferation and patterning within the developing pituitary placode. Clinical data indicate that abnormal Thyroid Hormone (TH) levels during the embryonic and fetal periods can have long-term implications for thyroid system regulation. The mechanisms by which embryonic TH levels affect the ontogeny of thyrotropes and the maturation of negative feedback regulation remain a mystery. The zebrafish embryo provides a powerful system to study the embryonic roles of TH, as the components of the hypothalamic-pituitary-thyroid (HPT) axis are largely conserved across vertebrate species and hormone levels can be easily manipulated in the absence of a blood-placental barrier. We show that type 2 deiodinase (Dio2), the primary enzyme needed to convert T4 to the more potent T3, is expressed in a few cells of the developing pituitary placode starting at 24 hours post fertilization (hpf). This is followed by the emergence of TSH expressing cells 4 hours later. We show that thyrotrope cell numbers increase dramatically between 32 hpf and 6 days post fertilization (dpf). Exposure to TH (T4) at different times during this period led to a dose-dependent reduction in thyroptrope cell numbers. Interestingly, a subpopulation of thyrotropes (~20%) was resistant to T4 treatments, regardless of dose. A 24 hour pulse of T4 exposure between 2 dpf and 3 dpf, followed by a 5 day wash-out, also led to a dramatic decrease in thyrotrope cell numbers, indicating that short exposure to altered T4 concentrations at early stages can have profound long term effects on pituitary development. Our results indicate that overexposure to TH during a critical period of pituitary patterning and differentiation impairs normal thyrotrope development and may have long-term implications for thyroid axis regulation postnatally. Importantly, the incidence of congenital hypothyroidism has doubled in the last 30 years, and defects in central thyroid regulation are likely involved. Our studies suggest that early embryonic stressors, including abnormal TH levels, may contribute to this growing problem. Nothing to Disclose: KNT, ROK

Pub #	P1-648
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	Genetic Markers of Thyrotrope Differentiation, Hypertrophy and Proliferation Identified by Gene Expression Profiling in Glycoprotein α-Subunit (CGA) Knock-Out Mice
Author String	P Gergics, ML Brinkmeier, F Castinetti, SA Camper University of Michigan, Ann Arbor, MI

Body

The mechanism whereby anterior pituitary cells become specialized in hormone production is a central focus for the field. Strides have been made in the identification of critical transcription factors and signaling pathways. The functional role for many of these has been determined by analysis of spontaneous and genetically engineered mouse mutants. Despite these discoveries there are significant gaps in our understanding. For example, *Pou1f1* is necessary for the lineage that is comprised of thyrotropes, somatotropes and lactotropes, yet the factors that permit the sub-specialization within this lineage have not been completely defined. We took advantage of a mouse knockout of *Cga*, the gene encoding alpha GSU, the common subunit of TSH, LH, and FSH, as an entrée to identification of factors that regulate thyrotrope differentiation and proliferation. These knockout mice exhibit severe hypothyroidism and hypogonadism due to the inability to secrete bioactive TSH, LH and FSH (1). Adult mutant pituitary histology reveals profound thyrotrope hypertrophy and hyperplasia that ultimately results in thyrotrope adenomas (2, 3). The other cells of the *Pou1f1* lineage are reduced in number, but there are no changes in gonadotropes or corticotropes. RNA isolated from pituitaries of normal and mutant 2 and 12-month-old mice was transcribed into cDNA and analyzed for expression of over 18,100 genes. We detected expected expression changes such as reduced *Cga* and increased *Trhr* and *Gata2* transcripts (4). Differentially expressed genes were filtered using statistics, literature data, and gene ontology terms, resulting in approximately 70 candidate genes for further analysis. These genes fall in the broad categories of transcriptional regulation, protein folding and secretion, and pro- and anti-apoptotic cellular pathways. We used real-time PCR to confirm differential expression of intriguing candidate genes including *Isl1* and a member of the cAMP response element binding protein family. *Isl1* is essential for development of the pituitary primordium, and recently it was discovered to be important in thyrotrope function (5, 6). The cAMP response element binding proteins regulate the TRH response of the *Tshb* gene (7). These findings are proof of principle that this approach reveals a new pool of novel regulators of thyrotrope cell genesis and proliferation, and could shed light on pituitary adenoma formation and progression.

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2. Stahl et al., Endo 140:1884, 1999
3. Brinkmeier et al., Mol Endo 15:2129, 2001
4. Charles et al., Mol Endo 20:1366, 2006
5. Takuma et al., Development 125: 4835, 1998
6. Castinetti et al., in preparation.
7. Hashimoto et al., J Biol Chem 275:33365, 2000.

Sources of Research Support: NICHD 34283 awarded to SAC.

Nothing to Disclose: PG, MLB, FC, SAC

Pub #	P1-649
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	Family Members CREB1 and CREM Control Thyrotropin-Releasing Hormone (TRH) Expression in the Hypothalamus
Author String	F Chiappini, KR Vella, FD Ye, AN Hollenberg Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA
Body	Thyrotropin-releasing hormone (TRH) is synthesized and secreted by the paraventricular nucleus (PVN) of the hypothalamus and prompts secretion of thyroid-stimulating hormone (TSH) by the pituitary, which promotes the synthesis and release of thyroid hormone (TH) from the thyroid. Furthermore, negative feedback by TH represses TRH and TSH production, thus establishing the hypothalamic-pituitary-thyroid (HPT) axis. In addition to TH, the melancortin 4 receptor has been implicated in to the increase of TRH synthesis potentially *via* the PKA- cAMP response element binding protein (CREB1) pathway. To evaluate the role of CREB1 in TRH regulation, CREB1 was deleted from PVN neurons using *Cre*-recombinase under the control of SIM1 (single minded 1) gene to generate the CREB$^{[Delta]SIM1}$ mouse. As expected, loss of CREB1 was compensated for by an up-regulation of its family member CREM. Interestingly, TRH mRNA expression was increased in the PVN of these mice compared to wild type (WT) controls. To explore the possibility that CREM is responsible for the up-regulation of TRH in CREB$^{[Delta]SIM1}$ mice, we co-transfected a TRH promoter-luciferase reporter with either CREB or two isoforms of CREM; CREMα and CREM[tau]2. While cAMP-mediated stimulation was similar in the presence of each of these family members, the CREM isoforms increased basal reporter activity by 3.5-fold. Thus, we hypothesized that the deletion of CREB1 and subsequent up-regulation of CREM might be implicated in the regulation of the HPT axis. To test this, CREB$^{[Delta]SIM1}$ and WT mice were fed either a chow diet or a PTU/Low iodine diet (PTU) to render them hypothyroid. To induce hyperthyroidism, half of the mice receiving the PTU diet were given four daily injections of T3 (2[mu]g/100g BW). As previously demonstrated, TRH mRNA expression in the PVN is increased in CREB$^{[Delta]SIM1}$ mice on a chow diet. Hypothyroid CREB$^{[Delta]SIM1}$ mice also have a significant increase in TRH mRNA expression compared to hypothyroid WT mice. This difference disappears between CREB$^{[Delta]SIM1}$ and WT mice when they are supplemented with T3. Interestingly, TSH, T4 and T3 serum levels are similar between CREB$^{[Delta]SIM1}$ and WT mice in all conditions. In conclusion, deletion of CREB1 and up regulation of CREM increases TRH expression in the PVN under both euthyroid and hypothyroid conditions. However, this up-regulation of TRH mRNA does not appear to regulate the HPT axis but could potentially affect pathways that are influenced by TRH production. Sources of Research Support: NIH Grant DK078090 awarded to ANH. Nothing to Disclose: FC, KRV, FDY, ANH

Pub #	P1-650
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	RXR Isoforms Exert Differential Effects on T3-Dependent Repression of Trh Transcription
Author String	BA Demeneix, S Decherf, I Seugnet, MS Clerget-Froidevaux CNRS UMR 7221 MNHN, Paris, France
Body	Thyroid hormone receptors (TR) control transcription of positively regulated target genes through heterodimerization with 9-cis-retinoic acid receptors (RXR) on positive thyroid hormone responsive elements (pTREs). RXRs improve binding of the TR-RXR heterodimer to pTREs, stimulating transcription. However, RXR actions on negative TREs are unclear. Indeed, the single half-site configuration of many negative TREs does not favor the binding of a TR-RXR complex, and thus whether RXR isoform specificity is of importance in such regulations is unknown. In a set of functional studies targeting the mouse hypothalamus, we analysed the effects of RXR subtypes in the negative transcriptional regulation by tri-iodothyronine (T3) of the thyrotropin-releasing hormone gene (Trh), the regulator of the hypothalamo-pituitary-thyroid axis. First, double-hybrid screening of a hypothalamic paraventricular nucleus cDNA bank revealed specific, T3-dependent interaction of TRs with the RXRβ isoform. Second, in vivo chromatin immuno-precipitation revealed recruitment of RXRs to the TRE-site 4 region of the Trh promoter in the absence of T3. Next, somatic transgenesis deploying overexpression and knock-down approaches in the mouse hypothalamus further delineated isoform-specific roles for RXRs. Overexpression of RXRα heightened T3-independent transcription from the Trh promoter, whereas RXRβ overexpression abrogated this activity with a loss of further repression in the presence of T3. RXRγ was without effect. Thus, each RXR isoform has specific roles in modulating the negative regulation of the Trh gene by T3. These results provide insight into understanding the actions of these different TR heterodimerization partners, particularly within the context of a negatively regulated gene. Sources of Research Support: EU contracts : Crescendo, Pioneer. Nothing to Disclose: BAD, SD, IS, MSC-F

Pub #	P1-651
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	Evidence for GABA-Mediated Effects on TRH-Producing Neurons in the Hypothalamic Paraventricular Nucleus of TRH-GFP Mice
Author String	G Wittmann, J Menyhert, E Sanchez, P Singru, T Fuzesi, V Molnar, Z Liposits, B Gereben, C Fekete, J Maguire, RM Lechan Tufts Medical Center, Boston, MA; Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary; Instituto Nacional de Psiquiatría Ramón de la Fuente Mu[ntilde]iz, México City, Mexico; Tufts University School of Medicine, Boston, MA
Body	Previously, we demonstrated that in addition to innervation by α-MSH-, AGRP-, NPY- and CART-containing axon terminals, hypophysiotropic TRH neurons in the hypothalamic paraventricular nucleus have a massive GABAergic innervation that establish symmetric synapses, suggesting an inhibitory function. As a first step to characterize the importance of GABA signaling in the regulation of the hypothalamic-pituitary-thyroid axis in rodent models, we performed electrophysiology and dual-labeling histochemical studies using immunofluorescence and in situ hybridization to establish the mechanisms by which hypophysiotropic TRH neurons are regulated by GABA, and to identify specific $GABA_A$ receptor subunits expressed in these neurons. Whole-cell voltage clamp recordings were made from hypophysiotropic TRH neurons in TRH-Cre x Z/EG reporter mice in which Cre-mediated excision of a loxp-STOP cassette leads to expression of GFP selectively in TRH neurons. The TRH-Cre transgene was prepared from a BAC clone containing the Mus musculus TRH genomic sequence along with 118.9 kb of 5' flanking sequence and 80.5 kb of 3' flanking sequence, and the Cre recombinase coding sequence inserted +65 bp from the translational start site in exon 2 of the TRH gene. Double-labeling immunofluorescence/in situ hybridization histochemistry in the TRH-Z/EG mice showed >85% of neurons expressing GFP in the hypothalamic paraventricular nucleus contain TRH mRNA. Electrophysiological recordings from TRH-GFP neurons revealed that these mice receive robust GABAergic inhibition and that TRH-GFP neurons are regulated by both tonic and phasic GABAergic inhibition. Of the $GABA_A$ receptors screened, $GABA_A\gamma2$ was highly expressed and identified in approximately 40% of hypophysiotropic TRH neurons in ad lib feeding animals. These data indicate that hypophysiotropic TRH neurons are regulated by both synaptic and extrasynaptic GABAergic inhibition, and that the $GABA_A\gamma2$ subunit is a major component of GABAergic inhibition in these neurons. Sources of Research Support: NIDDK 37021. Nothing to Disclose: GW, JM, ES, PS, TF, VM, ZL, BG, CF, JM, RML

Pub #	P1-652
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	The Use of Recombinant Adeno-Associated Virus to Overexpress Iodothyronine Deiodinase III in the Hypothalamic VMN
Author String	HC Greenwood, JR Counsell, E Richardson, W Dhillo, A Boelen, G Williams, D Bassett, SR Bloom, JV Gardiner Imperial College London, London, UK; University of Amsterdam, Amsterdam, Netherlands; Imperial College London, London, UK
Body	The hypothalamo-pituitary-thyroid (HPT) axis regulates systemic levels of thyroid hormones (TH) via a negative feedback loop mediated by the hypothalamic arcuate and paraventricular nuclei. However, the effect of TH in other hypothalamic nuclei are yet to be elucidated. Activation and inactivation of TH is mediated by the iodothyronine deiodinases (D1, D2 and D3). D2 deiodinates tetraiodothyronine (T4) to produce the active T3 hormone, whilst D3 inactivates T3. Administration of T3 directly into the hypothalamic ventromedial nucleus (VMN) increases food intake in rats. A plasmid containing the D3 gene (pTRCGW-D3) was transfected into the JEG-3 human placental choriocarcinoma cell line and concentrations of T3 in the cell media measured by radioimmunoassay. This transfection resulted in reduced T3 concentration in the cell media compared to cells transfected with a control plasmid not expressing D3. Recombinant adeno-associated virus (rAAV) was then generated using pTRCGW-D3, with a viral titre capable of over-expressing D3 (rAAV-D3). Anaesthetised male Wistar rats were bilaterally administered rAAV-D3 (n=10), or rAAV-green fluorescent protein (rAVV-GFP) (n=11) as a control, into the VMN. Animals were transferred from a normal chow diet (NCD) onto a 60% fat diet (HFD) on day 17 post-surgery, and the study continued until 72 days post-surgery. Quantitative PCR confirmed that hypothalamic *D3* mRNA was 10-fold higher following treatment with rAAV-D3 compared to the rAAV-GFP treated group ($P<0.001$). Plasma free T3 and free T4, were unchanged confirming that systemic thyroid hormone status was unaffected. These data confirm an increase in hypothalamic *D3* following rAAV-D3 administration into the rat VMN. This model is currently undergoing extensive metabolic phenotyping to determine the effects of inactivation of T3 in the VMN on energy homeostasis. Sources of Research Support: Biotechnology and Biological Sciences Research Council (BBSRC) research grant award; Integrative Mammalian Biology (IMB) Capacity Building Award; FP7-HEALTH-2009-241592 EurOCHIP grant; NIHR Biomedical Research Centre Funding Scheme. Nothing to Disclose: HCG, JRC, ER, WD, AB, GW, DB, SRB, JVG

Pub #	P1-653
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	Identification of TRH Responsive Neurons in the Hypothalamic PVN and in Amygdala after Exploration of the Open Field Test
Author String	M Gutierrez-Mariscal, E Sanchez, J-L Charli, P Joseph-Bravo Instituto de Biotecnología, UNAM, Cuernavaca, Mexico; INPRFM, Cd de México, Mexico

Body

Thyrotropin releasing hormone (TRH) synthesized in hypophysiotropic neurons participates in energy homeostasis; in amygdala TRH modulates anxiety behavior (1) Despite the known inhibitory effect of high glucocorticoids or stress in HPT-axis activity, it is not inhibited by various stressful behavioral models (elevated plus maze, open field [OF], water maze). The inhibitory effect of stress on HPT axis is observed in models like immobilization or restraint but may be overcome by a task performance that involves increased locomotion. The response however varies depending if animals are tested during light or dark phases. As the highest stimulatory response was detected in the amygdala of rats sacrificed 15 min after exposure to the OF during the dark, we sought to identify the TRHergic responsive neurons by in situ hybridization (ISH) of amygdale and PVN regions. Male Wistar rats were subjected to inverted light cycle 3 weeks before the experiment performed 2h after lights were off. Animals were exposed 5 min to the OF under dim light conditions, and sacrificed 15 min later. Corticosterone and TSH concentrations were quantified in serum and behavior recorded (Smart system). As before, corticosterone levels increased 2 fold. The rostral (-1.3 mm Bregma, Paxino), mid (-1.56), and caudal zones (-1.8 mm to -2.04) of the parvocellular PVN, and the amigdala (-1.6 to -3.30 mm) were identified by brightfield microscopic analysis of crystal violet stained- no hybridized contiguous sections. Hybridization was performed in every fourth coronal section (18 [micro]m) with UTP labeled cRNA proTRH probe (2); TRH mRNA levels showed a tendency to increase in anterior and medial PVN, more pronouncedly in the anterior zone but only values in middle PVN correlated negatively with anxiety levels. In amygdala, TRH mRNA is expressed in medial, basolateral (BLA) and posteromedial cortical nuclei but signal is much lower compared to that in PVN, or lateral hypothalamus; increased proTRH expression was detected in the anterior sections of the cortical nuclei. The anterior cortical nuclei of the amygdala is related to olfactory systems, rich in gluatmatergic neurons, responds to ethologically relevant tests and is a critical area in processing fear stimuli (3); the specific stimulation of TRHergic areas in these nuclei is consistent with TRH anxiolytic role.

(1) Gutiérrez-Mariscal et al., Psychoneuroendocrinology 2008; 33:198
(2) Sánchez et al., Endocrinology 2008; 149:4329
(3) Knapska et al., 2007; Physiol Rev 87:1113

Sources of Research Support: Financed by CONACYT: 83363.

Nothing to Disclose: MG-M, ES, J-LC, PJ-B

Pub #	P1-654
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	Type 3 Deiodinase (D3) Is Present in Neurosecretory Granules of Hypophysiotropic Neuron Axon Terminals in the Median Eminence of the Rat Hypothalamus
Author String	I Kallo, B Vida, Z Bardoczy, P Mohacsik, A Zeold, AC Bianco, C Fekete, Z Liposits, B Gereben Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary; Faculty of Information Technology, Pázmány Péter Catholic University, Budapest, Hungary; Miller School of Medicine, University of Miami, Miami, FL
Body	Thyroid hormone levels in the brain are tightly controlled by the deiodinases, including the hypothalamus where hypophysiotropic neurons are regulated by T3. The type 3 deiodinase (D3) inactivates thyroid hormone by inner ring deiodination and reportedly plays a role in the negative regulation of TRH secretion. However, the localization and subcellular distribution of D3 protein in specific hypothalamic neuronal systems is not known. To characterize the distribution of D3 in the hypophysiotropic system of the rats, we employed immunocytochemistry and an antibody directed against the C-terminal portion of D3. Very intense D3-immunoreactivity was detected in the external zone of the hypothalamic median eminence, a region known to contain axon terminals of parvocellular neurons. The specificity of immunolabelling was confirmed by the lack of immunoreactivity after staining with antigen-preabsored serum and also by western blot analysis. The D3 protein was located in axon varicosities. Immuno-electronmicroscopy revealed D3-containing neurosecretory granules of 80-120 nM diameter in hypothalamic neurons suggesting that D3 might function and travel in these organelles from the soma to the axonal compartment. Specific double-labeling immunofluorescence visualized by laser-scanning confocal microscopy revealed that the D3 protein is present in the axon terminals of different neurosecretory systems. D3 was found in subpopulations of TRH, CRH, GnRH and GRH axons but could not be detected in axons of the somatostatin-containing and magnocellular neurons. In parallel, no D3 was found in large neurosecretory granules and axon-varicosities of the magnocellular system by electronmicroscopy. The present findings reveal a novel aspect of the hypothalamic thyroid hormone homeostasis and suggest neuron-type specific intracellular trafficking of the D3 protein in the rat hypothalamus. The intense axonal presence of D3 only in selected neurosecretory systems suggests that local T3 regulation via D3 is highly specialized and cell-specific. The data indicates that D3 and thyroid hormone inactivation potentially play a role in metabolism, stress, growth and reproduction. Sources of Research Support: Hungarian Research Fund (OTKA) K81226 and K73002. Nothing to Disclose: IK, BV, ZB, PM, AZ, ACB, CF, ZL, BG

Pub #	P1-655
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	The -258 A/G Type 2 Deiodinase Gene Polymorphism Is Associated with a Differential Response in the Pattern of Thyroid Hormone Secretion after TRH-Stimulated TSH Release
Author String	MY Peltsverger, PW Butler, SM Smith, JD Linderman, AT Alberobello, OM Dubaz, Y Guevara, MC Skarulis, C Cochran, F Pucino, FS Celi NIH, Bethesda, MD
Body	Background: The rise in circulating levels of thyroid hormone (TH) following the acute thyrotropin (TSH) stimulation is in part mediated by the Type 2 deiodinase (D2) which modulates the intrathyroidal conversion of thyroxine (T4) to triiodothyronine (T3). We have previously demonstrated that a common D2 gene polymorphism (Thr92Ala) is associated with a blunted rise in T3 following the TSH surge secondary to intravenous (IV) administration of thyrotropin-releasing hormone (TRH), consistent with a reduction of the intrathyroidal deiodination of T4 (1). Another common D2 variant (-258 A/G) has been associated with an increase in transcription of the gene and in D2 activity (2). The aim of the current study is to characterize the effects of the -258 A/G D2 gene polymorphism on the pattern of TH secretion following the TRH-mediated acute rise in TSH. Methods: Secondary analysis of a previously described dataset (1). Forty-five volunteers who underwent an IV injection of 500 [mu]g of TRH with serial measurements of serum total T3 (TT3), free T4 (fT4) and TSH over 180 minutes were genotyped by PCR-RFLP for the -258 A/G polymorphism. Results: The study population was composed of 26 subjects with the -258 A/A genotype, age 32.8±10.4 (16 f, 10 m), and 19 subjects with the -258 G/x genotype, age 31.1±10.9 (11 f, 8 m). No significant differences between genotypes were noted in baseline serum TSH, fT4, or TT3 levels. Compared to -258 A/A group, the -258 G/x group showed a significantly lower TRH-stimulated increase in serum fT4 at 120' (0.203±0.020 vs. 0.124±0.0210 ng/dL, $p<0.01$) and at 180' (0.283±0.0187 vs. 0.203±0.020 ng/dL, $p<0.01$). No differences between groups were observed in TT3 at 120' (46.6±4.1 vs. 44.9±3.5, $p=0.769$) and at 180' (49.5±2.67 vs. 52.5±3.56, $p=0.490$). Similarly, no differences were observed between the two genotypes in TSH AUC (1611±757.4 mcIU/mL/min vs. 1558±571.3 mcIU/mL/min, $p=0.798$). Haplotype analysis of the Thr92Ala/-258 A/G D2 polymorphisms did not demonstrate any significant difference among the subgroups. Conclusion: Compared to the A/A genotype, D2 -258 A/G polymorphism is associated with a blunted rise of FT4 following TRH-mediated TSH secretion, while the increases in TT3 serum levels were similar. These findings indicate that the -258 A/G polymorphism is associated with a shift in the reaction product/substrate ratio consistent with an increase in D2 enzymatic activity. (1)Butler PW et al., Thyroid. 2010; 20:1407-12 (2)Coppotelli G et al., Thyroid. 2006; 16:625-32 Sources of Research Support: Intramural Research Program of the NIDDK-NIH; ClinicalTrials.gov Identifier NCT00812149. Nothing to Disclose: MYP, PWB, SMS, JDL, ATA, OMD, YG, MCS, CC, FP, FSC

Pub #	P1-656
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	Lacking Thyrostimulin in Mice Does Not Abolish the Acute Inflammation-Induced Decrease in Serum Thyroid Hormones Despite Pronounced Effects on Tissue Deiodinase Expression
Author String	A Boelen, CJJ van Zeijl, OV Surovtseva, J Kwakkel, M van Beeren, WM Wiersinga, E Fliers Academic Medical Center, Amsterdam, Netherlands
Body	Thyrostimulin, a putative glycoprotein hormone consisting of the GPA2 and GPB5 subunit, has been reported to activate the thyroid stimulating hormone receptor (TSHR). The observation that proinflammatory cytokines stimulate GPB5 transcription suggested a role for thyrostimulin in the pathogenesis of the nonthyroidal illness syndrome (NTIS), which is incompletely understood at present. In the present study, we induced acute inflammation by lipopolysaccharide (LPS, bacterial endotoxin) administration to $GPB5^{-/-}$ and WT mice to evaluate the role of thyrostimulin in altered peripheral thyroid hormone metabolism during NTIS. In addition to serum thyroid hormone concentrations, we studied deiodinase type 1, 2 and 3 (D1, D2 and D3) mRNA expression and activities in liver, white adipose tissue (WAT) and brown adipose tissue (BAT). The LPS-induced decrease of serum free T_4 and free T_3 index and the increase of the inflammation marker interleukin (IL)-1β mRNA were similar in $GPB5^{-/-}$ and WT mice. Although the LPS-induced hepatic D1 mRNA decrease was similar in $GPB5^{-/-}$ and WT mice, the decrease in D1 activity was more pronounced in $GPB5^{-/-}$ mice (*P*[le]0.01). Hepatic D3 mRNA and activity decreased to a similar extent in $GPB5^{-/-}$ and WT mice after LPS administration. LPS resulted in increased D2 mRNA expression in WAT of WT mice (*P*<0.001) but there was no effect in $GPB5^{-/-}$ mice. D2 activity was below detection limit in all WAT specimens of both strains. LPS resulted in a decrease in WAT D3 mRNA expression that was similar in $GPB5^{-/-}$ and WT mice. In BAT, an increase in D2 mRNA and activity was observed shortly after LPS administration in both $GPB5^{-/-}$ and WT mice. However, the LPS-induced decrease of D3 mRNA in BAT of WT mice was absent in BAT of $GPB5^{-/-}$ mice (*P*[le]0.05). LPS administration resulted in decreased TSHR mRNA expression in WAT of both $GPB5^{-/-}$ and WT mice while the LPS-induced suppression of TSHR mRNA was significantly smaller in BAT of $GPB5^{-/-}$ mice compared to WT (*P*[le]0.05). In conclusion, lacking GPB5 -and thus thyrostimulin- during acute illness results in differential altered peripheral thyroid hormone metabolism in mice without affecting the LPS-induced decrease of serum thyroid hormone levels. A role for the TSHR in this effect is unlikely. Sources of Research Support: Grant from MSD and the GPB5 -/- mice were generated by Lexicon Genetics. Nothing to Disclose: AB, CJJvZ, OVS, JK, MvB, WMW, EF

Pub #	P1-657
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	Up-Regulation of Type 2 Deiodinase in the Lung May Protect Against Acute Lung Injury in the Mouse
Author String	O Barca-Mayo, XH Liao, C Di Cosmo, L Moreno-Vinasco, MS Wade, S Sammani, T Mirzapoiazova, JGN Garcia, S Refetoff, RE Weiss University of Chicago, Chicago, IL; University of Illinois, Chicago, IL; University of Chicago, Chicago, IL
Body	Thyroid hormone (TH) metabolism, mediated by type 1, 2 and 3 deiodinases (D1, D2, D3) is profoundly affected by acute illness. We examined the role of TH metabolism in ventilator induced injury (VILI) of mice lungs. Mice exposed to VILI compared to spontaneously breathing mice (SB) recapitulated the serum TH findings of acute illness, namely a 16% ($p<0.05$) decrease in T3 and reduction of TSH to <10 mU/L and a 55% increase in rT3 ($p<0.05$). To understand the effect of lung TH metabolism, D1, D2, and D3 were measured in response to VILI. mRNA expression of D2 showed a 6.4 ± 1.8 fold increase ($p<0.01$) and D2 enzymatic activity was increased 2.2 ± 0.6-fold ($p<0.05$). D1 and D3 did not change. Using D2 knock out mice (D2KO) we determined whether the increase in D2 was an adaptive response. Similar changes in serum TH hormone levels were observed in D2KO as WT mice. D2KO mice exhibited increased susceptibility to VILI compared to WT, quantified by chemokine and cytokine mRNA induction (increases in IL6, IL-1β. CXCL1, TNFα, CXCL2 and decreases in HSP1, Serpine 3G, MMP9 and Retlng). Histological analysis of VILI-mediated alterations in lung demonstrated worsening alveoli integrity in D2KO compared to WT. These data suggest that increase lung D2 is protective to VILI. Similar findings of increased cytokines and chemokines were found in hypothyroid wild type (WT) mice treated with VILI compared to euthyroid WT mice. VILI-exposed D2KO mice displayed 43% reduction in lung D1 and a 2.4 ± 0.5 fold increase in lung D3 mRNA. We conclude that the induction of D2 during acute lung injury in the VILI mouse model constitutes a protective mechanism against acute damage, which could be due to maximize the cellular T3 levels. Nothing to Disclose: OB-M, XHL, CDC, LM-V, MSW, SS, TM, JGNG, SR, REW

Pub #	P1-658
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	Mct8 Deficient Mice Have Increased Energy Expenditure and Reduced Fat Mass That Is Abrogated by Deiodinase 1 Deletion
Author String	C Di Cosmo, X-H Liao, AM Dumitrescu, H Ye, RE Weiss, S Refetoff University of Chicago, Chicago, IL; University of Chicago, Chicago, IL; University of Chicago, Chicago, IL
Body	Children with loss-of-function mutations of the *MCT8 gene lose weight even when nutrition is adequate. Changes in blood makers compatible with thyrotoxicosis suggests that this may be due to T_3 excess in peripheral tissues. Mct8 deficient mice (Mct8KO) replicate the human thyroid phenotype including markers indicating increased thyroid hormone action on peripheral tissues. As direct measurements of metabolism are lacking in both humans and mice, we used Mct8KO mice to determine whole-body energy homeostasis. Dual Energy X-ray Absorptiometry (DEXA) showed that, compared to wild-type (Wt) mice, Mct8KO mice were significantly leaner (24.8 ± 0.4 vs 27.1 ± 0.4 grams), had less fat mass (15.0 ± 0.8 vs 18.6 ± 1.2 % of total mass) and more lean mass (85.0 ± 0.8 vs 81.4 ± 1.2 % of total mass). When housed in metabolic cages, the Mct8KO mice had significantly higher total energy expenditure (TEE) (19.6 ± 0.8 vs 16.9 ± 0.7 Kcal/h/Kg BW), food (0.177 ± 0.004 vs 0.145 ± 0.007 g/g BW/day) and water intake (0.132 ± 0.009 vs 0.106 ± 0.006 ml/g BW/day), while their total activity (XT) (798 ± 78 vs 769 ± 97 Arbitrary Units (AU)/day) was not different. To determine the role that high serum T_3 levels could have on the hypermetabolism associated with MCT8 deficiency, we studied mice deficient in both Mct8 and deiodinase 1 (Mct8D1KO), in which serum T_3 i not different from that of Wt mice. Compared to the D1KO littermates, the Mct8D1KO had similar total body mass (26.8 ± 1.5 vs 27.6 ± 0.7 grams), fat (20.7 ± 1.6 vs 20.4 ± 0.7 % total mass), and lean mass (79.3±1.6 vs 79.6±0.7 % total mass). TEE (15.2 ± 0.4 vs 15.4 ± 0.4 Kcal/h/Kg BW), food intake (0.142 ± 0.009 vs 0.144 ± 0.008 g/g BW/day), water intake (0.100 ± 0.014 vs 0.085 ± 0.007 ml/g BW/day), and XT (759 ± 66 vs 694 ± 7 AU/day) were also similar in these two genotypes. All these parameters were comparable to those of Wt animals.* *These studies demonstrate that in MCT8 defects, failure to maintain normal weight despite adequate calory intake is due to increased energy expenditure associated with high serum T_3 levels. Inhibition of T_3 production by D1 corrects body mass and the metabolic alterations caused by MCT8 deficiency.* *Sources of Research Support: NIH grants DK15070, DK07011 and DK20595; Smile foundation with support from the Sherman family.* *Nothing to Disclose: CDC, X-HL, AMD, HY, REW, SR*

Pub #	P1-659
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	Distribution of the Thyroid Hormone Transporter OATP1c1 mRNA in the Rat Brain and Its Regulation by Bacterial Lypopolysaccharide
Author String	G Wittmann, SS Nouriel, P Mohacsik, B Gereben, RM Lechan Tufts Medical Center, Boston, MA; Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary; Tufts University School of Medicine, Boston, MA

Body

The organic anion transporting polypeptide 1c1 (OATP1c1) was originally characterized as a high affinity thyroxine (T4) transporter expressed by brain endothelial cells. A recent immunocytochemical study (1), however, demonstrated that the OATP1c1 protein is also present in the vascular endfeet of astrocytes and in hypothalamic tanycytes. This observation raised the possibility that OATP1c1 may be the primary T4 transporter of both astrocytes and tanycytes that express the type 2 deiodinase (D2) selenoenzyme, the major activating deiodinase in the brain that converts T4 into T3, the form of thyroid hormone that can effectively ligand the thyroid hormone receptor. The need for deeper understanding of glial thyroid hormone transport is emphasized in a recent study showing the importance of glia-mediated paracrine effects on T3-dependent neuronal gene expression (2). To further investigate the distribution and cell-type specific expression of OATP1c1 in different brain regions, localization of OATP1c1 mRNA was studied in rat forebrain sections using isotopic and fluorescent in situ hybridization. OATP1c1 mRNA was ubiquitously expressed in endothelial cells of the brain, and also in GFAP-positive astrocytes in the cortex and hippocampus, but was almost entirely absent from astrocytes in the amygdala, thalamus and hypothalamus. The highest concentration of OATP1c1 mRNA was identified in tanycytes and epithelial cells of the choroid plexus. Since bacterial lypopolysaccharide (LPS) is a potent regulator of D2 activity and mRNA expression, we determined whether LPS also regulates the expression of OATP1c1 in vivo. Within 9 hours of intraperitoneal LPS administration, an almost complete loss of the OATP1c1 hybridization signal was observed in blood vessels throughout the brain. In contrast, OATP1c1 mRNA expression in astrocytes, tanycytes and choroid plexus epithelial cells was unchanged. These data suggest that the D2-expressing cells of the brain, such as tanycytes as well as cortical and hippocampal astrocytes, are capable of T4 uptake via the OATP1c1 transporter. They also demonstrate cell-type specific regulation of OATP1c1 by endotoxin treatment, and suggest that T4 transport through the blood-brain barrier might be compromised during infection.

(1) Roberts et al., Endocrinology 2008; 149:6251
(2) Freitas et al., J Clin Invest 2010; 120:2206

Sources of Research Support: NIDDK-37021.

Nothing to Disclose: GW, SSN, PM, BG, RML

Pub #	P1-660
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	Thyroid Hormone Analog and ENDO- and Xenobiotic Substrate Preference of High-Affinity Thyroxine Transporter OATP1C1
Author String	JA Schneider, DE Westholm, GW Anderson, JN Rumbley University of Minnesota, Duluth, MN; The College of St Scholastica, Duluth, MN
Body	Organic anion transporting polypeptides (Oatps) are part of the solute carrier (SLCO) superfamily, which include 12 human OATPs paralogs. Oatps contain 12 trans-membrane domains, which mediate the uptake transport of a broad range of compounds in a sodium independent manner. These compounds include bile salts, hormones, steroid conjugates, organic dyes, anionic oligopeptides, and xenobiotics. Oatps are expressed in a variety of tissues which include; intestine, liver, kidney, blood brain barrier (BBB), heart, testis, placenta, lung. Our group has been studying Oatp1c1 a high affinity thyroxine (T4) transporter expressed primarily in the BBB and testis. To determine the molecular characteristics for high T4 specificity, inhibition of Oatp1c1 T4 transport by several thyroid hormone derivatives is being investigated, e.g. T4, T3, rT3, T2, T1, and T4amine. Establishing Oatp1c1 thyroid hormone preference has implications for the delivery of thyroid hormone agonists to the brain in the treatment of neurological disorders such as Allan-Herndon-Dudley syndrome. This syndrome is due to mutations in the MCT8 transporter responsible for delivery of T3 to the neurons and leads to disruption of normal development of the brain. Oatp1c1 inhibition studies have revealed IC50 values for the following derivatives; T4: 0.0047[micro]M, T4 acetic acid: 0.013 [micro]M, T4 amine: 0.128[micro]M, T3: 15[micro]M, rT3: 0.12[micro]M, T2: 248 [micro]M, T1 amine: 13.4 [micro]M. In addition, we are interested defining Oatp1c1 substrate preference more broadly with a goal to utilize Oatp1c1 as a BBB transporter of novel neurologically active drugs. We present IC50 values for a number of additional potential Oatp1c1 substrates. A compilation of these results are used to generate an Oatp1c1 specific pharmacophore and comparative molecular field analysis (CoMFA). Pharmacophore analysis determines a set of necessary features on the compounds in reduced representation. CoMFA determines the correlation between the biological activity (IC50) and 3D shape, electrostatic, hydrophobic, and hydrogen bonding properties. CoMFA displays areas vital for transport of the measured set of molecules (training set) with which one will be able to predict the inhibitor potential of novel compounds. The measured compounds and corresponding IC50 values include: BSP: 0.81 [micro]M, Estrone-3-sulfate: 2.47[micro]M, 4-hydroxytamoxifen: 33.1 [micro]M, simvastatin: 15.89 [micro]M, taurocholate: 29.05 [micro]M, mifepristone: 6.7 [micro]M, probenecid: 42.9 [micro]M, WY-14643: 15.1 [micro]M. Nothing to Disclose: JAS, DEW, GWA, JNR

Pub #	P1-661
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	BAT Thermogenic Deficiency in Thyroid Hormone Receptor Alpha-Null (*Thra-0/0*) Mice: Evidence for a Cellular Defect Caused by Underexpression of *Thra*-dependent Novel Genes Needed for T_3 Activation of UCP1
Author String	P Sherchan, W Ramadan, P Pelletier, O Sideleva, JE Silva Baystate Medical Center, Springfield, MA; University of Vermont, Colchester, VT
Body	TRa0/0 mice are cold intolerant due to impaired brown adipose tissue (BAT) thermogenesis, although all major genes known to be involved respond normally to adrenergic stimulation. This suggests either a defect in brown adipocytes (BATcytes) to *activate* UCP1 or an *intrinsic defect in the mitochondria*. Therefore, we examined comparatively the oxygen consumption rate (OCR, surrogate of thermogenesis) in mature, intact BATcytes or isolated mitochondria from wild type (WT) or *Thra-0/0* mice, at baseline or in response to various stimuli as well as the pattern of gene expression of BATcytes from both genotypes. Neither cAMP production nor lipolysis (glycerol release) to adrenergic stimulation was affected by the genotype. BATcytes OCR was examined in the presence of oligomycin to measure only uncoupled respiration. Results are expressed per 10^6 cells, corrected by prohibitin to document similar mitochondrial content, which was virtually identical in 3 of 4 experiments. *Thra-0/0* cells showed: baseline OCR 40-50% lower in *Thra-0/0* BATcytes ($P<0.05$); 1 mM norepinephrine increased OCR 4.5-fold in WT but only 1.4 fold in *Thra-0/0* BATcytes; the subsequent addition of 4 mM oleate stimulated OCR 44% in *Thra-0/*0 but *not* in WT BATcyte ($P <0.05$). Overall, stimulation to NE and oleate was 60-70% reduced in *Thra-0/0* BATcyte ($P<0.0001$). Experiments in isolated mitochondria showed no significant differences in baseline OCR, in suppression by GDP or in stimulation by oleate, while FCCP did not further increase respiration indicating full uncoupling. Most prominent differences in gene expression were the absence or extreme underexpression of fatty acid binding protein 1 (FABP 1), transthyretin and ApoA1 in *Thra-0/0* BATcytes. Other genes were significantly under- or over-expressed in *Thra-0/*0 mice, but to a lesser degree. FABP1, TTR and ApoA1were underexpressed since the development of BAT (14 d fetuses). Such differences were not seen in the *Thra-0/*0 mice livers (predominantly *Thrb*). In WT mice, all 3 genes were reduced in hypothyroidism, and stimulated by T_3 (4 mg/100/d x5d; 8-fold production rate), but not T_4 replacement or KB141, a *Thrb*-selective agonist. Conclusions: Defective BAT thermogenesis in *Thra-0/0* mice is due to an intrinsic defect of BATcytes, due to the failure of *Thra-0/0* BATcytes to express genes that require *Thra* for thyroid hormone stimulation. We suggest these genes are needed to provide fatty acids and retinoic acid for UCP1 activation. Nothing to Disclose: PS, WR, PP, OS, JES

Pub #	P1-662
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	Embryonic and Neonatal Iron Deficiency Impairs Thyroid Hormone-Dependent Gene Transcription in the Neonatal Rat Hippocampus
Author String	TW Bastian, J Anderson, SJ Fretham, JR Prohaska, MK Georgieff, A Charging Hawk, GW Anderson University of Minnesota Duluth, Duluth, MN; University of Minnesota, Minneapolis, MN; University of Minnesota Duluth, Duluth, MN
Body	Micronutrients play a critical role during late mammalian brain development. Copper (Cu), iron (Fe), and thyroid hormone (TH) deficiencies lead to similar defects in late brain developmental processes including reduced myelination of axons and aberrant hippocampal structure and function, suggesting that these micronutrient deficiencies share a common mechanism that contributes to these derangements. Studies in rodents and humans indicate that Cu and Fe deficiencies affect the hypothalamic-pituitary-thyroid axis, leading to altered thyroid hormone status. We recently demonstrated that embryonic/neonatal Cu and Fe deficiencies reduce circulating and brain TH concentrations in neonatal rats. We hypothesize that the reductions in TH concentrations associated with these deficiencies will affect TH-dependent gene expression and contribute to the derangements in brain development observed in Cu- and Fe-deficient rats. To test this hypothesis we rendered pregnant Sprague-Dawley rats Cu-, Fe-, or TH-deficient from early gestation through weaning. Metal deficiencies were induced through Cu- or Fe-deficient diets. Mild or moderate TH deficiencies were induced by addition of either 1 or 3 ppm propylthiouracil (PTU) in the drinking water. Total thyroxine (T4) and triiodothyronine (T3) concentrations were subsequently measured in the serum and brains (T3 only) of rat pups at postnatal (P) day 10. Iron deficiency reduced serum total T3 by 34% ($p < 0.05$), serum total T4 by 47% ($p < 0.05$) and whole brain T3 by 18% ($p < 0.05$) at P10. Hippocampal mRNA expression was assessed for several genes known to be TH-responsive in the developing brain. Fe deficiency reduced Parvalbumin (Pvalb) and Myelin basic protein (Mbp) P10 hippocampal mRNA levels by 37% ($p < 0.05$) and 41% ($p < 0.05$), respectively. Fe deficiency also resulted in trends towards altered P10 hippocampal mRNA expression for several other genes including Myelin-associated oligodendrocyte basic protein (Mobp) and Type II deiodinase (Dio2). These results indicate that some of the brain defects associated with Fe deficiency may be mediated through altered thyroidal status and the concomitant alterations in TH-dependent hippocampal gene transcription. We are currently assessing the expression of additional TH-responsive genes in P10 Fe- and TH-deficient hippocampi and cerebral cortices. Sources of Research Support: National Insitutes of Health Grant 5RO3HD055423-02. Nothing to Disclose: TWB, JA, SJF, JRP, MKG, ACH, GWA

Pub #	P1-663
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	New Insights into the Effects of 3-Iodothyronamine (T1AM) on Metabolism in Mice from Cavity Ring Down Spectroscopy (CRDS)
Author String	G Chiellini, J Haviland, H Reliand, D Butz, FM Assadi-Porter, TS Scanlan, R Zucchi University of Pisa, Pisa, Italy; University of Wisconsin, Madison, WI; Oregon Health and Science University, Portland, OR
Body	The 3-iodothyronamine (T1AM) is an endogenous thyroid hormone derivative that is found in vertebrate tissues as well as in the circulatory system, and has physiological effects opposing those of thyroid hormone (1). Administration of T1AM to rodents results in rapid and profound reduction in body temperature, heart rate and metabolism. Since its discovery, the pharmacology experiments with T1AM have exclusively involved single, high-dose T1AM administration while following biological effects that occur rapidly, generally peaking within 1 h after injection. In particular, it was recently discovered that single doses of T1AM administered to rodents dramatically switch fuel utilization away from carbohydrates and toward lipids (2). The aim of the present study is to analyze the metabolic response to chronic, low-dose T1AM administration in mice. To evaluate both the short-term and the long-term effects of T1AM, we injected mice daily for one week with a low-dose (10 mg/Kg body weight) of T1AM and followed changes in body weight over three weeks. A significant weight loss was associated with T1AM administration on a high-calorie diet. After T1AM withdrawal mice regained only part of lost weight in the following weeks, indicating long-lasting effects of this natural molecule. Real-time sampling of breath carbon in exhaled CO2 (13CO2/12CO2 delta value) by cavity ring down spectroscopy (CRDS) is a noninvasive method excellent for monitoring weight loss and daily substrate utilization for energy expenditure (3). Shifts in 13CO2/12CO2 delta value shortly after administration of T1AM challenged animals indicate a switch from carbohydrate to lipid metabolism, leading to high levels of weight loss. The weight loss was found not to be associated with a decrease in appetite during both short and long durations of time. In conclusion, metabolic switching leading to lipid mobilization by T1AM may provide a new opportunity for development of this endogenous compound as an effective human weight-loss drug. (1) Ianculescu, AG and Scanlan TS, Molecular BioSystems. 2010; 6:1338 (2) Braulke LJ et al., Journal of Comparative Physiology B Biochemical Systemic and Environmental Physiology 2008; 178:167 (3) Butz DE et al. , Rapid Communications in Mass Spectrometry 2009; 23:3729 Nothing to Disclose: GC, JH, HR, DB, FMA-P, TSS, RZ

Pub #	P1-664
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	Role of LDL Oxidation on Non-Genomic Thyroid Hormone Action in Human Endothelial Cells
Author String	R Vicinanza, G Coppotelli, C Malacrino, T Nardo, B Buchetti, L Lenti, FS Celi, S Scarpa Sapienza University of Rome, Rome, Italy; Karolinska Institutet, Stockholm, Sweden; National Institutes of Health, Bethesda, MD
Body	The crucial role of TH in regulation of lipid metabolism, cardiac contractility, and systemic vascular resistance (SVR) has been well established, but little information is currently available on the modulation of non-genomic TH action on peripheral endothelial function. While hypothyroidism is associated with an increase in the SVR and in low-density lipoprotein (LDL) serum levels, oxidized-LDL (oxLDL) may in turn adversely affect the endothelium by inducing the expression of adhesion molecules, ultimately promoting atherosclerosis. Mounting evidence indicates that TH modulates endothelium-dependent vasodilatation by activating the endothelial nitric oxide (NO) synthase (eNOS) via PI3K/Akt non-genomic signaling pathway. Since oxLDL can also inhibit Akt activation, we investigated the effect of native LDL (nLDL) or oxLDL on non-genomic TH action in Human Endothelial Cells (HUVEC). When HUVEC were treated with 100 nM T3, western blot analysis showed a significant increase of Akt phosphorylation at ser.473 and eNOS phosphorylation at ser.1177. Further, when HUVEC were treated with oxLDL, an inhibition in Akt phosphorylation was observed, mimicking the inhibitory effect of Wortmannin. Pretreatment with the antioxidants, Vitamin E (150 [mu]M) and Vitamin C (150 [mu]M), prevented the inhibitory action of oxLDL on Akt phosphorylation. Finally, an inhibition in T3-mediated NO and cyclic guanosine monophosphate (cGMP) production, after incubation with oxLDL, was also observed ($p<0.0001$). In conclusion, we demonstrate that LDL oxidation may contribute to a blunt in the non-genomic TH action on vascular endothelium, suggesting that oxLDL, aside from inducing the atherosclerotic process, may also promote a mechanism of peripheral, tissue- and pathway-specific resistance to TH. Sources of Research Support: Sapienza University of Rome and in part by the Intramural Research Program of the NIDDK-NIH. Nothing to Disclose: RV, GC, CM, TN, BB, LL, FSC, SS

Pub #	P1-665
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	The Role of LAT1 Expression in TH-Mediated Development Using a LAT1-Knockout Mouse Model
Author String	F Mitchell, Y-B Shi, P Taylor, A Ibrahim National Institutes of Health, Bethesda, MD; University of Dundee, Dundee, UK; University of Dundee, Dundee, UK
Body	Thyroid hormones are required for normal development in vertebrates and must be transported into cells in order to access nuclear receptors and effect their genomic actions. The LAT1 gene encodes the light-chain transport subunit of a heterodimeric glycoprotein-associated transporter complex that accepts both branched-chain amino acids (BCAA) and thyroid hormones (TH) as substrates. LAT1 is widely expressed in the mammalian system and is thought to play a critical role in maintaining BCAA and TH balance in many tissues. LAT1 has been shown to transport TH in a number of tissues and could therefore have a significant role in TH-mediated development. To enable study of the LAT1 protein in a physiological context, particularly during development, we have generated a conditional LAT1-knockout mouse line using the Lox-P system. In these mice the wild-type LAT1 gene has been substituted with a transgene where a region containing the start codon is flanked by Lox-P sites (FLOXed); these FLOX animals appear to be normal. When Cre-recombinase is co-expressed, the FLOXed region of the LAT1 gene is excised and so transcription cannot take place. The resulting LAT1+/- animals are viable and fertile but no LAT1-/- animals have been produced suggesting that LAT1 expression is required for embryonic development. This is consistent with the finding that knockout of the LAT1-associated heavy-chain CD98 is embryonic lethal in mice. We are currently using a number of techniques to study the phenotype of the LAT1+/- mice. Preliminary data shows that these mice are around 10% smaller than their FLOX littermates, with no measurable change in food-intake. Quantitative RT-PCR data confirms that the expression of LAT1 in a number of tissues including the brain and heart is significantly reduced. Uptake of the BCAA phenylalanine into diaphragm is significantly reduced by over half in LAT1+/- mice and BCAA concentrations in both liver and skeletal muscle are significantly reduced from control, as determined by HPLC. These results are consistent with the observed reduction in LAT1 mRNA expression being translated into reduced functional expression of the LAT1 transport subunit. Our current research is focused on determining the role of LAT1 in TH-controlled development at both a molecular (eg. regulation of TH-target genes) and tissue (eg. changes in intestinal morphology) level. Nothing to Disclose: FM, Y-BS, PT, AI

Pub #	P1-666
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	Down-Regulation of Tumor Necrosis Factor alpha Promoter by Thyroid Hormone
Author String	AA Amato, GM Santos, M Togashi, FA Milton, LA Simeoni, FdAR Neves University of Brasilia, Brasilia, Brazil; Medical Research Council, Cambridge, UK
Body	Several chronic conditions, such as kidney disease and heart failure, result in multiple abnormalities in thyroid hormone (TH) physiology, including reduced serum thyroxine and triiodothyronine (T3) levels with unexpectedly normal circulating thyrotropin levels. A number of clinical studies have shown that reduced serum T3 levels represent a negative prognostic index and are strongly correlated to the elevation of circulating systemic inflammatory markers, including C-reactive protein and proinflammatory cytokines such as interleukin-6 and tumor necrosis factor α (TNF-α). Whether decreased serum T3 levels are only an indicator of this systemic inflammatory state or there is a causal relationship between them is still an unanswered question. To address this issue, we investigated the effect of TH and of TH receptor (TR) on the activity of TNF-α promoter. To address this issue, we investigated the effect of TH and of TH receptor (TR) on the activity of TNF-α promoter on human U937 pro-monocytes using reporter gene assays. We found that both TR beta 1 and TR alpha 1 activated TNFα promoter in the unliganded state, and that T3 significantly down-regulated its activity in a concentration-dependent manner. We also showed that T3-dependent down-regulation of TNF-α promoter by TR required the DNA binding domain (DBD) of the receptor, which was recruited to an inhibitory element in the region between -125 and -82 of TNF-α promoter. In order to investigate a direct interaction between the DBD of TR and TNF-α promoter, we carried out equilibrium binding experiments using fluorescence anisotropy assays, which indicated that TR bound directly to the region between -125 and -82 of TNF-α promoter, with a dissociation constant of 257 nM. These results suggest that T3 down-regulates TNF-α promoter through TR in an on-DNA mechanism. It is possible therefore that T3 might directly regulate the inflammatory response and that this effect could be implicated in the correlation between reduced serum T3 levels and the proinflammatory state seen in chronic kidney disease. Nothing to Disclose: AAA, GMS, MT, FAM, LAS, FdARN

Pub #	P1-667
Session Information	POSTER SESSION: BASIC - Hypothalamic-Pituitary-Thyroid Axis: Thyroid Hormone Metabolism, Cellular Uptake & Action (1:30 PM-3:30 PM)
Title	The [lsquo]Prohormone[rsquo] T4 Is Transcriptionally Active in Neuroblastoma Cells
Author String	WE Visser, SMA Swagemakers, Z Ozgur, WFJ van IJcken, PJ van der Spek, TJ Visser Erasmus MC, Rotterdam, Netherlands; Erasmus MC, Rotterdam, Netherlands; Erasmus MC, Rotterdam, Netherlands
Body	Thyroid hormone (TH) is crucial for the development and function of many tissues, in particular the brain. The thyroid gland predominantly secretes T4, whereas T3 is produced by the deiodinases D1 and D2 in peripheral tissues. The genomic actions of TH are mediated via binding of T3 to the TH receptor (TR). In this view, T4 largely serves as a prohormone, from which T3 is generated. This paradigm is mainly based on studies showing that T3 has a 10-100-fold higher affinity for the TR than T4. However, in humans, serum FT4 is ~3-fold higher than of serum FT3. Furthermore, intracellular concentrations of T3 and T4 are also determined by cellular TH transporters and deiodinases. Thus, it may be reasoned that under specific conditions T4 can reach sufficient intracellular levels to bind to the TR, which results in transcriptional activity. We tested the hypothesis that physiological concentrations of T4 have transcriptional activity. SHSY-5Y neuroblastoma cells were cultured in DMEM/F12-medium containing 0,1% BSA with or without 1 10 and 100 nM T3 or T4 (equivalent free hormonal fractions are 15% and 3% for T3 and T4, respectively). After 2-48 h, RNA was harvested and gene expression profiles were generated with Affymetrix Plus 2.0 chips qPCR was used for validation of differentially expressed genes. In parallel, deiodinase activity was assayed in intact cells. Only D3 activity was measured, excluding autocrine signaling by T3 produced from T4 by D2. At a false-discovery rate of 5% and a fold-change of 1.5, we detected 31 transcripts regulated by 10 nM T3. Interestingly, 14 transcripts were induced by 10 nM T3 as well as 10 nM T4. Several genes responded with the same fold induction to T3 or T4 treatment. Even treatment with 1 nM T3 or T4 affected gene expression. Obviously, the present results support the well-known role of the bioactive T3 in modulating gene expression. Furthermore, our data demonstrate that T4 is biologically active under physiological conditions at the transcriptional level. This may imply that T4 is not merely a prohormone. The intrinsic biological activity of T4 may have advances in circumstances where cellular T3 levels are insufficient. Dependent on the differences in cellular TH transporter equipment and/or deiodinase activities T4 may play a direct role in determining the cellular transcriptome. Nothing to Disclose: WEV, SMAS, ZO, WFJvI, PJvdS, TJV

Pub #	P1-668
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	IGF-IR or FGF-Like Stimulatory Autoantibodies in Different Subtypes of Thyroid-Associated Ophthalmopathy
Author String	MB Zimering, AG Gianoukakis, JJ Shin, J Zaitz, EA Nunez, TJ Smith Veterans Affairs New Jersey Healthcare System, East Orange, NJ; University of Michigan, Ann Arbor, MI; UMDNJ-New Jersey Medical School, Newark, NJ; Harbor-UCLA Medical Center, Los Angeles, CA
Body	Insulin- like growth factor 1 (IGF-1) receptor antibodies have been implicated in the pathogenesis of thyroid associated ophthalmopathy (TAO) via effects on IGF-1 receptors the expression of which increases in TAO-derived, orbital fibroblasts. Fibroblast growth factor (FGF)- like autoantibodies (AA) have been associated with endocrine neoplasms. We used endothelial cell (EC) proliferation assays to test whether a subset of Graves' disease (GD) IgG AA may share activities with FGF. Protein A eluate fractions of serum from 6/12 uncomplicated patients with GD exhibited significantly increased growth stimulatory activity in EC, mean: 151 $\pm$ 13% compared to control cells incubated (for 4 days) with medium 199 and 10% FCS. Mean EC growth promoting activity in GD sera IgG (123 $\pm$ 35%, n=12) significantly exceeded activity in normal sera o those from other autoimmune conditions (93 $\pm$ 10%, n =10, P = .02). GD EC stimulatory AA activity was not associated with anti-TPO, anti-TG, or TSI titer, and it occurred in both patients with (n=4) and without (n=2) TAO. One of 12 patients with GD tested had EC inhibitory activity (59%) and the highest titer of anti-TG AA A patient with TAO and severe extra-ocular muscle (EOM) involvement displayed high titer EC AA activity (143-176%) that was significantly neutralized (~70%) by monoclonal anti-IGF-1R antibodies. A patient with longstanding, euthyroid GD complicated by type 2 diabetes, macular edema & focal segmental glomerulosclerosis presented (during taper of high dose glucocorticoids) with globe prolapse secondary to orbital fat hypertrophy. His plasma contained low titer inhibitory (57%) and high titer stimulatory AA activity (143%) which was completely neutralized by anti-bFGF antibodies. Similar low titer inhibitory and high titer stimulatory AA was detected in longstanding adult type 1 diabetes with autoimmune thyroiditis and euthyroid GD with orbital fat and EOM hypertrophy. These data suggest a possible association between EC inhibitory and/or (FGF-like) autoantibodies and a subset of TAO. Although the mechanism is unclear, dexamethasone and bFGF promoted adipose-derived mesenchymal stromal cell expansion *in vitro* suggesting that glucocorticoids and FGF-like AA might promote orbital fat expansion. In euthyroid GD with globe prolapse, color Doppler imaging revealed a [ldquo]thyroid inferno[rdquo] pattern. It remains uncertain whether FGF-like, anti-IGF-1R or other GD IgG contribute to intra- or extra-thyroidal angiogenesis. Nothing to Disclose: MBZ, AGG, JJS, JZ, EAN, TJS

Pub #	P1-669
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Thyroxine Autoantibody in a Patient with Hashimoto Thyroiditis and Cryoglobulinemia
Author String	EM Alford, C Potter, MI Hu The University of Texas MD Anderson Cancer Center, Houston, TX; Baylor College of Medicine, Houston, TX
Body	Introduction Autoimmune thyroid disorders are relatively common, with approximately 1% of the general population affected and as many as 5-10% of reproductive age women affected. Most commonly in iodine-sufficient regions, anti-thyroperoxidase (TPO Ab) and anti-thyroglobulin antibodies (Tg Ab) account for chronic autoimmune thyroiditis. Autoantibodies directed against either T4 or T3 are rare causes of elevation of TSH. Here, we present a patient with Hashimoto's thyroiditis and symptomatic hypothyroidism and elevated TSH but with paradoxically elevated free T4. Case Presentation A 42 year old female with history of marginal zone leukemia, hepatitis C, and cryoglobulinemia vasculitis presented 4 years ago with more than 130 pound weight gain and fatigue. She was evaluated by her primary care physician and diagnosed with hypothyroidism with an elevated TSH. She was started on thyroid hormone replacement with improvement of symptoms. She rapidly lost weight and was found to be over replaced, and her dose was decreased. However, because of continued elevation of TSH, she was referred for further evaluation. Repeat thyroid hormone levels showed TSH 17.28 mcu/ml (range 0.5-5.5) with free T4 >5 ng/dl (range 0.9-1.8) and total T3 78 ng/dl (range 80-190). She was clinically hypothyroid with cold intolerance, fatigue, weight gain, and dry skin. Because of this unclear overall picture, further analysis was performed. This showed patient had elevated TPO Ab 13,850 IU/ml (range <35) and Tg Ab 158,400 IU/ml (range <40), consistent with Hashimoto's thyroiditis. However, as this did not explain her thyroid function studies, further testing for presence of anti-T4 and anti-T3 antibodies was performed. She was found to have anti-T4 antibody but no anti-T3 antibody. After being placed on higher dose of levothyroxine, free T4 equilibrium dialysis was 1.4 ng/dl (range 0.8-2), while the standard free T4 was > 5 ng/dL and concomitant TSH of 1.81 mcu/ml. Conclusion Several factors are known to interfere with thyroid hormone assays, including medications and medical conditions such as AIDS and Hepatitis C. To our knowledge, this is the first report of a patient with cryoglobulinemia producing an anti-T4 antibody leading to an elevated free T4 by standard testing. Treatment is guided by normalization of the TSH and free T4 by equilibrium dialysis. When the clinical picture and the laboratory assays appear incongruous, other causes for abnormal thyroid hormone assays should be explored. Nothing to Disclose: EMA, CP, MIH

Pub #	P1-670
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Predictors of Resistance to Radioiodine Ablation for Graves Disease
Author String	C Norman, R Bahn, Y Durski, M Stan Mayo Clinic, Rochester, MN; Mayo Clinic, Rochester, MN
Body	Introduction: Current studies suggest that up to 20% of patients with Graves disease treated with radioiodine ablation (RAI) fail the first treatment and require a subsequent dose. This is more frequent in patients with larger goiters or severe hyperthyroidism. We assessed several factors as potential predictors of initial RAI ablation failure in patients with Graves' disease. Patients/Methods: 202 patients treated with RAI at Mayo Clinic in 2005 and 2006 were included in the study. Patient data was collected by retrospective chart review and included demographics, baseline thyroid function tests, thyroid uptake, presence or absence of Graves' ophthalmopathy (GO), prior use of antithyroid drugs (ATD), RAI dose per gram of thyroid and thyroid status at subsequent follow-up visits. Results: Demographics: 202 patients (160 females, mean age 50, range 19-86), received 15.5 ± 5.8 mCi RAI, delivering 255.3 ± 10.2 [micro]Ci RAI/gm tissue for a mean thyroid size 35.8 ± 11.2 gm. RAI was repeated in 23 patients (11.4%), with 3 patients requiring a third treatment. We found main predictors of repeat RAI ablation to be 1) severity of hyperthyroidism (FT4), and 2) prior treatment with ATD. Mean FT4 (ng/dl) for single RAI versus repeat RAI treatment: 3.25 ± 0.16 vs. 5.41 ± 1.01, p=0.0017). Odds ratio (OR) of repeat RAI if patients received ATD prior to RAI is 2.91 (CI: 1.18-7.17, p =0.03). We noted a trend for repeat RAI therapy with higher uptake (52.9 ± 1.5 % for single RAI vs. 60.9 ± 4.6% for repeat RAI, p=0.09) or prior PTU use (OR =2.2, p=0.28) that failed to reach statistical significance. Repeat RAI therapy was independent of thyroid size (35.6 ± 0.9 gm vs. 37.78 ± 2.6 gm, p=0.41), duration of treatment with ATD (in days) before RAI (319 ± 84 vs. 403 ± 146, p=0.67), 24 hr RAI uptake (52.9 ± 1.5 % vs. 60.9 ± 4.6 %, p=0.09), or dose of RAI in mCi given (16.7 ± 5.8 vs. 15.2 ± 4.4, p=0.65). Factors not associated with increased risk of repeat RAI include GO at baseline, OR = 2.12 (0.83 - 5.43, p=0.12), development of new/ worse GO following RAI, OR= 2.01 (0.61 - 6.58, p=0.27); and smoking, OR = 0.96 (0.31 - 3.02, p=0.94). Conclusion: We found the severity of hyperthyroidism (FT4 level) and pretreatment with antithyroid medication be to factors associated with need for repeat RAI treatment. Our data suggests that patients with these characteristics might benefit from administration of higher doses of RAI at initial treatment. Nothing to Disclose: CN, RB, YD, MS

Pub #	P1-671
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Novel Autoantibody Identification in Thyroid Autoimmune Disease
Author String	JS Mammen, LC Rosen, PW Ladenson, A Rosen Johns Hopkins Bayview, Baltimore, MD; Johns Hopkins, Baltimore, MD; Johns Hopkins, Baltimore, MD
Body	Autoantibodies against thyroid peroxidase, thyroglobulin and the TSH receptor are found in many patients with thyroid autoimmune disease but provide limited prognostic information, for example about the risk for Graves Ophthalmopathy, or the risk of long-term hypothyroidism in post-partum thyroiditis. Recent advances in understanding autoimmunity suggest that antibodies target proteins which are upregulated during the disease process, thereby amplifying and propagating the autoimmune response. We hypothesized that novel autoantibodies targeting antigens upregulated during thyroid regeneration could be involved in the pathogenesis of thyroid autoimmune disease. In order to identify such antigens, we prepared cell lysates from fresh human thyroid follicle cells isolated from surgical goiter samples. Autoantibodies recognizing antigens specifically expressed in these lysates were defined by immunoblotting lysates of these cells adjacent to Hela cell lysates. Screening over 100 patients with autoimmune thyroid disease in this way, several bands were detected in the sera of multiple patients that represented novel antibodies directed against thyroid-specific autoantigens in follicular cell lysates but not Hela cell lysates. These thyroid-specific antigens, recognized by sera from patients with autoimmune thyroid disease were then identified by mass spectroscopy after preparative 2D gel electrophoresis. The first such antigen we pursued was a ~25KD protein found in 10% of our cohort, which was identified as PEBP-1, a negative regulator of Raf and NFkB signal transduction pathways. Immunofluorescence in normal thyroid and an autoimmune mouse model demonstrates staining of PEBP-1 in a subset of follicular cells in small immature follicles. This suggests that in some patients with autoimmune thyroid disease, the immune system may target those cells responsible for the regeneration and repair of the damaged gland, potentially providing a mechanism for the propagation of autoimmune disease in these patients. This in turn suggests targeting this pathway in the development of novel therapeutic interventions. Nothing to Disclose: JSM, LCR, PWL, AR

Pub #	P1-672
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Clinical Value of Thyroid Ultrasonography in Patients with Subacute Granulomatous Thyroiditis
Author String	H-C Kang, S-J Kim, J-H Yun, H-K Kim Chonnam University Medical School, Gwangju, Republic of Korea
Body	**Background :** Subacute granulomatous thyroiditis (SAT) is an inflammatory thyroid disease characterized by transient thyrotoxicosis and thyroid pain. The reports describing the ultrasonographic changes in patients with SAT are not common, although there have been increasing use of thyroid ultrasonography (US) in diagnosing and monitoring of thyroid pathology. The aim of this study was to evaluate the value of thyroid US in acute stage of SAT and in the follow-up period. **Methods :** The study group included 30 patients with SAT diagnosed on clinical and cytological grounds. All patients were evaluated by ultrasonography equipped with 10 MHz probe at presentation and followed-up for up to 2 years. Thyroid volume (TV) was calculated by the formula of ellipse using data obtained from the thyroid US. Clinical and laboratory parameters were compared in ultrasonographically distinct groups. **Results :** Ill-defined hypoechoic nodular lesion was the most common finding in patients with SAT. 53.3% of the patients showed nodular involvement and the remainder showed diffuse involvement of one or both thyroid lobes. Fine needle aspiration cytology (FNA) was diagnostic of SAT in all cases and US-guided FNA was very helpful in patients with nodular SAT. Among clinical and laboratory parameters, diffuse involvement group showed higher ESR (78.6±27.6 *vs.* 51.2±30.0 mm/hr) and female preponderance. Initially, the TV was increased (15.4±5.3 ml) and it rapidly decreased to near normal value at 1 month and then further decreased to the nadir (6.2 ±3.2 ml) at 1 year. Four patients with TSH elevation showed persistent thyroid atrophy at 2 years after the initial SAT episode. **Conclusion :** We believe that thyroid ultrasonography has a useful supporting role in the initial diagnosis and follow up of patients with SAT. It provides useful information regarding the degree of anatomical involvement and facilitates FNA by providing US-guidance. Additionally it allows determination of TV which might have some implications in determining the disease activity and the possibility of later development of permanent hypothyroidism. Nothing to Disclose: H-CK, S-JK, J-HY, H-KK

Pub #	P1-673
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Rhabdomyolysis Post Treatment of Graves Hyperthyroid Patients: A Case Series
Author String	C Seow, W Hoi, R Dalan Tan Tock Seng Hospital, Singapore
Body	Background Myopathy has been reported in hyperthyroid patients after initiation of thioamides, total thyroidectomy and I-131 therapy. We report three patients who developed rhabdomyolysis after rapid decline in thyroxine levels upon initiation of treatment. Clinical Case Series Case 1: A 21 years Filippino lady presented with Graves' Disease with fT4 of 56 pM (RI: 8-21) and TSH of <0.01 mIU/L. She developed myalgia 4 weeks after starting carbimazole (CMZ) at a dose of 20mg daily. Investigations at this time: fT4 11 pM, TSH <0.01 mIU/L, CK 4645 U/L (RI: 40-200), aldolase 14 U/L (RI: 3-10 U/L), Urine myoglobin <21 ug/L. After intravenous hydration and reduction of CMZ dose to 5mg daily, myalgia resolved in about a week. However, CK still remained elevated at 720 U/L 10 weeks later. She is still on active follow up and we are monitoring her CK. Case 2: A 32 years Chinese man with background Charcot Marie Tooth Disease presented with Graves' disease with a fT4 of 52.1 pM and a TSH of 0.01 mIU/L. He developed myalgia 3 weeks after initiating CMZ 20 mg daily. At this time his fT4 was 8.3pM, TSH<0.01 mIU/L, CK 2184 U/L. After hydration and dose reduction of CMZ to 5mg daily, his symptoms subsided. The CK normalized to 161 U/L 10 weeks later with complete resolution of symptoms. Case 3: A 49 years Chinese man with Graves Disease was referred for I-131 therapy. Prior to therapy, his fT4 was 30 pmol/L (RI: 8-21) and TSH <0.05 mIU/L. Eight weeks after receiving 18mci of I-131 he developed severe myalgia. At this time his fT4 was 9 pM, TSH<0.01mIU/L, CK 31012 U/L, aldolase 31 U/L and Urine myoglobin <21 ug/L. EMG showed no electrophysiological evidence of primary muscle disease. Symptoms of myalgia improved with hydration and thyroxine replacement. However, CK normalized only after 4 months. Conclusion Relative hypothyroidism from rapid correction of thyrotoxicosis has been proposed as the main mechanism for the myopathy. Relative hypothyroid-associated myopathy may be overlooked given its presentation only after starting treatment for hyperthyroidism and therefore may be attributed to the side effects of thioamides. Most case studies reported rapid normalization of CK concentrations in weeks but in this case series, it may take up to 4 months for the CK concentrations to normalize completely. Increased awareness of this entity with slower correction of a patient's thyrotoxicosis may help to avert the occurrence of this event. Nothing to Disclose: CJS, WHH, RD

Pub #	P1-674
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Changes of TgAb Epitope Pattern after Iodine-131 Therapy for Graves Hyperthyroidism
Author String	F Latrofa, D Ricci, L Montanelli, B Mazzi, M Tonacchera, C Ceccarelli, F Lippi, F Bianchi, F Brozzi, P Santini, A Pinchera, P Vitti University of Pisa, Pisa, Italy
Body	Serum thyroglobulin (Tg) autoantibodies (AbTg) epitope pattern is restricted in patients with Hashimoto's thyroiditis and Graves' Disease. We evaluated whether it is changed by 131-I treatment for Graves' hyperthyroidism. Sera of 34 GD patients were collected at the time of 131-I treatment (time 0) and 3 (34 patients), 6 (30 patients) and 12 (16 patients) months thereafter (mo 3, mo 6 and mo 12, respectively). To prevent Graves' Ophthalmopathy exacerbation, all patients received oral prednisone (initial daily dose: 0,5 mg/kg, tapering ofl in 3 months). TgAb titers were evaluated by AIA-Pack, Tosoh Bioscience, Japan. Serum TgAb binding to Tg was inhibited by a panel of four recombinant human TgAb-Fab, recognizing Tg epitope regions A-D. The ability of single TgAb-Fab and that of the 4 TgAb-Fab pool to inhibit the binding of serum TgAb to Tg was evaluated in ELISA and using $^{125\text{-}I}$Tg binding, respectively. Percent of Tg binding inhibition was calculated comparing the binding of serum TgAb in presence of each TgAb-Fab (or the TgAb-Fab pool) with that in its absence. Results were compared by paired samples T test. Results. In comparison with concentration at time 0 (926±1783 IU/ml) (mean±SD), TgAb were significantly higher at mo 3 (2123±3273 IU/ml) (p=0.042) and at mo 6 (1473±2226 IU/ml) (p=0.036) and comparable at mo 12 (1117±2317 IU/ml). The inhibition at time 0 was 61±15 (mean±SD) for TgAb-FabA, 38±33 for TgAb-FabB, 36±25 for TgAb-FabC and 16±22 for TgAb-FabD. In comparison with time 0, inhibition by TgAb-FabA was significantly lower at mo 3 (57±18) (p=0.045). Inhibition by TgAb-FabD was lower at mo 12 (9±21) than at mo 6 (13±16) (p=0.01). Inhibition by TgAb-FabA and D at the other time points ant by TgAb-FabB and C at any time point was usually lower than at time 0, although not at a statistically significant level. In comparison with time 0 (65±20), inhibition by TgAb-FAb pool was significantly lower at mo 3 (55±20) (p=0.035) and mo 6 (54±23) (p=0.039) and similar at mo 12 (60±24). Our data show that the increase of TgAb concentration following 131-I treatment for Graves' hyperthyroidism is associated with a transient spreading of TgAb epitopes. Nothing to Disclose: FL, DR, LM, BM, MT, CC, FL, FB, FB, PS, AP, PV

Pub #	P1-675
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Remarkable Association between Acute Ischemic Stroke and Cerebral Venous Thrombosis with Autoimmune Thyrotoxicosis
Author String	CC Porto-Silva, TM Vidotto, FA Carvalho, GS Silva, JR Sa, RMdB Maciel, JRM Martins Universidade Federal de São Paulo - UNIFESP/EPM, São Paulo, Brazil; Universidade Federal de São Paulo - UNIFESP/EPM, São Paulo, Brazil
Body	Background: Many abnormalities of blood coagulation have been described in patients with thyroid dysfunction. Here, we described a concomitant and unusual association between ischemic and thrombotic events occurring in a patient with Graves' disease and antiphospholipid antibodies syndrome (APS). Clinical case: A 21-yo woman presented to the ER with right arm weakness and aphasia that were progressive in the last 7 days. She was healthy until 6 months before when she noticed thyrotoxic symptoms. Physical examination showed a large goiter, extremities shivering and tachycardia. A brain MRI/MRA showed a left frontal infarct and an occlusion of the left middle cerebral artery. Transthoracic echocardiogram, CSF analysis, and carotid Doppler ultrasonography were unremarkable. She started on methimazole and propranolol. Laboratory exams at admission were as follows: TSH<0.05[micro]UI/mL; FT4=5.8ng/dL; positives thyroid receptor and antithyroglobulin antibodies. Electrolytes and renal function were normal. Viruses serology were all negatives. Hematologic exams showed an increased factor VIII activity >600% (RR:60-150%) and positive antiphospholipid antibodies, but other exams such as Leiden V factor, homocysteine levels, antithrombin III, rhematoid factor, anti-DNA native, anti-nucleus Hep-2, cryoglobulines and anti-ENA were all normal. Seven days after her admission, she had a sudden decrease in the level of consciousness and was taken to the ICU. A new brain MRI/MRA showed thrombosis of the sagittal, left transverse and sigmoid sinuses. The patient spent 17 days on the ICU receiving iodine, methimazole, prednisone, propranolol and heparin. She was discharged from the hospital presenting only expression aphasia, without motor deficits, taking oral anticoagulant. Conclusion: Thyrotoxicosis has been described as a risk factor for ischemic and cerebral venous thrombosis in some reported cases. However, the concomitant association of arterial and venous thrombosis is an extremely rare situation. There are indications that genetic predisposition can explain the presence of APS in patients with autoimmune thyroid disorders, and it has been suggested that some anticardiolipin antibodies may act as thyrotropin receptor-stimulating antibodies. This case represents a remarkable condition where a hypercoagulability state, represented by thyrotoxicosis, APS and coagulation test disorder should be suspected, especially when occurring in a younger subject. (1) J Clin Endocrinol Metab, July 2007, 92 (7): 2415-2420 Nothing to Disclose: CCP-S, TMV, FAC, GSS, JRS, RMdBM, JRMM

Pub #	P1-676
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	The Utility of TSH Receptor Antibody (TBII) as a First-Line Test in the Management of Hyperthyroidism -- A Large Retrospective Observational Study
Author String	AC Madathil, JH Parr, ML Andrew, S Wahid, S Razvi Gateshead Health NHS Foundation Trust, Gateshead, UK; South Tyneside NHS Foundation Trust, South Shields, UK; South Tyneside NHS Foundation Trust, South Shields, UK

Body

Background

Hyperthyroidism affects 2-3% of the general population. The initial diagnostic evaluation of patients with hyperthyroidism is variable ranging from very little diagnostic evaluation to full range of biochemical, immunological and radiological investigations. The diagnosis of cause of hyperthyroidism has implications for the management and prognosis of the disease. Therefore, studies investigating appropriate initial diagnostic tests in hyperthyroidism are required. This may lead to a pragmatic and simplified diagnostic pathway for patients with hyperthyroidism.

Objectives

1.Is measurement of TBII as first line investigation in all patients with hyperthyroidism efficient?
2.What cut-off of TBII should be used to diagnose Grave's disease (GD)?

Methodology

This was a retrospective observational study. All consecutive patients with first episode of hyperthyroidism referred to our endocrinology unit in the last 8 years were included. They underwent a fixed protocol for investigation of hyperthyroidism i.e. thyroid peroxidase antibody, TBII, thyroid technetium uptake scan and US scan of thyroid; regardless of the initial clinical suspicion of the etiology of hyperthyroidism. The outcome for the study was to estimate the sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) of TBII in diagnosing GD using two different cut off values >1.0 IU and >1.5 IU.

Results

A total of 478 patients (83.5% women) with mean age of 52 years were available for analysis. GD was diagnosed in 57% of patients, toxic nodular goiter in 32.7% and thyroiditis in the remaining 10.3%. Sensitivity, specificity, PPV and NPV for TBII with a cut off >1.5 IU for GD were 87.1%, 93.7%, 95% and 84.7%, respectively. And that for TBII with cut off >1.0 IU were 90%, 87%, 90% and 86.5%, respectively. The prevalence of TBII negative GD was 12.8%.

Discussion

In this largest series to date, we found that a TBII value of >1.5 IU has high probability of accurately diagnosing GD (PPV 95%). But it did not reliably exclude GD given lower NPV of 84.7%. On the other hand TBII value of >1.0 IU had lower accuracy in diagnosing GD. We suggest that TBII should be used as the first line test in the diagnostic pathway of hyperthyroidism and only those patients whose TBII levels are <1.5 IU need further tests to elucidate the etiology of hyperthyroidism. This could reduce costs and inconvenience in a significant majority of hyperthyroid patients.

Nothing to Disclose: ACM, JHP, MLA, SW, SR

Pub #	P1-677
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	[ldquo]Normal[rdquo] TSH Caused by Heterophile Antibodies Delays Diagnosis in Two Patients with New-Onset Graves Disease Associated with Autoimmune Polyglandular Syndrome Type 2
Author String	A Omer, LV Rao, M Safran, MC Blendea University of Massachusetts, Worcester, MA; University of Massachusetts, Worcester, MA
Body	**Background**: TSH is the key parameter to evaluate thyroid function and screen for thyroid disease. Although rare, interference of heterophile antibodies in the TSH assay results in falsely elevated, decreased or inappropriately normal values, thus delaying diagnosis of thyroid disease. We present two cases of Autoimmune Polyglandular Syndrome Type 2 (APS2), both with new onset Graves' disease, who had non-suppressed serum TSH levels by Beckman Access chemiluminescence assay. Both had suppressed TSH by a different manufacturer (Roche) chemiluminiscence assay. **Case 1:** A 28 years old female known with autoimmune adrenal insufficiency was evaluated for 20 lbs weight loss, new goiter and mild proptosis. Initial TSH was normal at 2.03 mIU/L (n:0.28-3.89 mIU/L). Anti-thyroid antibodies were negative. Within 6 months, she lost another 20 lbs. Serum FT4 was 4.32 ng/dL (n:0.58-1.64 ng/dL) and TT3 was 635 ng/dL (n:87-178 ng/dL). However, TSH level by Beckmann assay was still normal, at 0.32 mIU/L. Serum RF and human antimouse antibodies (HAMA) were negative. She had elevated thyroid stimulating immunoglobulins (TSI) at 377% (n<129%). A TSH by enzyme immunoassay (EIA) was found to be suppressed at 0.01 IU/mL. **Case2:** A 21 years old female with vitiligo was evaluated 8 months postpartum for weight loss. One month prior to the visit she had a FT4 of 4.10 ng/dL, TT3 of 566 ng/dL and TSH of 0.02 mIU/L. She had positive anti-thyroperoxidase antibodies of 217 IU/mL (n:<35 IU/mL). At the time of the visit, FT4 was 3.65 ng/dL, TT3 was 391 ng/dL, but TSH by Beckmann assay was normal at 0.41 mIU/L. Her TSI was elevated at 215% (n<129%), and the radioactive iodine uptake was 47% (n: 8-30%). A repeat TSH by Roche assay was appropriately suppressed at 0.01 mIU/L. **Clinical lessons**: In case of discrepancy between clinical presentation suggesting thyrotoxicosis and [ldquo] normal[rdquo] TSH levels, the presence of heterophile antibodies is suspected. Repeat TSH by alternative methods can help identify these cases, thus avoiding delays in diagnosis and treatment. Patients with APS2 might represent a subgroup more prone to heterophile antibody interference, as illustrated in our two cases. This might be due to the presence of polyreactive immunoglobulins, encountered frequently in autoimmune disease. A full thyroid panel should be considered when screening for thyroid disease in these patients. To our knowledge, this is the first report of association between heterophile TSH antibodies and APS2. Nothing to Disclose: AO, LVR, MS, MCB

Pub #	P1-678
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Phenotypic Expression of Euthyroid Graves Disease: A Case Series
Author String	E Chng, R Dalan Tan Tock Seng Hospital, Singapore
Body	**Introduction:** Euthyroid Graves' disease (EGD) is characterized by clinical thyroid eye disease (TED) accompanied by normal circulating thyroid hormone levels (TFT) and no prior treatment of hyperthyroidism. It is rare, representing 8 to 21 percent of TED cases. We describe the phenotypic expression, biochemical features and outcomes of 6 cases of EGD. **Case reports:** **Case 1:** A 45 year old lady presented with proptosis and CT orbits confirmed TED. She initially had subclinical hyperthyroidism (free T4 12pmol/L (8-21), TSH 0.13mIU/L (RI:0.34-5.6)) while her TRAb was 3.6IU/L (RI<1.7). During 6 months of follow-up, she remained euthyroid. **Case 2:** A 58 year old gentleman presented with vertical diplopia. CT orbits confirmed TED. He remained euthyroid during follow-up (initial free T4 15pmol/L (RI: 8-21), TSH 0.52mIU/L (RI:0.34-5.6)) and his TRAb was 1.1IU/L (RI:<0.4). **Case 3:** A 35 year old gentleman presented with dry eyes and proptosis. He remained euthyroid throughout follow-up (initial free T4 12pmol/L (RI:8-21), TSH 0.49mIU/L (RI:0.34-5.6)). Although his TRAb was 1.4IU/L (RI:<1.7), TSI was elevated at 1946% (50-179). **Case 4:** A 45 year old gentleman with diplopia initially had subclinical hyperthyoidism (free T4 14pmol/L (10-20), TSH <0.01mIU/L (0.29-3.77)) but was euthyroid on subsequent follow-up of 4 years. His TRAb was 2.2IU/L (<1.7). **Case 5:** A 54 year old gentleman with proptosis had TED confirmed on CT orbit. He was euthyroid (initial free T4 16pmol/L (10-20), TSH 0.51mIU/L (0.4-3.98), TRAb 6.2IU/L (<1.7)) for the first five years of follow-up before developing hyperthyroidism (free T4 14pmol/L (10-20), TSH 0.07mIU/L (0.29-3.77), TRAb 3.4IU/L (<1.7). **Case 6:** A 60 year old gentleman with history of treated Graves' 40 years ago presented with ophthalmoplegia. CT orbit confirmed TED. His TFTs were normal (initial free T4 12pmol/L (8-21), TSH 0.95mIU/L (0.34-5.6) whilst his TRAb was <0.4IU/L (<0.4). **Discussion and Conclusions:** Although Graves' disease is more common in females, EGD seems to have a male predeliction. They all had mild to moderate features of TED and 5/6 patients remained euthyroid throughout a mean follow-up of 16 months. Their TRAb levels tend to be normal or just mildly raised suggesting that while the antibodies against TSHR may be the inciting event, antibody and T lymphocyte reactivity against some other eye muscle antigen may separately and independently lead to TED. Nothing to Disclose: EC, RD

Pub #	P1-679
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Comparison of the Effects of Near-Total Thyroidectomy and Radioiodine Therapy on the Evolution of Patients with Graves Disease
Author String	S Kautbally, O Alexopoulou, D Maiter St Luc University Hospital UCL, Brussels, Belgium
Body	*Introduction*: Radioiodine therapy and thyroid surgery are the two second-line treatments mostly prescribed in Graves' disease (GD), at least in Europe. Both of them have advantages and side-effects and the treatment's choice mainly depends on medical staff expertise and disease characteristics. Until now, there is no clear consensus regarding the optimal radical treatment of GD. *Objectives*: This retrospective study compared the effects of near total thyroidectomy (NTTx) and radioiodine therapy (RAI) on the clinical evolution of GD patients and on the course of thyroid hormone (TSH, FT_4, FT_3) and thyroid stimulating immunoglobulin (TSI) concentrations. The occurrence of side-effects was also studied. *Patients*: We studied 80 patients with a proven GD previously treated with anti-thyroid drugs and requiring either RAI (one single dose of 5-10 mCies of I^{131}; mean ± SD: 8.3 ± 1.7; n=40) or NTTx (n=40) as a second-line therapy performed between 2000 and 2006 at the Cliniques Universitaires St Luc. General characteristics of both groups were not different at diagnosis, except for a larger goiter (24.3 vs. 18.7 ml), a higher FT3 concentration (20.0 vs. 11.1 pg/ml) and a higher prevalence of active Graves' orbitopathy (GO) (15/40 vs. 7/40) in the NTTx group as compared to the RAI group ($p<0.05$). The follow-up lasted between 12 and 36 months. *Results*: A permanent correction of hyperthyroidism was observed in 97% of the patients in the surgical group but only in 73% of patients treated with radioiodine ($p = 0,006$). NTTx was followed by a rapid and constant decrease in TSI levels during the first 9 months with stabilization thereafter. In contrast, after radioiodine therapy a surge of TSI levels was observed during the first 6 months followed by a slow decrease over the next 18 months. At the last available visit, 60% of the patients in the RAI group had still positive TSI as opposed to only 18% in the surgery group ($p < 0,001$). Active GO was observed in 17 patients at the time of radical treatment and its prevalence improved after NTTx (from 33 to 10%) while it worsened after RAI (from 10 to 18%; $p = 0,004$ vs. surgery). *Conclusion*: This study shows that near total thyroidectomy is more efficient than radioiodine to induce rapid and permanent correction of hyperthyroidism, remission of the auto-immune disease and improvement of Graves' orbitopathy. Nothing to Disclose: SK, OA, DM

Pub #	P1-680
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Hyperthyroidism and Thyroid Autoimmunity Induced by Sorafenib in Metastatic Renal Cell Cancer
Author String	C Konca Degertekin, U Coskun, F Balos Toruner, S Buyukberber, M Akturk, U Demirci, M Arslan Gazi University Faculty of Medicine, Ankara, Turkey; Gazi University Faculty of Medicine, Ankara, Turkey
Body	**Background** Sunitinib and Sorafenib are novel agents, that are small molecule inhibitors of a family of thyrosine kinase receptors and they are approved for the treatment of cancers such as advanced renal cell carcinoma. Thyroid dysfunction occurs with the use of these drugs, most frequently in the form of hypothyroidism. However, not much is known about the thyrotoxic effect of these agents. The cases with a suppressed TSH reported previously had findings consistent with destructive thyroiditis showing decreased idodine uptake. **Clinical Case** We are presenting a 58-year-old man with metastatic renal cell cancer, who experienced overt hyperthyroidism after a short course of sorafenib treatment. He had no history of thyroid disease, his previous thyroid function tests were normal and thyroid autoantibodies were negative. He developed symptoms of hyperthyroidism 4 weeks after initiation of sorafenib. He had tachycardia, fine tremor, brisk deep tendon reflexes and a smooth, non-tender, slightly enlarged thyroid gland on examination. No abnormality was found in eye examination. TSH level was suppressed [0.011 IU/ml (0,55 - 4,78)] and serum fT4 and fT3 levels were elevated [8.14 ng/dl (0,89 - 1,76) and 17,3 pg/dl (2,3 - 4,2), respectively]. Tc-99 scintigraphy showed a homogeneously increased absorption; four and twenty-four hour uptakes were 49,5% and 70,1%, respectively suggesting increased endogenous thyroid hormone production rather than destructive thyroditis. He also developed high titers of thyroid autoantibodies [anti-TG: 2500 U/ml (<60); anti-TPO: 2723 U/ml (<60); TSH-receptor antibody: 24,6 U/L (0-14)]; thyroglobulin levels were not elevated [4.76 ng/mL (1,6-59,9)]. The patient started his treatment with methimazole (20 mg/day) and propranolol and he was switched from sorafenib to sunitinib. In 12 weeks time, his hyperthyroidism resolved and the patient developed persistant hypothyroidism requiring levothyroxine therapy. He is still being followed up on 150 mcg/day levothyroxine treatment for 7 months without significant symptoms. **Conclusion** This is the first case of hyperthyroidism induced by thyrosine kinase inhibitor sorafenib demonstrating increased uptake and endogenous hyperproduction. The development of thyroid autoimmunity after thyrosine kinase inhibitor therapy is also noteworthy. Nothing to Disclose: CKD, UC, FBT, SB, MA, UD, MA

Pub #	P1-681
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	A Rare Case of Sarcoidosis Involving the Thyroid Gland in a Patient with Graves Hyperthyroidism
Author String	R Chandra, R Kant, EI Krug Sinai Hospital of Baltimore/Johns Hopkins University, Baltimore, MD; Sinai Hospital of Baltimore/Johns Hopkins University, Baltimore, MD

Body

Introduction: Sarcoidosis (S) affects thyroid gland in less than 2% of cases. It was first described in 1938 (1) Typically it is associated with hypothyroidism. Graves' disease (GD) in patients with S involving thyroid is extremely rare.

Case presentation: 59-y.o. otherwise healthy woman presented with hyperthyroidism and multiple thyroid nodules. Her TSH level was 0.011 mcIU/ml (0.500-4.00), Free T4 -1.47 ng/dl (0.80-1.70), Total T3 of 227 ng/dl (84-172), TSI of 164 % (<130), anti TPO antibody of 10.9 IU/ml (0.00-35.0). Thyroid scan and I-123 uptake revealed heterogeneous 24 hour uptake of 25%, and a dominant nonfunctional nodule at the base of right lobe. Thyroid ultrasound revealed multinodular goiter. Initial FNA of the dominant nodule was non-diagnostic. Treatment with methimazole was initiated. The patient returned for follow-up in 1 year with complains of occasional palpitations and nervousness. She stopped taking methimazole after 2 weeks of treatment due to headaches on daily basis. Repeat work-up showed T3 of 199 ng/dl, TSI of 145 %, anti TPO antibody of 17.9 IU/ml, undetectable anti-thyroglobulin antibody and stable heterogeneous nodules on thyroic ultrasound. She was started on PTU 50 mg TID. Follow-up laboratory work up showed TSH of 0.784 mcIU/ml, Free T4 of 0.99 ng/dl, Total T3 of 165 ng/dl. Repeat thyroid FNA revealed cellular lesion on the right suspicious for follicular neoplasm. Due to bilateral disease subtotal thyroidectomy was performed. Surgical pathology demonstrated multiple noncaseating granulomas within the thyroid bed. Chest x-ray revealed bilateral reticulo-nodular infiltrates. Chest CT scan demonstrated diffuse mediastinal adenopathy with diffuse bilateral reticulonodular infiltrates with innumerable bilateral 1-2 mm nodules. Her angiotensin converting enzyme level was 65 U/L (12-68).

Discussion: The association of S and thyroid autoimmunity has been reported by several studies, with a wide range of variability (3-6). The percentage of antithyroid antibodies ranged from 1.3 to 54.5% in different studies (4,7). Most cases present with hypothyroidism (7). GD and thyroid involvement of S is rarely reported in literature (9-13). In our asymptomatic patient S was not suspected prior to thyroidectomy. She required surgery due to suspicious cytology results. Patients with known S presenting with hyperthyroidism may be treated with anti-thyroid medication or I-131 ablation (7).

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Nothing to Disclose: RC, RK, EIK

Pub #	P1-682
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Long-Term Continuous Methimazole or Radioiodine Treatment for Hyperthyroidism
Author String	F Azizi, V Yousefi, A Bahrainian, F Sheikholeslam, M Tohidi, Y Mehrabi Research Institute for Endocrine Sciences, Tehran, Islamic Republic of Iran
Body	**Background:** Treatment of hyperthyroidism with radioiodine may increase mortality from vascular causes and cancer. It also causes hypothyroidism with high occurrence of subclinical hypo- and hyperthyroidism during thyroid hormone replacement therapy. **Methods:** We enrolled 239 patients with diffuse toxic goiter who had recurrence of hyperthyroidism. One hundred and four patients were randomized into two, the methimazole (MMI) and the radioiodine treatment groups. The remaining 135 patients voluntarily enrolled into two groups. From all patients 59 MMI and 73 radioiodine-treated hypothyroid on thyroxine therapy completed follow up. Numbers of occurrence of thyroid dysfunction during the years of follow up were recorded. Thyroid function tests, serum lipids and lipoproteins echocardiography, bone mineral density and 7 neuro-psychology tests were evaluated at final visit. **Results:** In the radioiodine as compared to MMI treated patients, during a mean of 14 years of follow up, there were more times of elevated TSH>5 mU/L (adjusted relative risk= RR 1.23; 95% confidence interval [CI], 1.04-1.47) and increased triglycerides>150 mg/dl (RR 2.20; CI, 1.34-3.62), HDL-cholesterol<40 mg/dl (RR 3.46; CI, 1.40-8.53), early diastolic annular velocity<12.2 cm (RR 3.91; CI, 1.42-10.74) and decreased early diastolic to annular velocity ratio <6.7 (RR 7.14; CI 1.38-34.48). The MMI group scored better in neuropsychology tests including mood, direction, logical memory, repeated numbers and intelligent quotience than did the radioiodine one. **Conclusion**: Methimazole was superior to radioiodine therapy in patients with diffuse toxic goiter when mood, cognition, cardiac function and occurrence of thyroid dysfunction were compared in patients treated with long term MMI to radioiodine-induced hypothyroid patients on thyroxine treatment. Nothing to Disclose: FA, VY, AB, FS, MT, YM

Pub #	P1-683
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Hyperthyroidism Associated with the Use of Everolimus in the Treatment of Metastatic Renal Cell Carcinoma: The First Reported Case in the Literature
Author String	HN Abou Assi, JM Perkins Duke University Medical Center, Durham, NC
Body	**Background:** Metastatic renal cell carcinoma (mRCC) treatment has progressed over the past 2 decades from cytokine-based immunotherapy to novel selective therapies targeting key molecules associated with RCC development and metastasis. These new agents can be divided into two categories: 1) VEGFR directed therapies including tyrosine kinase inhibitors (TKIs) and 2) mTOR inhibitors including everolimus and temsirolimus. Hypothyroidism has been described as one of the side effects associated with TKIs. To date mTOR inhibitors-induced thyroid dysfunction has not been reported in the literature. **Clinical Case:** Our patient is a 65 year-old man with history of RCC status post left nephrectomy in 10/2006, and cryoablation for local recurrence in 6/2009. Treatment with sunitinib was initiated in 10/2009 for disease progression. His disease continued to progress after 4 cycles. He was started on Everolimus 10mg po daily in 4/2010. Data prior to his transfer of care to our institution showed normal TSH and FT4 in 3/2006. His TSH level has been mildly suppressed since 9/2008 with normal FT4 levels. In 7/2010 his TSH was significantly suppressed to 0.07uIU/ml (0.34-5.66) with elevated FT4 at 1.82ng/dl (0.52-1.21) during which he was asymptomatic. Thyroid uptake and scan showed normal homogeneous uptake at 15%, antimicrosomal antibodies and thyroid stimulating immunoglobulins were undetectable and thyroid ultrasound revealed sub centimeter nodules. He had no history of neck radiation and family history revealed a daughter with hyperthyroidism and one with hypothyroidism. Repeat thyroid profiles continued to reveal suppressed TSH with slightly elevated FT4. He remained asymptomatic and has not required antithyroid medications. With the exception of hyperlipidemia and a rash, he has tolerated everolimus therapy well with no further disease progression. **Conclusion**: mTOR has recently been shown to regulate cell survival and normal thyroid cell function in vivo and in vitro by participating in the control of thyroid iodide uptake. Everolimus increased iodide uptake in normal rat thyroid in vivo making it a potential agent for use in thyroid cancer not only by decreasing tumor cell viability but also by improving the efficacy of radioactive iodine therapy. This may explain why our patient had normal uptake. To the best of our knowledge, this is the first reported association of mTOR inhibitors with hyperthyroidism. The mechanism and implications are unclear at this time. Nothing to Disclose: HNAA, JMP

Pub #	P1-684
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Low-Dose Anti-Thyroid Medication in Graves Disease: Assessment of the Efficacy Based on a Long-Term Follow-Up (Interim Report)
Author String	HR Bazrafshan, F Fitz, M Steinmair, C Reichl, M Beheshti, W Langsteger Golestan University of Medical Sciences, Gorgan, Islamic Republic of Iran; PET - CT Center LINZ, St Vincent's Hospital, Linz, Austria
Body	Background: The optimal dosage of anti-thyroid medications for the treatment of Graves' disease remains a matter of controversy. As a historical cohort, we assessed the rate of response to the treatment by low dose regimen in a sample of 119 randomly selected patients with graves' disease. Methods: All patients were treated with anti-thyroid drugs at the initial dose of 20 mg/day of methimazole (MMI). Then, doses were gradually decreased, and finally discontinued when the patients were able to maintain euthyroid (normal FT4 and TSH) for at least 12 months with the minimum maintenance dose. After discontinuation of drugs, FT4, FT3 and TSH were measured every one month for the first 3 months and every 3 months for the next 12 months and then performed 2, 5, and 10 years following treatment to confirm continuous remission. Results: Out of 119 patients (19 male and 100 female; mean age: 54.67±15.72), 88 (73.9%) of patients had pure Graves' disease and 31 (26%) had Graves' disease with an additional nodule. 70 (66.1%) patients achieved remission after treatment. 28 (26.4%) and 8 (7.5%) patients required surgical and iodine therapy to help complete control signs and symptoms, respectively. Normal TSH was established in 34%, 34.6%, 44%, 47.2%, 52.8%, 58.5% and 66% during 3rd, 6th, 9th, 12th, 24th, 60th and 120th months, respectively. There were no significant differences between the female in disease remission compared to male patients. Conclusion: The dose of anti-thyroid drugs for the treatment of Graves' disease can safely be kept to the minimal required with almost equal efficacy to that of the high dose regimen. This will provide the best balance between possible risks and benefits. Nothing to Disclose: HRB, FF, MS, CR, MB, WL

Pub #	P1-685
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Subacute Thyroiditis Presenting as Acute Psychosis: A Case Report and a Literature Review
Author String	KA Lee, HY Jin, HS Baek, TS Park Research Institute of Clinical Medicine, Chonbuk Natinoal University Hospital, Jeonju, Republic of Korea
Body	Most cases of thyrotoxicosis or hyperthyroid-related psychosis have been described in patients who have Graves' disease or toxic multinodular goiter. We herein report on an 18-year-old male with subacute thyroiditis presenting as acute psychosis. An 18-year-old male was taken to the emergency department by his parents due to abnormal behavior, agitation, and increased activity. One week prior to this visit, he reported experiencing dizziness, headache, and sore throat and took a cold medicine that included a NSAID (non-steroidal anti-inflammatory drug). The patient reported that he subsequently developed painful discomfort in the anterior aspect of his neck, subsequent worsening led to additional features of psychomotor agitation, sexual hyperactivity, and paranoid ideas. Unusual behaviors were also observed by his family members and friends. Laboratory findings included an ESR of 36 mm/hr(N: 0-9mm/hr), a freeT4 of 100.0pmol/L(N: 11.5-22.7pmol/L), and a TSH of 0.018uU/ml (N: 0.35-5.5uU/ml), with negative anti-TPO(thyroid peroxidase) antibody and anti-TSH receptor antibody finding. A Tc-99m pertechnetate scan revealed homogenously reduced activity in the thyroid gland (20 minute Tc-pertechnetate uptake= 3.1). These results were compatible with subacute thyroiditis, and symptomatic conservative managements was initiated. The patient's behavioral abnormalities and painful neck symptoms gradually resolved and his thyroid function steadily recovered. Although a primary psychotic disorder should be strongly considered in the differential diagnosis, patients with an abrupt and unusual onset of psychotic symptoms should be screened for thyroid abnormalities. Furthermore, transient thyroiditis should be considered as a possible underlying etiology along with primary hyperthyroidism. Nothing to Disclose: KAL, HYJ, HSB, TSP

Pub #	P1-686
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Allelic Frequency of -318C/T, A49G and CT60 CTLA-4 and R620W PTPN22 Polymorphisms in a Brazilian Pediatric Population with Graves Disease and Hashimoto Thyroiditis
Author String	MR Bedin, E Trarbarch, G Guerra Junior, D Damiani, S Marui Disciplina de Endocrinologia-Faculdade de Medicina-USP, São Paulo, Brazil; Instituo da Crian[ccedil]a-Faculdade de Medicina-Universidade de São Paulo, São Paulo, Brazil; Faculdade de Ciencias Médicas-Universidade Estadual de Campinas-Unicamp, Campinas, Brazil
Body	Background: Graves disease (GD) and Hashimoto's thyroiditis (HT) are the two main types of autoimmune thyroid disease (AITD). Some genes and their polymorphisms, as -318C/T, A49G and CT60 CTLA-4 (cytotoxic T lymphocyte antigen-4) and R620W PTPN22 (protein tyrosine phosphatase, non-receptor type 22 are associated with the development of both GD and HT. However, the relevance of these polymorphisms in the genetic susceptibility to AIDT remains controversy, particularly in childhood presentation. Objective: To determine the allelic frequency of polymorphisms in CTLA4 and PTPN22 genes in a pediatric cohort with GI or HT. Methods: We analyzed CTLA-4 (-318C/T, A49G and CT60) and PTPN22 (R620W) genotype polymorphisms by real time PCR in 75 children with AIDT: 35 GD (23 females and 12 males) and 40 HT (34 females and 6 males). GD diagnosis was determined by clinical and biochemical hyperthyroidism. HT was determined by antithyroid antibodies positive (antithyroperoxidase >100 UI/mL; IFMA) with or without thyroid dysfunction. For comparison, 80 adults with normal thyroid function and absence of AITD were also genotyped. The presence of these polymorphisms was correlated with clinical presentation, age of AITD onset, sex and race and compared to control adult group. Results: The mean age at diagnosis of GD was 9.5 years (SD 3,2; range 5-18.1) and of TH was 10.1 years (SD 3; range 4.4-17.5). The majority of patients (60.6% of GD and 62.5% of TH) were Caucasians. Exophthalmia was found in 57% of GD patients. All polymorphisms were in Hardy-Weinberg equilibrium. Allelic frequencies of pathogenic CTLA4 polymorphisms among GD, HT and adult control were similar: -318C/T Allele T 9%, 12% and 16%, A49G Allele G 50%, 50% and 40% and CT60 Allele G 67%, 69% and 60% in GD, HT and control individuals, respectively. We also found no difference in R620W PTPN22 polymorphism: Allele G 90%, 90% and 96% in GD, HT and control individuals, respectively. When allelic compositions of these polymorphisms were compared with clinical presentations (age of onset, sex and race) between GD and HT, no significant differences were found. Conclusions: Our results support the lack of association between polymorphisms in CTLA4 and PTPN22 genes and GD or HT, particularly in pediatric patients. Sources of Research Support: FAPESP 2009/17327-7. Nothing to Disclose: MRB, ET, GG-J, DD, SM

Pub #	P1-687
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Frequently Recurring Shoulder Dislocation In Hyperthyroid Graves Disease: Remission with Anti-Thyroid Drug Therapy
Author String	AS Sacerdote, G Bahtiyar Woodhull Medical Center, Brooklyn, NY; SUNY Downstate Medical Center, Brooklyn, NY; NYU School of Medicine, New York, NY; St George University School of Medicine, St George, Grenada
Body	Hyperthyroid Graves' disease is known to cause such connective tissue abnormalities as opthalmopathy, dermopathy, and pre-tibial myxedema. Hyperthyroidism of any cause may result in hyperthyroid myopathy with muscular pain and weakness. Joint abnormalities have not previously been reported in hyperthyroidism. The patient is a 28 year old Filipino man who gives a history of both right and left shoulder dislocations, sometimes happening spontaneously during sleep, occurring almost every month since the age of 16. In Endocrine clinic he complained of increased hair loss, palpitations, and photophobia. He had a pronounced stare and moderate exopthalmos.. Hyperthyroidism was diagnosed in 02-2008 when his TSH by chemiluminescence was <0.004 mIU/L [0.35-5.50], total T4 by immunoassay was 25.8 [mu]g/dl [4.5-10.9], T3 resin uptake by spectrophotometry was 36.9% [22.5-37.0], total T3 by immunoassay was 309.7 ng/dl [60.0-181.0]. Thyroid stimulating immunoglobulin [TSIG] by bioassay was 152% [≤ 125]. The patient was treated with propylthiouracil and metoprolol with gradually improving symptoms and thyroid function tests. He became euthyroid in 7-09 and TSIG normalized at the same time. His shoulder dislocations became less frequent and he has not experienced any since 7-09. Joint dislocation appears to be an extremely rare complication of hyperthyroid Graves' disease which is responsive to anti-thyroid drugs. It. is unknown whether remission from this process is due to becoming euthyroid or is a result of improvement in the autoimmune process. Hyperthyroid Graves' disease should be considered in the differential diagnosis of joint dislocation. Nothing to Disclose: ASS, GB

Pub #	P1-688
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Optimal Use of Thyroid Antibody Assays in the Identification of Autoimmune Thyroid Disease
Author String	D Kariyawasam, LL Chuah, Y Karim, G Swana, B McGowan, P Carroll Guy's and St Thomas' NHS Foundation Trust, London, UK; Guy's and St Thomas' NHS Foundation Trust, London, UK
Body	Background: A variety of thyroid antibody assays are used in the diagnosis of autoimmune thyroid disease (AITD). Commonly both thyroid peroxidase (TPOab) and thyroglobulin antibodies (TGab) are measured but the added value of testing two markers has not been established. Method: We retrospectively collected clinical and laboratory data on 500 consecutive patients who had thyroid autoantibodies requested from a specialist endocrine department of a tertiary hospital from December 2008-October 2010. TPOab and TGab were simultaneously analysed using the FIDISTM multiplex bead assay (BMD, Marne La Vallee, France). Results: There were 399 (79.8%) females and 101 (20.2%) males in the cohort, aged 43.5±15.3, (mean±SD) years. 163 (32.6%) patients had Graves' disease and 118 (23.6%) had Hashimoto's thyroiditis. The other diagnoses included thyroid nodules 101 (20.2%), other autoimmune diseases e.g. Type 1 diabetes 58(11.6%), primary hypothyroidism 41(8.2%)and transient thyroiditis 19 (3.8%). From the 163 patients with Graves' disease 103 (63.2%) had TPOab, 64 (39.3%) had TGab. Of the 118 patients with Hashimoto's thyroiditis, 104 (88.1%) were positive for TPOab and 74 (62.7%) positive for TGab. Conclusion: TPOab testing was superior to TGab assay in identifying patients with both Graves' disease and Hashimoto's thyroiditis. Although there may be a rationale in reserving TGab testing as a second-line test in patients testing negative for TPOab, this would miss 8.6% of Graves' and 11.9% of Hashimoto's thyroiditis patients. The multiplex assay tests both antibodies concurrently and dual testing provides increased sensitivity for AITD. The higher cost of the multiplex assay would be offset by the need to test TGab separately in the anti-TPO negative patients, which constitute 36.8% of Graves' disease and 11.9% of Hashimoto's patients. Nothing to Disclose: DK, LLC, YK, GS, BM, PC

Pub #	P1-689
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Pericarditis and Thymic Hyperplasia in a Patient with Graves Thyrotoxicosis
Author String	AF Shwayhat, MKM Shakir, PW Clyde National Naval Medical Center, Bethesda, MD; Naval Medical Center San Diego, San Diego, CA
Body	The association of thyrotoxicosis and thymic hyperplasia is well recognized, and there is also a lesser known association between thyrotoxicosis and pericarditis. We present a case of Graves' thyrotoxicosis, pericarditis, and thymic hyperplasia co-existing in the same patient. A 24 year-old male presented with acute pleuritic chest pain as well as a 5-month history of progressive weight loss, heat intolerance, and anxiety. Medical history was significant for current cigarette smoking. Physical exam revealed an anxious male with heart rate of 124 beats per minute, bilateral lid lag, stare, and proptosis, but with intact extraocular muscles. A diffusely enlarged non-tender goiter was present, and cardiac exam demonstrated tachycardia without murmurs, gallops, or rubs. ECG finding were consistent with acute pericarditis. Laboratory results included normal serum chemistry values and normal blood counts. Thyroid studies confirmed the diagnosis of Graves' hyperthyroidism: thyrotropin < 0.01 mIU/mL ; free T_4 4.5 ng/dL (0.89-1.76 ng/dL); free T3 21 pg/mL (2.4-4.2 pg/mL); thyroid stimulating immunoglobulins 176% (normal range, < 126%); and thyroperoxidase antibody titer 909 IU/mL (normal < 2 IU/mL). Radioactive iodine uptake could not be performed because the patient received iodine contrast with CT imaging. Technetium scan revealed a large thyroid gland with homogeneously increased radiotracer uptake consistent with Graves' disease. There was no uptake of technetium in the mediastinum. A magnetic resonance imaging confirmed an anterior mediastinal mass measuring 8.5 cm x 10.0 cm x 1.3 cm. Transthoracic echocardiogram revealed no evidence of pericardial effusion. The patient's pericarditis and thymic hyperplasia resolved following treatment with radioactive iodine and corticosteroids. Conclusion: This is the first reported case of a patient with Graves' thyrotoxicosis and thymic hyperplasia also presenting with pericarditis. Pericarditis may be a previously unrecognized complication of Graves' disease. Further immunologic studies elucidating the nature of the relationship between pericarditis and thymic hyperplasia in the setting of Graves' disease need to be performed. (1) Sugar SJ. Arch Intern Med. 1981;141:1242. (2) Budavari et al. Mayo Clin Proc. 2002;77:495-499. Nothing to Disclose: AFS, MKMS, PWC

Pub #	P1-690
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	The Prevalence of Autoimmune Disorders in Turner Syndrome (TS)
Author String	VK Bakalov, LS Gutin, J Zhou, CA Bondy NICHD, National Institutes of Health, Bethesda, MD
Body	To determine the prevalence of autoimmune disorders among adults with TS in relation to X- chromosome dosage and parent of origin, we reviewed medical records of 209 consecutive women with TS, age 20 to 67 years, participating in a natural history study sponsored by NICHD. All participants had a 50 WBC high resolution karyotype by G-banding. The parental origin of the single normal X-chromosome was determined by X-chromosome polymorphisms. Spontaneous hypothyroidism (assumed Hashimoto's thyroiditis) was present in 80/209 or 38.3%, Relative Risk (RR) compared to NHANES data for the US female population (1) was 6.3-8.8. Diagnoses of ulcerative colitis (5/209, 2.39%, RR 4.15-23.5), Crohn's disease (4/209, 1.9%, RR 3.68-24.4), and celiac sprue (6/209, 2.9%, RR 1.08-15.2) were also significantly increased. Diagnoses that do not appear increased were: psoriasis (7/209, 3.3%, RR 0.57-2.96; Graves' disease (4/209, 1.9%, RR 0.35-4.8); Type 1 DM (2/209, 0.96%, RR 0.4-8.7); rheumatoid arthritis (2/209, 0.96% RR 0.27-3.8); Lupus (2/209, 0.96%, RR 0.94-26-2). Logistic regression analysis showed no association between presence of thyroid autoimmunity (AITD) and non-thyroid autoimmune diseases (p=0.40). Iso-chromosome Xq karyotype (in >50% of the cells) was associated with higher prevalence of AITD (16/26, 61.5%) and non-thyroidal autoimmune diagnoses (8/26, 30.7%) compared to [ldquo]pure[rdquo] 45,X (in >90% of the cells) (50/123, 40.6%, p=0.042 for AITD, and 13/123, 10.6%, p=0.017 for non-thyroid autoimmunity respectively, logistic regression analysis). The parental origin of the X-chromosome was not associated with a risk for thyroid (p=0.72) or non-thyroid autoimmunity (p=0.97). Studies utilizing proteomics are currently in progress trying to characterize alterations in immune mediators related to X-chromosome gene dosage in groups of TS and 46,XX women. In summary, women with TS have increased risk of several autoimmune diseases including Hashimoto's thyroiditis, inflammatory bowel disease and celiac sprue compared to the US female population. The risk is highest in those with Isochromosome-Xq, i.e. monosomy for Xp combined with trisomy for Xq. Hollowell JG, et al. J Clin Endocrinol Metab. 2002;87:489 Nothing to Disclose: VKB, LSG, JZ, CAB

Pub #	P1-691
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Thyroid Storm after Radioactive Iodine Ablation for Graves Disease
Author String	A Jabbour, AW Nuristani, MH Horani, W Allabban Banner Good Samaritan Hospital, Phoenix, AZ; Alsham Endocrinology, Chandler, AZ
Body	The patient is 19 Y/O Caucasian female with history of Graves's disease untreated for two years presented to the hospital with symptoms of thyrotoxicosis. She was referred to an outpatient clinic for radioactive iodine ablation. She did not receive any antithyroid medication prior or after her ablation, except for high dose beta blockers(Propranolol 80 mg Q8 hours). Ten days after she received her dose, she presented again with severe dyspnea, anxiety, palpitations and insomnia. Her physical examination revealed a heart rate of 140, respiratory rate of 40, blood pressure 130/92 and her oxygen saturation 95% on 5L. She was alert and oriented, anxious and in respiratory distress. Exophthalmia was present, with erythema around periorbital area. Her lung examination showed coarse breathing sounds, with crackles at the bases of both lungs. Heart examination showed tachycardia with a rate of 120-140 BPM. Neurological exam revealed tremor, no focal deficit. Laboratory data showed white blood count 13.5, hemoglobin 10.8, TSH < 0.01, free T3 20.37, and free T4 6.4, normal renal function. Her urine toxicology screen was positive for THC. Few hours later patient developed pulmonary edema, respiratory failure and was intubated and admitted to the intensive care unit with a diagnosis of thyroid storm. She was started on PTU 300 mg every 4 hours, hydrocortisone 100 mg IV every 8 hours, propranolol 40 mg q.6 hours and esmolol drip to keep her rate below 120 BPM. The patient was extubated 24 hours after admission, and gradually she was weaned off of esmolol drip and was transferred to the telemetry unit. The patient left the hospital two days later against medical advice. She was given prescriptions for PTU 200 mg PO for 5 days and then 100 mg PO for 25 days, propranolol 80 mg every 8 hours and prednisone slow taper. It is well known that I 131 causes abrupt rise in T3 and T4 from pre treatment levels.(1) , but reports of thyroid storm after radioactive ablation in hyperthyroidism are seldom found in the literature. and it was deemed safe to administer I 131 in patients with severe hyperthyroidism without the fear of thyroid storm (2) Physicians should be aware of the risk of thyroid storm when treating patients with radioactive iodine, especially in centers where it's preferred as the first option, without pretreatment with antithyroid medication. This could lead to acute thyroiditis or thyroid storm with possible disastrous outcomes. 1. Lancet. 1975 Oct 4;2(7936):635-7. 2. Ann Nucl Med. 2006 Jul;20(6):383-5. Nothing to Disclose: AJ, AWN, MHH, WA

Pub #	P1-692
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Riedel Thyroiditis Associated with Systemic Fibrosclerotic Disease: Two-Year Treatment with Tamoxifen
Author String	SI Deutsch, LV Castro Jozami, MA Bustamante, MC Basta, MA Della Sala, CE Calvar Hospital JA Fernández, Buenos Aires, Argentina; Hospital JA Fernández, Buenos Aires, Argentina; Hospital JA Fernández, Buenos Aires, Argentina
Body	Our aim is to report a patient with a Riedel's thyroiditis (RT) and multisystemic fibrotic disease, successfully treated with surgery and tamoxifen (TXF). We present a 58 year old woman with a four month history of severe anterior neck pain, rapidly enlarging goiter and hoarseness. The thyroid gland was painful during palpation, stony hard, increased in size, multinodular, hypoecoic and heterogenous on US and bilateral, small adenopathies were present. Cytology of dominant nodule on left lobe, measuring 34 x 24 mm informed a colloid nodule. She was clinically and biochemically euthyroid. The ESR was 36 mm with a normal blood cell count. TPO Ab were negative, I131 uptake was 2, 11 and 24% at 1, 24 and 48 h respectively, with a cold nodule in left lobe. A considerable right tracheal deviation was observed in the neck x-ray. A short course high dose glucocorticoid (GCC) treatment was ineffective and on Sept 2008 a thyroidectomy with a modified left neck lymph node resection was performed. Extensive fibrosis involving sternocleidomastoid, scalene muscles, neurovascular bundle and esophagus was found and a permanent hypoparathyroidism and left vocal cord paralysis developed. The histological study informed RT. Post-surgical studies showed no abnormal tissues in neck, but several lesions with soft tissue density in contact with pleura, bilateral anterior chest wall and pre-vertebral retro-peritoneum contacting the psoas muscle, surrounding the right ureter and anterior iliac artery. The right kidney was uronephrotic and non-functioning in the radiorenogram. The patient refused to receive GCC and 10 mg/day of oral TXF was started in Nov 2008. She has since been in good health, continues with TXF, with no lesions in neck and evident regression of the retro-peritoneal lesions. The right kidney is small and non-functioning. No changes were seen in the thoracic lesions describec in the initial CT scans. The etiology of RT is unknown, but there is strong evidence that it can be the thyroid manifestation of a multifocal fibrosclerotic disease. Reports of the use of TXF in non-steroid responsive cases or in patients with severe steroid adverse reactions suggest that TXF may decrease fibroblast collagen production through the inhibition of TGF-β activity. We suggest that in patients with known RT a trial treatment with TXF could avoid extensive and complicated surgeries, with considerable less toxicities than GCC. The duration of this treatment remains to be established Fontaine S et al., Thyroid 2005; 1:85 Sadao Iwakura MS et al. Arq Bras Endocrinol Metab 2004; 6:903 Nothing to Disclose: SID, LVCJ, MAB, MCB, MADS, CEC

Pub #	P1-693
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Spectrum of Thyroid Dysfunction in Interferon-α-Treated Hepatitis C-Positive Patients
Author String	C Moran, A Melvin, M Nicholls, P Mallon Mater Misericordiae University Hospital, Dublin, Ireland; Mater Misericordiae University Hospital, Dublin, Ireland
Body	The reported incidence of thyroid dysfunction associated with interferon-α (IFNα) treatment for hepatitis C infection varies widely between countries.[1] To our knowledge the spectrum of thyroid dysfunction in IFNα-treated Irish patients has not been described outside of an anti-D-related, hepatitis C-infected cohort. We reviewed thyroid function tests (TFTs) from all Hepatitis C positive patients undergoing IFNα treatment over a 2 year period. 64 patients were included (41 male, 23 female). The majority of patients were Irish (48), the remainder were Eastern European (12), Italian (2), Mongolian (2). 11 patients (17.2%) developed abnormal TFTs (9 female, 6 Irish). The mean age was 35 years (range 20-60). The incidence of thyroid dysfunction was 12.5% for Irish patients, 33% for Eastern Europeans. Mean treatment duration at time of diagnosis of thyroid dysfunction was 18 weeks. Transient subclinical hypothyroidism occurred in 3 patients; thyroid stimulating hormone (TSH) levels rangec from 4.3-7.1mIU/L (RR 0.3-4.0mIU/L) and none required treatment. Two patients developed hypothyroidism requiring eltroxin, one of whom was profoundly hypothyroid (TSH 83.6mIU/L, FT4 7.9pmol/L, TPO Ab positive). Two developed transient hypo- and hyperthyroidism respectively, which spontaneously resolved. Two patients developed thyrotoxicosis due to destructive thyroiditis (TSH <0.1mIU/L, FT4 18-26 pmol/L, TPO Ab positive) with subsequent transient hypothyroidism, requiring temporary eltroxin treatment. The remaining two patients developed transient hyperthyroidism, later reverting to permanent hypothyroidism requiring eltroxin. We detected clinically relevant thyroid dysfunction at a frequency similar to previous studies.[1] Thyroid dysfunction was more common in female patients and those of Eastern European origin. All patients continued IFNα treatment and euthyroid status was achieved with medical intervention. These data suggest that frequency of TFT testing could possibly be reduced in male patients and may need to be increased in Eastern European patients. Those who develop thyroid dysfunction can be managed without discontinuation of IFNα therapy. (1) Costelloe S, Wassef N, Schulz J, Vaghijian T, Morris C, Whiting S, Thomas M, Dusheiko G, Jacobs M, Vanderpump M (2010) Thyroid dysfunction in a UK hepatitis C population treated with interferon-α and ribavirin combination therapy. Clinical Endocrinology 73, 249-256 Nothing to Disclose: CM, AM, MN, PM

Pub #	P1-694
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Serum TSH and Antithyroid Peroxidase Antibodies (TPOAb) in Obese German and Kuwaiti Patients
Author String	A Essam, K Nestler, M Taisseer, M Shabaan, D Klingmueller Armed Forces Hospital, Kuwait, Kuwait; University Hospital, Bonn, Germany; Armed Forces Hospital, Kuwait, Kuwait
Body	Context: TSH and prevalence of TPOAb may relate to ethnicity or geographic location. Environmental factors may also play a role in these differences. Therefore, we measured these thyroid parameters in German and Arabic patients who presented at our clinic due to obesity. Methods and subjects: We measured by automated immunoassay TSH, free T4 and thyroid peroxidase antibodies (TPO-Ab) in 172 obese (BMI > 30) subjects: 49 German men (mean +/- SEM age, 41.1 +/1 1.9 years), 68 German women (age: 39 +/- 1.5 years) 17 Kuwaiti men (age: 42.1 +/- 2.6 years) and 38 Kuwaiti women (age: 36.0 +/- 2.0 years). Results: TSH was high (> 4 [micro]U/ml) in 0 German men, 7.2% of German women, 5.8% of Kuwaiti men and 21% of Kuwaiti women. It was in the upper normal range (2.5 - 4 [micro]U/ml) in 11% of German men, 28 % of German women, 11.7 % of Kuwaiti men and 44.7 % of Kuwaiti women and low (< 0.4 [micro]g/ml) in 6.1% of German men, 1.5% of German women, 5.8% of Kuwaiti men and 2.6% of Kuwaiti women. TPOAb was positive (>34 IU/ml) in 8.1 % of German men, 14.7% of German women, 23.5% of Kuwaiti men and 39.5% of Kuwaiti women. TPOAb was significantly higher ($p < 0.002$) in Kuwaiti (998 + 255 IU/ml) than in German (122 +/- 38 IU/ml) subjects and also significantly higher ($p < 0.01$) in Kuwait women (1149 +/- 341 IU/ml) compared to German women (115 +/- 63 IU/ml). TSH and prevalence of TPOAb of the German population are similar to the Caucasian U.S. population. Conclusion: Prevalence of antithyroid antibodies and TSH is greater in females, increases with age, and is more widespread in Kuwaiti than in German and Caucasian American subjects. To date, it remains unclear whether this is due to genetic factors or environmental influences. Nothing to Disclose: AE, KN, MT, MS, DK

Pub #	P1-695
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Myxedema Coma and Hepatic Encephalopathy Are Non-Identical Twins: Myxedema Coma with Hepatic Encephalopathy & Graves Ophthalmopathy -- A Rare Initial Presentation of Severe Hypothyroidism
Author String	M Trabolsi, L Rifai, R Sanghani Advocate Christ Medical Center/University of Illinois at Chicago UIC, Oak Lawn, IL; Advocate Christ Medical Center/University of Illinois at Chicago UIC, Oak Lawn, IL
Body	**INTRODUCTION:** Myxedema coma is a life threatening presentation of hypothyroidism. Coexistence with hepatic encephalopathy is rare but must be considered if there is inadequate response to the therapy. **CASE REPORT:** A 49 year old male with alcoholic liver cirrhosis was found unresponsive and brought to the ER. He was obtunded, hypotensive, bradycardic and hypothermic. Physical examination revealed spider angiomas and ascites. Laboratory studies revealed hypoalbuminemia, hyperammonemia 172mcg/dl and elevated liver enzymes AST, ALT and ALKP. He was placed in warming blankets. Treatment was directed for hepatic encephalopathy. Stress IV steroid were given. Because of persistent bradycardia and coma, myxedema coma became a concern. Thyroid hormone levels came back diagnostic for myxedema coma with a TSH 253 [mu]U/mL, total T4 0.8 mcg/dl, total T3 < 0.20 ng/ml. Further analysis revealed elevated antimicrosomal, antithyroglobin antibodies, thyroid stimulating immunoglobulin and normal TSH receptors antibodies(TRAB). Initial bolus of IV levothyroxine along with low dose liothyronine were given and resulted in a rapid improvement in the mental status and vital signs. Repeat physical examination revealed significant bilateral exophthalmos and conjunctival injection. Thyroid was not palpable. He was maintained on IV levothyroxine for the first 2 weeks to allow for resolution of gut wall myxedema and the effect of lactulose. He was switched to oral levothyroxine on discharge. **DISCUSSION:** To the best of our knowledge, this is the first reported case of initial presentation of myxedema coma associated with GO in a patient with hepatic encephalopathy. Hypothyroidism and liver cirrhosis share common clinical features and rarely coexist. Altered mental status, edema, elevated liver enzymes, cholestatic jaundice, hypotension, hypothermia and hyperammonemia can present in both(1,2,3). GO is almost always associated with hyperthyroidism and rarely hypothyroid or euthyroid patients with a prevalence of 3%(3,4,5). The mechanism is related to antibody-mediated reaction against TSH receptors in the retro-orbital connective tissues. Previous studies have shown that hypothyroid patients with GO has less severe symptoms, more asymmetric disease and lower levels or even negative TRAB compared to hyperthyroid patients(4,6,7). Upon diagnosis of primary hypothyroidism thorough physical examination should be performed to detect any signs of GO to prevent vision threatening complications. 1. Wall CR. Myxedema coma: diagnosis and treatment. Am Fam Physician. Dec 1 2000;62(11):2485-90. 2. Rimar D, Kruzel-Davila E, Dori G, et al. Hyperammonemic coma--barking up the wrong tree. J Gen Intern Med. Apr 2007;22(4):549-52. 3. R.Malik and H. Hodgson. The relationship between the thyroid gland and the liver. QJmed, oxfordjournals 2002. 4. A K Eckstein, C L[ouml]sch, D Glowacka, et al. Euthyroid and Primarily Hypothyroid Patients Develop Milder and Significantly More Asymmetrical Graves Ophthalmopathy. Br J Ophthalmol. 2009;93(8):1052 [copy] 2009 BMJ Publishing Group Ltd. 5. Jasmina Ciric, Milos Zarkovic, Biljana Beleslin, et al. Hypothyroid Graves' ophthalmopathy: a case report, Endocrine Abstracts (2007) 14 P370 6. Cakir M. Euthyroid Graves' ophthalmopathy with negative autoantibodies. J Natl Med Assoc. 97:1547-1549, 2005. 7. Paunkovic J, Paunkovic N. Does autoantibody-negative Graves' disease exist? A second evaluation of the clinical diagnosis. Horm Metab Res.38:53-56, 2006. Nothing to Disclose: MT, LR, RS

Pub #	P1-696
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Serum Triiodothyronine to Thyroxine Ratio in a Child with T3-Predominant Graves Disease
Author String	Y Miyoshi, N Namba, M Tachibana, Y Kiyohara, M Ito, K Ozono Osaka University Graduate School of Medicine, Suita, Japan; Kuma Hospital, Kobe, Japan
Body	Patients with 3,5,3'-triiodothyronine (T3) -predominant Graves' disease (GD) have increased serum T3 and normal or even low serum thyroxine (T4) levels during treatment with antithyroid drugs (ATDs), and enhanced iodine metabolism in the thyroid gland is presumed to cause the discordant T3 overproduction. Serum T3 to T4 ratio is reported as an useful predictor of outcome in adult patients. We thus evaluated T3 to T4 ratio in pediatric patients with GD and control children. Patients: A 7 year-old girl was referred to the clinic because of goiter and exopthalamus, and ATD therapy (methimazole: MMI) was started. She visited our hospital at 11 years old. She preferred maintenance of medication in our hospital, although operation was recommended in the previous clinic. Laboratory data were as follows (on 25mg/day MMI): WBC 2960 /[mu]l, Neu 49.0 %, AST 19 U/L, ALT 13 U/L, T-Cho 149 mg/dl, TSH <0.03 [mu]U/ml (0.40-3.80), FT4 0.7 ng/dl (0.9-1.6), FT3 7.8 pg/ml (2.0-3.4), TgAb 3.0 U/ml (0.0-0.3), TRAb >40 IU/l (<1.4), TPOAb >30 U/ml (<0.3), Tg >400 ng/ml (0-45), TSAb 1569 % (<180). Ultrasound of the thyroid gland demonstrated diffuse goiter and increased blood flow. Estimated thyroid volume has increased from 27 to 180ml during the therapy. Her present data were as follows (on 20mg/day MMI): TSH 0.37 [mu]U/ml, FT4 0.2 ng/dl, FT3 5.5 pg/ml, TRAb >40 IU/l, TSAb 1617 %. Results: Her serum T3 (pg/ml) to T4 (ng/dl) ratio has increased during the ATD therapy: before the therapy; not available because FT3 and FT4 were over the range, one month later; 3.78, 4years later; 22.5, 5 years later; 27.5. In our hospital, the average ratio of 48 pediatric patients with ordinary type of GD was 3.3 (2.2 - 5.3) before the therapy, and it has decreased to 3.0 (on MMI) and 2.8 (on PTU) three months after the initiation of ATDs. The median ratio in 278 control children was 2.7 (2.6 - 3.0): 2.7 (< 1year old), 3.0 (1-4y), 2.7 (4-8y), 2.8 (8-12y), 2.9 (12-16y), 2.6 (16-20y). Conclusion: ATD blocks the formation of thyroid hormone by the thyroid gland, and most often used to treat pediatric patients with GD in Japan. It was difficult to manage the child with T3-predominant GD using ATD presumably due to enhanced type 1 and type 2 iodothyronine deiodinase in the thyroid gland. We are now planning total thyroidectomy in near future. The serum T3 to T4 ratio is also a useful predictor of the outcome of ATD therapy in pediatric patients with GD. Nothing to Disclose: YM, NN, MT, YK, MI, KO

Pub #	P1-697
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Plasma Selenium, Iodine Excretion and Prevalence of Autoimmune Thyroiditis in Omnivores, Vegetarians and Vegans
Author String	R Gaertner, S Pohl, T Hildbrand, O Adam University of Munich, Munich, Germany; University of Munich, Munich, Germany
Body	OBJECTIVE: Severe combined iodine and selenium deficiency is the cause of destructive thyroiditis and myxoedematous cretinism. Mild selenium as well as iodine deficiency might be one cause of the development of autoimmune thyroiditis. The prevalence of autoimmune thyroiditis was determined in omnivores, vegetarians and vegans because these three groups might have different selenium and iodine intake. METHODS: A prospective cross-sectional study was performed to determine plasma selenium, TSH, free thyroid hormones, thyroid specific autoantibodies (TPOAb and TgAb) in 99 omnivores, 101 vegetarians and 83 vegans with unknown thyroid diseases. In addition thyroid ultrasound was done in all subjects. For recruitment of the study population newspaper announcement and flyers in specific restaurants were distributed. The study was approved by the local ethic committee. RESULTS: Mean age of the omnivores was 43 y, vegans and vegetarians 39 y, 70% in all three groups were females. The median selenium plasma level in omnivores was 83.2 [micro]g/L (range 49-189), in vegetarians 68.9 [micro]g/L (range 36.9-114.5) and in vegans 56.7 (range 21.7-125.7), significantly lower compared to omnivores. The median iodine excretion was 50.7 [micro]g/l (range 3.47-253) in omnivores, 33 [micro]g/L (range 2-305) in vegetarians and 13 [micro]g/L (range 0.3-168) in vegans. Elevated TPOAb and TgAb as well as hypoechoic pattern of the thyroid indicating autoimmune thyroiditis were detected in 10% of omnovores, 14% in vegetarians and 16% in vegans. In 4% of omnivores, 3% in vegetarians and 4% in vegans TSH was above 4 mU/L. CONCLUSION: Combined nutritional selenium and iodine deficiency might contribute to the development of autoimmune thyroiditis. In vegetarians as well as vegans the plasma selenium levels and also iodine excretion was significantly lower and far below the reference range compared to omnivores. The prevalence of autoimmune thyroiditis was higher in vegetarians and vegans, but failed significance because of low numbers Vegetarians and vegans should be supplemented with iodine and selenium. Nothing to Disclose: RG, SP, TH, OA

Pub #	P1-698
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Clinical and Laboratory Evaluation of Patients with Graves Disease after Treatment with 20 mCI of Iodine-131
Author String	ACW Xavier, L Caetano, D Aimore, AP Liberati, V Junqueira, SMP Vechiatti, SR Correa-Silva, RA Guerra Servidor Publico Municipal Hospital, São Paulo, Brazil; Servidor Publico Municipal, São Paulo, Brazil; Servidor Publico Hospital, São Paulo, Brazil
Body	Graves' Disease (GD) is an auto-imune comorbity that affect 0,5% of population. There are three options of treatment, anti-thyroid drugs, iodotherapy and surgery. None of them is the ideal, because they don't act direct in the etiopatology of the disfunction. Nowadays exist many controverses about what the best radiodine dose to use. Many autors agree that hypotyreoidism ins't a side efect, but a accept and desire of treatment. There ar two ways of terapy. To use a calculated dose or to use a fixed dose, generally between 10 and 15 mCi. There is a recomendation from Royal College of Physician of London to use 20 mCi at least for a second dose, but in our Knowledge, there aren't trials that evaluate the efects of theses dose in a series of pacients. Aim: To evaluate the eficacy of treatment in patientes with GD after 6 months of 20 mCi I131, the side effects, the clinical and laboratorial behaviour and to compare the eficacy of treatment in 6 and 12 months. Methods: We reviewed patients of Servidor Publico Municipal Hospital, these patientes were observed every 15 days until 6 moths after iodine treatment with 20 mCi, looking clinical and laboratory parameteres. After one year they were revisited and checked the sucess of therapy. Results: Outcome in 6 months: There were 34 pacients, 29 (85,29%) became hypotyroidism, 5 (14,7%) kept in hypertyroidism. Side effects: The side efects were cervical pain and weight gain with this averade: 5,7 Kg in one year ($p< 0,001$). Clinical and Laboratorial behaviour in 6 months: The most comon symptoms were: irritability (40%); heat intolerance (36%) and difficulty sleeping (22,7%). These symptoms desapeared in 75 days after treatment. To evaluate laboratorial findings, we exclude pacients that used metimazol. There were a cleary trend of elevation of t4l in the evaluate of 30 days and decrease after 60 days. The statistical analisis show a significant diference between these times (30 and 60), $p=0,004$. Comparing outcome in 6 and 12 months: The success of the treatment (absence of hyperthyroidism), was observed 86,95% with six months and 95,65% in one year. Conclusion: With this treatment we achieve a successfull rate higher at six months and after one year when comparing with other doses, without side efects. To sum up, we believe that the 20 mCi dose could be interesting for treatment of graves disease without the need to calculate or estratificate doses. Nothing to Disclose: ACWX, LC, DA, APL, VJ, SMPV, SRC-S, RAG

Pub #	P1-699
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Thyrotoxicosis Presenting as a Stroke
Author String	M Kaur, NM Goldenberg UC Health - University Hospital, Cincinnati, OH
Body	Patients with thyrotoxicosis often present with tachycardia, confusion, febrile illness. We report a patient with Graves' disease who presented with stroke and pulmonary embolism. 51 year old female with history of Hypothyroidism was brought to the ER after she was found unconscious. On arrival, she had GCS of 3 and required intubation. Vital signs showed; T 102.2F, HR 121, RR 16, and BP-90/70-140/70. Neck exam showed an enlarged goiter. EKG showed ST elevations and patient went for urgent cardiac catheterization that showed clean coronary arteries. MRA of the head showed diffuse narrowing of bilateral posterior cerebral arteries without focal stenosis identified. CT scan of the head at the time of admission showed possible stroke. Repeat study few days into admission showed evolving bilateral parieto-occipital infarcts with mild mass effect. CT Chest showed a small pulmonary embolus and was treated with heparin. She was nonsmoker, not on estrogen and did not have any known risk factors for thrombo-embolic events including no personal or family history of prior stroke or blood clots. Her Factor VIII was 223% (50-150), protein C and S activity were low and ATIII was borderline low. Thyroid profile showed suppressed TSH and Free T4 of 6.78ng/dL (0.61-1.76) U/L. Since she was documented to have history of hypothyroidism, etiology of above lab abnormalities was initially unclear. Thyroglobulin was 125 ng/ml (0.5-55), TRABS 6.50IU/L (0.0-1.76), which confirmed Graves' disease. Further inquiries revealed that she had stopped levothyroxine due to new onset of hyperthyroidism five months prior to admission. She was prescribed Methimizole but never took it. Hyperthyroidism was treated with PTU and SSKI, her numbers improved, including protein C levels. ProteinS levels completely normalized. Stroke left the patient blind in both eyes. Thyrotoxicosis is known to cause elevated Factor VIII, enhanced clearance of Vitamin K dependent clotting factors like protein C, S as seen in our patient. These changes could explain the PE and stroke in this case. It is important to consider thyrotoxicosis as a cause of coagulopathy in otherwise healthy patient with stroke and PE. Thyrotoxicosis should be quickly treated to avoid any further clotting events. Nothing to Disclose: MK, NMG

Pub #	P1-700
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Disease: Autoimmunity (1:30 PM-3:30 PM)
Title	Turner Syndrome and Thyroid Disease in Adult Life
Author String	M Alves, M Bastos, J Santos, A Vieira, S Gouveia, J Saraiva, C Moreno, M Carvalheiro Hospitais da Universidade de Coimbra, Coimbra, Portugal
Body	**BACKGROUND:** Susceptibility to autoimmune diseases is common in Turner syndrome (TS). Autoimmune thyroiditis is one of the most proeminent syndrome-associated diseases, with an estimated prevalence of 50% in middle age patients. **OBJECTIVE:** To study the prevalence and characteristics of thyroid disease among women with TS. **METHODS:** In patients with TS the following parameters were evaluated: age, BMI, thyroid disease and age at diagnosis of thyroid disease. This was defined by any abnormality in thyroid hormonal values (TSH and FT4), peroxidase (TPO) and thyroglobulin (Tg) antibodies and/or thyroid ultrasound (US) (simple, uni or multinodular goiter, thyroiditis signs - hypoechogenic, heterogeneous, ill-defined gland). **RESULTS:** We evaluated 70 patients with TS followed in our department. Their mean age was 31.2 years, with a mean BMI of 25.9 Kg/m^2. Most patients (n=43, 61.4%) had documented thyroid disease. This subgroup had a mean age of 31.8 years, with BMI of 27.0 Kg/m^2. Only 2 (4.7%) patients had thyroid family history. The median age at diagnosis of thyroid disease was 20.9 years old. At presentation, 5 (11.6%) patients had subclinical hypothyroidism, 21 (48.8%) had frank hypothyroidism, 2 (4.7%) had subclinical hyperthyroidism and 15 (34.9%) were euthyroid with positive antibodies. Overall, 28 patients (65.1%) were positive for thyroid peroxidase (TPO) and/or thyroglobulin(Tg) antibodies. Levothyroxine treatment was needed in 84.6% patients with hypothyroidism (frank or subclinical). Ultrasound was performed on 34 (79.0%) patients. A diffuse goiter was revealed in 14 (41.2%) patients, nodular goiter in 12 (35.3%) and thyroiditis signs in 15 (44.1%) patients. **CONCLUSIONS:** Most women with Turner syndrome (61.4%) exhibited one or more thyroid abnormalities. The most prevalent disease was autoimmune thyroiditis with hypothyroidism. Thyroid evaluation should be performed regularly throughout life in all patients with Turner syndrome, allowing early detection and treatment. Nothing to Disclose: MA, MB, JS, AV, SG, JS, CM, MC

Pub # P1-701

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)

Title Diagnostic Model for the Detection of $BRAF^{V600E}$ Mutation by Using Receiver Operating Characteristic Curve of Pyrosequencing Analysis in Fine Needle Aspiration Biopsy Samples of Thyroid Nodules

Author String MK Kim, JU Lee, M-K Yeo, T Oh, S An, KS Kim, J-M Kim, M Shong, YS Jo
Chungnam National University Hospital, Daejeon, Korea; Daejeon St Mary's Hospital, The Catholic University of Korea, Daejeon, Korea; Chungnam National University School of Medicine, Daejeon, Korea; GenomicTree, Daejeon, Korea

Body

Context: Recently, tremendous efforts toward the development of sensitive technique to detect $BRAF^{V600E}$ mutation in fine needle aspiration biopsy (FNAB) samples have been devoted. However, currently developed quantitative and semi-quantitative methods such as dual-priming oligonucleotide (DPO)-based multiplex PCR have a potential to generate false-positive results.
Objectives: To eliminate this false-positive result, we decided to develop Receiver Operating Characteristic (ROC) curve and investigate the diagnostic accuracy of pyrosequencing, presenting quantitative data.
Design: Cytological diagnoses of 983 thyroid nodules were made according to the Bethesda system 2007. $BRAF^{V600E}$ mutation was analyzed by pyrosequencing and statistical analyses were performed.
Results: 902 out of 983 nodules were finally adopted to evaluate the diagnostic value of pyrosequencing. The number of malignancy pathologically confirmed was 192 and among those that of papillary thyroid cancer (PTC) was 180. By generating ROC curve, we defined the optimal cut-off value of mutant allele peak was 5.95 percent (Area under the curve: 0.849, Sensitivity: 0.55, 1-Specificity: 0). Applied this selective cut-off value, the number of $BRAF^{V600E}$ positive PTC was 99 (54.4% of total PTC). Diagnostic sensitivity and specificity of cytology alone to detect malignancy was 71.2% and 100%, respectively. Pyrosequencing improved this diagnostic sensitivity from 71.2% to 78.5% without any change of diagnostic specificity. When we considered Suspicious for Malignant as positive cytological outcome, pyrosequencing also improved diagnostic sensitivity from 95.8% to 96.9% and diagnostic specificity from 99.8% to 99.9%.
Conclusions: Pyrosequencing is effective method to detect $BRAF^{V600E}$ mutation in FNAB samples because we can determine the optimal cut-off value and improve the diagnostic sensitivity without unnecessary false-positive result.

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Nothing to Disclose: MKK, JUL, M-KY, TO, SA, KSK, J-MK, MS, YSJ

Pub #	P1-702
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Ultrasound Elastography a Valuable Method To Exclude Thyroid Malignancy
Author String	CMV Ghervan, D Dumitriu, C Botar-Jid, SM Dudea, V Muntean, IM Duncea University of Medicine and Pharmacy, Cluj-Napoca, Romania; University of Medicine and Pharmacy, Cluj-Napoca, Romania; University of Medicine and Pharmacy, Cluj-Napoca, Romania
Body	**Background and objective** One of the key features of thyroid gland cancer evaluated at palpation is the degree of firmness: malignant lesions tend to be much harder than benign ones. US elastography is combining the diagnostic advantages of high-frequency US examination and the accuracy of thyroid cancer diagnosis based on the lesion's stiffness. The aim of our prospective study was to evaluate the elastographic appearance of thyroid gland tumors and to explore the sensitivity and specificity of US elastography for differential diagnosis of thyroid cancer, with histopathologic analysis as a reference standard. **Materials and Methods**: A total of 34 patients were included in the study, presenting one or several suspicious thyroid nodules. Elastography was performed by the same examiner with the same settings of the machine. The nodules were classified in five classes of tissue stiffness. All the patients were operated and the results of elastography were compared with histopathologic results. **Results:** The 34 patients had 99 thyroid nodules that were investigated. 65 were soft in elastography (score 1-3) and 34 were hard (score 4-5). At pathological exam all the 65 soft nodules were benign and from the 34 hard nodules 17 were benign and 17 malignant. In 4 patients multiple malignant nodules were found. **Conclusion:** Elastography showed a sensitivity of 100% and a specificity of 79% in diagnosing malignant nodules. With a positive predictive value (PPV) of 50% and a negative predictive value (NPV) of 100% it seems more valuable in excluding malignancy than in affirming it Nothing to Disclose: CMVG, DD, CB-J, SMD, VM, IMD

Pub #	P1-703
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Preoperative Thyroglobulin as a Useful Predictive Marker to Differentiate Follicular Thyroid Cancer from Benign Nodules in Indeterminate Nodules
Author String	EK Lee, YJ Lee, HS Min, TS Kim, TH Kim, JS Ryu, YS Jung, K-W Chung, SK Kim National Cancer Center, Goyang, Korea
Body	Background: Thyroid nodules are very common. A few patients might have undergone unnecessary surgery due to indeterminate cytology results. Therefore this study was designed to find useful and simple predictive tools to differentiate malignant nodules from indeterminate thyroid nodules. Methods: We retrospectively enrolled 164 patients who had undergone thyroid surgery because of the results of indeterminate cytology in National Cancer Center. We reviewed patients' age at diagnosis, sex, preoperative biochemical markers (thyroglobulin (Tg), anti-Tg antibody, free T4 and TSH level), sonographic findings including tumor size, and pathological findings, which were analyzed by statistical methods. Additionally, we compared gene expression patterns between benign and malignant group using the general cDNA microarray technique with illumina HumanRef-8 v3 Expression BeadChip (Illumina, INc., San Diego, CA) in the patients who categorized in [ldquo]follicular neoplasm[rdquo] as preoperative diagnosis. Results: We could find several clinical and sonographical predictive factors showing significant differences; young age, male, larger tumor size, preoperative high Tg level, and hypoechoic nodule without hypoechoic rim on sonography increased cancer probability significantly in multivariate analysis. With cut-off value 187.3 ng/ml of Tg, sensitivity and specificity was 54.8% and 90.1%, respectively. AUC was 0.748 and *p*-value was <0.005. However, we could not elucidate significantly different genes in microarray analysis between benign and cancer group, unfortunately. Conclusion: Although microarray analysis did not show significant genetic differences between benign and malignant thyroid nodules, preoperative Tg levels had very high specificity in prediction of follicular thyroid cancer in the case of suspicious [ldquo]follicular neoplasm[rdquo]. Therefore, it may be a useful marker to differentiate follicular thyroid cancer from benign thyroid nodules in the cytologic diagnosis of indeterminate nodules. Sources of Research Support: National Cancer Center Research Grant No. 0910033-1. Nothing to Disclose: EKL, YJL, HSM, TSK, THK, JSR, YSJ, K-WC, SKK

Pub #	P1-704
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Interobserver and Intraobserver Agreement of Thyroid Ultrasound Elastography Using *In Vivo* Compression
Author String	D-J Lim, S Lou, M-H Kim, S-H Ko, J-S Bae, Y Kim The Catholic University of Korea, Seoul, Korea; The Catholic University of Korea, Seoul, Korea; University of Washington, Seattle, WA; University of Washington, Seattle, WA; Pohang University of Science and Technology, Pohang, Korea
Body	**BACKGROUND** Recent studies showed the potential feasibility of real-time ultrasound elastography (USE) in differential diagnosis of thyroid nodules. A major issue limiting more clinical use of USE is the interobserver variability when external compression is used (1). The objective of this prospective study was to investigate the interobserver and intraobserver reliability of real-time USE without external compression, but using the inherent carotid artery pulsation. **MATERIALS AND METHODS** Between November 2010 and January 2011, 35 patients aged 46.3+/-13.0 with 36 thyroid nodules were examined with conventional B-mode ultrasound and real-time USE using *in-vivo* compression. All enrolled malignant patients (n=25) were bound to thyroid surgery due to a proven malignancy in biopsy. Benign nodules (n=10) were confirmed by fine needle aspiration. Three endocrinologists with less than three months of experience in using ultrasound performed the USE exams on the same patient. Each endocrinologist performed the USE exam on each nodule twice. To perform an USE exam, the transverse view showing the largest diameter of a nodule was identified in B-mode, and data were acquired for ~4 seconds by placing the transducer over the thyroid without applying any external compression. The elasticity contrast index (ECI) quantitative metric was calculated. A larger ECI value indicates a higher probability of a nodule being malignant. Interobserver and intraobserver agreement was evaluated using Pearson's correlation analysis. **RESULTS** The size of all nodules ranged from 5 to 24 mm (median 12 mm). The intraobserver reliabilities were 0.88, 0.74, and 0.86 for observer 1, 2, and 3, respectively. The interobserver reliabilities were 0.84 (observer 1 and 2), 0.82 (observer 1 and 3), and 0.70 (observer 2 and 3), indicating substantial agreement between two observers. **CONCLUSION** Our study has shown substantial intraobserver and interobserver agreement in real-time USE using *in-vivo* compression and objective scoring method. Our real-time USE technique could minimize the operator dependency since it avoids and reduces the major sources of variability associated with external compression elastography. (1) Park et al., AJR Am J Roentgenol 2009; 193(5):W416-23 Nothing to Disclose: D-JL, SL, M-HK, S-HK, J-SB, YK

Pub #	P1-705
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Immediate Assessment of Fine Needle Aspirates of Thyroid Nodules by Telecytopathology
Author String	R Kasturi, KK Khurana, R Hopkins, I Swati, R Izquierdo SUNY Upstate Medical University, Syracuse, NY; SUNY Upstate Medical University, Syracuse, NY
Body	Background: We discuss our experience with onsite evaluation of ultrasound guided fine needle aspiration (USGFNA) of thyroid nodules using telecytopathology. We discuss inadequacy rate and diagnostic accuracy. Methods: This was a retrospective analysis of USGFNA of thyroid nodules performed by a single provider over a 9 month period. Real time images of Diff Quik stained cytology smears were obtained with an Olympus Digital camera attached to an Olympus CX41 microscope and transmitted via the internet by a cytotechnologist to a pathologist who communicated the preliminary diagnosis and sample adequacy. The inadequate specimen rate was compared between a group whose images were transmitted (n=45) vs. another group without onsite adequacy assessment (non-transmitted) (n=47). Four passes were obtained per nodule in the non-transmitted group versus predominantly 2 passes per nodule in the transmitted group. Results: A total of 92 nodules in 67 patients were aspirated with ultrasound guidance. The inadequate sample rate in the transmitted group was 13% and that of the non-transmitted group was 21%. There was no statistically significant difference in the inadequacy rates between the two groups (p=0.47). In the transmitted group, the cytology specimens of 3 patients that were initially deemed inadequate by the pathologist were considered adequate after 2 additional passes. We were able to discuss the malignant diagnoses with 2 patient who had papillary thyroid cancer immediately after the USGFNA. We were able to communicate the preliminary onsite diagnoses to all patients in the transmitted group and also triage 2 patients for surgery based on diagnosis of malignancy. Preliminary diagnosis was comparable to final diagnosis in 96% of cases. Conclusion: Immediate assessment of USGFNA via telecytopathology not only assured adequacy of the cytology sample but also permitted fewer passes per nodule. In 3 patients it saved a second trip to the endocrinologist for re-aspiration. This procedure allowed us to discuss the results of FNA immediately after the procedure with the patient thereby alleviating patient anxiety. Preliminary onsite assessment was highly accurate compared to final diagnosis. Direct visualization of the sample by the pathologist allowed reimbursement for adequacy assessment. The inadequacy rate between groups was not statistically significant and may be due to the small size of the study groups and the difference in the number of passes. Nothing to Disclose: RK, KKK, RH, IS, RI

Pub #	P1-706
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	The Use of a Set of Genetic Markers Helps to Discriminate Benign and Malignant Thyroid Nodules with a Fine Needle Aspiration Pattern of Follicular Proliferation in an Area of Borderline Iodine Deficiency
Author String	F Niccolai, P Agretti, T Rago, M Scutari, A Molinaro, A Candelieri, G Di Coscio, F Basolo, P Iacconi, P Miccoli, C Di Cosmo, A Pinchera, P Vitti, M Tonacchera University of Pisa, Pisa, Italy; University of Pisa, Pisa, Italy; University of Cosenza, Cosenza, Italy
Body	Objective: To evaluate a set of 6 recently proposed marker genes (TG, LGALS3, ADM3,TFF3, HGD1 and PLAB), and to investigate their diagnostic potential to distinguish thyroid nodules with microfollicular pattern of growth, starting from thyroid nodule material in a defined population living in a region with borderline iodine deficiency. Patients and methods: Ultrasound-guided fine-needle aspiration citology was performed for patients with thyroid nodules. One hundred and fifty-three thyroid samples obtained from 151 consecutive patients (112 females of medium age 45.4 ± 11.7 and 41 males of medium age 48.0 ± 8.6 years) were collected and included in the study. FNA samples were collected and total RNA extraction was performed. Quantitative gene expression studies were performed. To determine differences in mRNA expression of the 6 genes in the series of 56 benign thyroid nodules and 43 malignant thyroid nodules the median of each gene expression was evaluated. To perform a prediction of malignancy of the 54 microfollicular thyroid nodules, we adopted PART algorithm, a machine learning method for learning rules. All cDNA samples were also analyzed for V600E BRAF mutation. Results: The expression of TG, LGALS3, ADM3, TFF3, HGD1 and PLAB mRNAs was demonstrated in all 153 specimens. No significant differences among benign and malignant thyroid nodules were observed in TG LGALS3 and ADM3 expression. A significant decrease in TFF3 and HGD1 expression was observed in malignant thyroid nodules with respect to benign thyroid nodules, while an increase in PLAB expression was demonstrated in malignant thyroid nodule. Our results of the decision model of the three genes expression profile obtained at fine needle aspiration of the 54 aspirates of nodules with follicular pattern of growth was valid for 37 of 54 cases (68.5%), with a total of 8 False Positive (14.8 %) and 9 Negative predictions (16.6 %) with a Sensitivity of 43.7%, and a Specificity of 78.9%. The mutated form V600E of BRAF gene in the heterozygous state was demonstrated by direct sequencing in 19/43 (44%) malignant thyroid nodules, in 0/56 benign thyroid nodules and in only 1/54 (1.8 %) microfollicular thyroid nodules. Conclusions: The gene expression profiles of three genes (TFF3, HGD1 and PLAB) allowed a good prediction for the differentiation of benign thyroid lesions and thyroid cancer starting from cells of fine needle aspiration of thyroid nodules with a follicular pattern of growth. Nothing to Disclose: FN, PA, TR, MS, AM, AC, GDC, FB, PI, PM, CDC, AP, PV, MT

Pub #	P1-707
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Accuracy of Ultrasound Elastography in the Diagnosis of Thyroid Cancer in a Low-Risk Population
Author String	A Vidal-Casariego, A Jimenez-Perez, L Lopez-Gonzalez, MD Ballesteros-Pomar, B Perez-Corral, RM Alvarez-San Martin, A Urioste-Fondo, JJ Lopez-Gomez, V Roiz-Gaztelu, R Aguado-Garcia, I Cano-Rodriguez, JM Jimenez-Garcia de la Marina Complejo Asistencial Universitario de León, León, Spain; Complejo Asistencial Universitario de León, León, Spain
Body	**Introduction:** Stiffness has been associated to malignancy in prostate and breast, as well as thyroid. Ultrasound elastography (USE) objectively measures tissue elasticity, and previous surveys have described it as a technique with high sensitivity and specificity (>85%) for the detection of malignant thyroid nodules in high-risk populations (>15%). **Aim:** To assess the accuracy of USE in a population with high prevalence of nodular goiter but low-risk of malignancy (<5%). **Patients and method:** 128 consecutive patients with suspected nodular goiter were recruited in the clinics of Endocrinology. USE and ultrasound-guided fine-needle aspiration (FNA) were performed. When malignancy was suspected by FNA, surgery was recommended. Thyroid nodules were clalsiffied by USE according the criteria described by Ueno (Eizo Joho Medical 2004) and Fukunary (Medix 2007). Quantitative data are summarized as median (standard deviation), and categorical as percentage. Sensibility (S), specificity (Sp), and predictive values (PV+, PV-) were calculated. **Results:** 90.6% were female, with age 56.1 (14.7). Most patients had a single nodule (52.0%), followed by multinodular goiter (45.7%), and a few thyroiditis (2.3%). USE classified nodules as mostly elastic (69.5%), peripherally elastic (18.8%), non-elastic (8.6%), totally elastic (2.3%), and non-elastic periphery with intermedious inner (0.8%). FNA found a 59.2% of benign nodules, 6.4% of indeterminated, and 3.2% possibly malignant; there were 31.2% of no-valid FNA. After surgery 3 malignant nodules were confirmed (3.4% of valid citologies), all of them papillary carcinomas. All the malignant nodules were mostly elastic, as well as 75% of indeterminated nodules. This elastographic pattern has a S 5.6% (CI 95% 0.0-12.6), Sp 100.0% (CI 95% 98.4-100.0), PV+ 100.0% (CI 95% 78,6-96,5) and PV- 38.6% (CI95% 27.5-49.6) for the diagnosis of malignant nodules. **Conclusion:** In a low-risk population for thyroid cancer ultrasound elastography lacks of sensitivity for the diagnosis of malignant nodules. Nothing to Disclose: AV-C, AJ-P, LL-G, MDB-P, BP-C, RMA-S, AU-F, JJL-G, VR-G, RA-G, IC-R, JMJ-GdlM

Pub #	P1-708
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Estimating Levothyroxine Replacement Dose Requirements in Benign and Malignant Thyroid Disease
Author String	K Meinke Baehr, E Lyden, K Treude, J Erickson, W Goldner University of Nebraska Medical Center, Omaha, NE; University of Nebraska Medical Center, Omaha, NE
Body	***Background:*** Conflicting data on optimal thyroid hormone dosing after thyroidectomy exists. Levothyroxine (LT4) requirements can be affected by many variables; therefore, the usage of one standard weight-based formula (mcg/kg) may not be ideal in all settings. In this study we assessed LT4 dose requirements for benign and malignant thyroid disease and evaluated other variables that affect thyroid hormone dose. ***Methods:*** This retrospective study included all participants in the University of Nebraska Medical Center (UNMC) Thyroid Tumor and Cancer Collaborative Registry (TCCR), a registry including benign and malignant thyroid nodules. The study included patients from March 2008 through September 2010 that underwent total thyroidectomy and were on a stable dose of LT4 replacement. Anthropometric data (age, gender, actual weight, BMI, calculated ideal body weight), TSH, menopausal status, past medical history, and medication data were collected. Weight-based LT4 dose for benign and malignant etiologies were determined and other confounders were evaluated. ***Results:*** 246 patients were included (205 women, 41 men), with 48 (20%) benign and 198 (80%) malignant pathology. Actual weight-based LT4 dose was statistically different between benign and malignant etiology (P=0.002); however, there was no difference when using ideal body weight (P=0.24). Gender and menopausal status were also independent predictors of actual weight-based LT4 dose (P=0.002). There was no statistical difference in adherence, other medications, or concurrent medical illnesses between benign and malignant groups. The mean actual weight-based dose in men was similar to pre-menopausal women in both benign (1.8 [micro]g/kg for both men and pre-menopausal women) and malignant (2.2 [micro]g/kg vs. 2.1 [micro]g/kg) etiologies; however, post-menopausal women required less LT4 (benign 1.5 [micro]g/kg vs. malignant 1.7 [micro]g/kg). The ideal body weight-based LT4 dosage was not statistically different between groups. ***Conclusion:*** LT4 dosage following thyroidectomy, calculated using actual body weight, is dependent on diagnosis (benign vs. malignant), gender, and menopausal status. All of these factors may need to be considered for optimal LT4 dosage post-thyroidectomy. In contrast LT4 requirements using ideal body weigh are not statistically different. Nothing to Disclose: KMB, EL, KT, JE, WG

Pub #	P1-709
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	PAX8/PPARγ Rearrangement Detection Is Feasible in Routine Air-Dried Fine Needle Aspiration (FNA) Smears
Author String	C Ferraz, A Krogdahl, C Rehfeld, E Jensen, E Bosenberg, L Hegedus, R Paschke, M Eszlinger University of Leipzig, Leipzig, Germany; Odense University Hospital, Odense, Denmark; Odense University Hospital, Odense, Denmark
Body	Some of the inherent limitations of the sensitivity and specificity of FNA of thyroid nodules can probably be overcome by molecular analyses. PAX8/PPARg rearrangements have been identified in a high percentage of follicular thyroid carcinomas (FTCs) and follicular variant of papillary thyroid carcinomas (PTC) and also in follicular thyroid adenomas (FTAs) but have never been analyzed in routine air dried FNA smears. We analyzed the presence of PAX8/PPARg rearrangements in a series of 76 routine FNA smears and the corresponding formalin-fixed paraffin embedded (FFPE) tissues using RT-PCR assay. We carried out 3 separate reactions for each sample with specific probes and the following primer pairs: PAX8-EX8 forward, PPARg-Ex01 reverse; PAX8-EX9 forward, PPARg-Ex01 reverse; and PAX8-EX10 forward, PPARg-Ex01 reverse. PAX8-PPARg was detected by RT-PCR in 7 of 76 FFPE samples (9%) and in 6 of 76 FNA smear samples. In 4 samples it was possible to match FFPE to FNA (3 FA and 1 FTC). 3 rearrangement positive FFPE cases could not be detected in FNA, and 2 positive smear samples could not be identified in the corresponding FFPE. PAX8/PPARg was present in 2 of 6 (33%) FTC; 5 of 29 follicular adenomas (17%) and also in 1 Hurthle cell adenoma (n = 8 in total). No rearrangement was detected in Hurthle cell carcinomas (n=1), goiter (n=7) or PTC (n=25). The most frequent fusion variant was PAX8 exons 1-8 juxtaposed to PPARg exon 1 (55%), followed by PAX8 exons 1-9 juxtaposed to PPARg exon 1. The least frequent variant was PAX8 exons 1-10 juxtaposed to PPARg exon 1(16.7%). These results, for the first time, demonstrate the feasibility of extracting RNA from routine air dried FNA smears to detect PAX8/PPARg rearrangements with RT-PCR. The introduction of molecular analyses of routine air dried FNA smears in every day practice, comprising also other mutations, could provide substantial improvements for the diagnosis of thyroid cancer and thereby potentially also reduce the rate of diagnostic surgery. Nothing to Disclose: CF, AK, CR, EJ, EB, LH, RP, ME

Pub #	P1-710
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Cytological Changes Associated with Percutaneous Ethanol Injection in the Treatment of Thyroid Nodules
Author String	G Paz Filho, GC Schrut, FY Miasaki, TC Cavalcanti, H Graf, GA de Carvalho John Curtin School of Medical Research, The Australian National University, Canberra, Australia; Universidade Federal do Paraná, Curitiba, Brazil; Universidade Federal do Paraná, Curitiba, Brazil
Body	Background: Percutaneous ethanol injection (PEI) is an alternative therapy of thyroid nodules (TN). However some concern is raised on its carcinogenic effects. Objective: To evaluate the cytological and clinical changes caused by PEI in patients with benign TN. Patients/methods: Thirty-nine patients with TN (23.1% hyperfunctioning) were submitted to a median of 3 PEI sessions. After a median of 17 months, patients were evaluated. A new ultrasound-guided fine needle biopsy (US-FNB) was performed, and smears were analyzed after May-Grünwald-Giemsa staining. The diagnostic findings and cellular characteristics were compared before and after treatment. Results: Hyperthyroidism was completely or partially resolved in 66.7%. By ultrasound, the percentage of homogeneous nodules decreased from 64.0% to 38.4% (p=0.0235), and the mean nodule volume decreased from 13.4±12.2 cm3 to 5.3±5.1 cm3. There was an increase in the proportion of inconclusive results (from 2.5% to 18.9%). No malignant cases were observed. The proportion of moderate/intense macrophage infiltration decreased from 60% to 15%. Conclusions: PEI is an efficacious and safe therapeutic option, with no carcinogenic effects observed on cytological evaluations. Since it increases the proportion of inconclusive results from US-FNB, cytological findings after PEI must be evaluated with caution. Nothing to Disclose: GPF, GCS, FYM, TCC, HG, GAdC

Pub #	P1-711
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Association of Thyroid-Related Hormones and Blood Flow in the Development of Nodular Goiter -- Analysis of Long-Term Follow-Up Results
Author String	T Kunori Iwaki-kyoritsu General Hospital, Iwaki-shi, Japan
Body	Background: During the last two decades 1538 new patients with nodular goiter (NOD) were referred to our clinic. If confirmed as benign by fine needle aspiration biopsy (FNAB), a fourth of patients received follow-up examinations using ultrasonogram (US) and hormonal assay including thyroglobulin (Tg). However the long-term course of NOD is not always convincing, and often necessitates repeated FNAB because of unexpected occurrence of malignancy. In this study, the follow-up results were retrospectively analyzed to find factors associated with its development and prognosis. Patients: Patients with NOD (n=657: 2000-2009) were analyzed: 437 samples from 148 repeaters and 509 single samples; aged 56 (16-89, 538 female) and 58 (24-91, 125 male). They were followed up to 112 months (mean 24 months). Methods: Volume (VOL) and blood flow (BF) of nodules were estimated by US equipped color doppler echogram. Serum level of Tg, anti-Tg, TSH, free-T3 and -T4 were measured by radio-immunoradiometric assay, radioimmuno-assay. Statistic difference between two samples was tested by student-t. Correlation was proved by Chi-square test. Significance was determined by p-value (less than 0.05). Results: A: Development of nodules: 1) Vol increased in 65% of patients with 1.2 times (mean) larger compared with VOL at the first visits. 2) Tg (median 98 ng/ml) increased in 55% of patients; less than two fold higher in 41% and more in 14% of patients. Individual Tg level much varied and tended to have own ranges. No remarkable increase was found beyond 8 years. 3) VOL was correlated with Tg. 4) Tg/VOL(TVR) decreased in 47% and increased in 35% of patients. TVR significantly decreased as enlarging VOL. 5) Initial TVR values were not associated with subsequent changes of VOL. B: BF and changes of nodules: 1) Cystic formation with intra-nodular fluid was more observed as decreasing peri-nodular BF. 2) Increased peri-nodular BF in small nodules was often associated with high TSH. C: FNAB diagnosis: 1) Inadequate sampling (15%) decreased to 1/3 in multiple exams. 2) Upgrade (class 2 to 4) diagnosis occurred in two patients (1.4%) Conclusions: Most of NOD enlarged with increase of Tg in a few years but the subsequent growth was limited. BF seemed to contribute to the initial growth in high TSH condition. FNAB results warrant follow-up biopsy to ensure the prognosis. Nothing to Disclose: TK

Pub #	P1-712
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Benign Cystic Nodules May Have Ultrasonographic Features Mimicking Papillary Thyroid Carcinoma during Interval Changes
Author String	MR Kim, BG Choi, JH Kang, YB Lim, YK Jeon, SS Kim, BH Kim, YK Kim, IJ Kim Pusan National University Hospital, Pusan, Korea; Kim Yong Ki Internal Medicine Clinic, Pusan, Korea
Body	**Background:** It had been observed that some cystic nodules change morphologically with ultrasonographic (US) features suspicious for malignancy. The aim of this study was to evaluate the US characteristics of benign cystic nodules mimicking papillary thyroid carcinoma (PTC) during interval changes. **Methods:** Between January 2009 and October 2009, 26 patients with benign cystic nodules showing marked hypoechogenicity in US during the follow-up period were enrolled. During the same period, 38 patients with marked hypoechogenicity in US were enrolled for the PTC group. We evaluated the differences in US characteristics between the 2 groups. **Results:** Nodule size, margin, echogenic dot and vascularity were not significantly different between the 2 groups. Nodule shape was significantly different between the 2 groups with a lower prevalence of taller than wide in the benign cystic group. If echogenic dot was detected in benign cystic nodule, it was more than 1 mm in size without posterior acoustic shadowing unlike echogenic dots in the PTC group. **Conclusion:** Some of the benign cystic nodules may have suspicious malignant features on US during interval changes. A careful assessment of US findings and a previous history may be of value in discriminating them from PTC. Nothing to Disclose: MRK, BGC, JHK, YBL, YKJ, SSK, BHK, YKK, IJK

Pub #	P1-713
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Serum Thyroglobulin: A Tumor Marker Useful before and after Thyroidectomy in Suspected Thyroid Cancer
Author String	P Jallepalli, R Vidyasagar NRI Medical College/Hospital, China Kakani, India
Body	26 years old Asian Indian born with a goiter prematurely at 8th month of gestation with mental retardation, and mild hypothyroidism, treated with l-thyroxine 0.15mg QD, noted increasing size of goiter in last 4 months with dysphagia. US of neck and clinical exam pointed to nodularity. FNAC showed Hashimottos thyroiditis. Serum thyroglobulin(TGB) preop was 6500ng/ml. Subtotal thyroidectomy done by Dr.Vidyasagar as CT of neck showed retrosternal extension to 1.5cm along with high TGB in a male with nodularity raising a possibility of cancer. Post operatively pathology confirmed papillary hyperplasia and serum TGB dropped to 835 ng/ml and with TSH suppression with L-thyroxine 0.2mg QD, to 24.7, subsequently. RAI scan without L-thyroxine showing no residual thyroid. Authors strongly believe that TGB measurement is yet another important tool preoperatively in suspected cancer thyroid as multiple biopsies though desirable is not practical in some areas of the globe. FNAC of the lesion can be misleading or miss the lesion at times. Case is open for suggestions and discussion. Nothing to Disclose: PJ, RV

Pub #	P1-714
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Relationship between Thyroid Nodules and Adrenal Incidentalomas
Author String	S Isik, U Ozuguz, FK Kucukler, YA Tutuncu, D Berker, G Akbaba, HN Ozcan, A Arduc, Y Yalcin, S Guler Ministry of Health, Ankara Numune Research and Training Hospital, Ankara, Turkey; Ministry of Health, Ankara Numune Research and Training Hospital, Ankara, Turkey
Body	**Objective:** Several studies have been published on possible relationships between insulin resistance and growth factors with either non-functional adrenal tumors or thyroid nodules. The aim of our study is to determine the frequency of concomitant thyroid nodules in patients with adrenal incidentaloma and to evaluate the association of this disease with insulin resistance and metabolic syndrome (MetS). **Methods:** Files of 113 patients who were followed up with the diagnosis of non-functional adrenal incidentaloma were examined. One hundred-thirty five healthy individuals found to have no adrenal tumor by abdominal ultrasonography were selected as a healthy control group. Functional evaluations were performed for adrenal incidentalomas. Insulin resistance was evaluated through homeostasis model assessment insulin resistance. Patients were evaluated with respect to their thyroid ultrasounds, radioactive iodine uptake assessments and, if needed, thyroid scintigraphy and thyroid fine needle aspiration biopsy. **Results:** Mean age and gender distribution of patients with adrenal incidentaloma and the control group were similar. BMI of patients with adrenal incidentaloma were higher than the control group (29.9±4.8 vs. 27.1±4.1, $p<0.001$). While the rate of obesity in patients with adrenal incidentaloma was 46.9%, 33.3% of the control group was obese ($p=0.029$). Thyroid nodule was found in 33 (28.1%) individuals in the control group, whereas thyroid nodule was observed in 55 (48.6%) patients with adrenal incidentaloma. Thyroid hormone statuses of the adrenal incidentaloma patients with or without nodules were similar (euthyroidism 81.8%, 82.8%; hypothyroidism 12.7%, 13.8%; hyperthyroidism 5.5%, 3.4%, respectively). Two out of 3 patients who underwent thyroidectomy were diagnosed with papillary thyroid cancer; while one had simple nodular goitre. No relationship was found between the adrenal tumor volumes and thyroid nodule volume, the number of nodules, fasting blood glucose, and thyroid function tests of the patients. **Conclusion:** This study established thyroid nodule prevalence to be higher in patients with adrenal incidentaloma compared with the control group. Although two patients with adrenal incidentaloma had papillary thyroid carcinoma, data at hand is insufficient to estimate a relation between adrenal incidentaloma and increased risk of thyroid cancer. Nothing to Disclose: SI, UO, FKK, YAT, DB, GA, HNO, AA, YY, SG

Pub #	P1-715
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Descriptive Study of the Clinical Behavior of Multiple Endocrine Neoplasia 2A
Author String	R Oliva-Rodriguez, R Guerrero-Vazquez, MdC Tous, MA Martinez-Brocca, JM Martos-Martinez, A Leal-Cerro, E Navarro-Gonzalez Biomedicine Institute of Seville University Hospital Virgen del Rocio, Seville, Spain
Body	**Introduction** The knowledge gained in recent years about the biological expression of different mutations in the RET proto oncogene has allowed to establish strategies regarding the timing of prophylactic thyroidectomy, and the monitoring of this group of patients. **Objectives** 1. To analyze the clinical and biological characteristics of patients with multiple endocrine neoplasia 2A (MEN 2A) treated at the University Hospital Virgen del Rocio (Seville, Spain). 2. To compare these data with those reported in the literature to assess whether screening strategies for pheochromocytoma and primary hyperparathyroidism proposed in mutation carriers are valid in our population. **Material and method** Descriptive retrospective study of 84 patients from 12 families with MEN 2A treated at the University Hospital Virgen del Rocío from 1983 until January 2011. **Results** We present the results of 84 patients (46 women and 38 men) from 12 families with an average follow-up of 11,48 ± 7,88 years and a current average age of 42.34±20.91 years. 20 patients were index cases and 64 were diagnosed by screening. The average age of diagnosis was 46.55±17.45 and 26.72±17.43 years for the index cases and the cases diagnosed by secreening, respectively. The mutations found in the proto oncogene RET were: C634Y 71 (9 families), C634 R 9 (3 families), C618R 4 (2 families). 98,7% of patients had thyroid disease (medullary thyroid carcinoma or C cell hyperplasia), 45.5% had pheochromocytoma and 5.2% had primary hyperparathyroidism. No cases of cutaneous lichen amyloid or Hirschsprung[acute]s disease were found in our series. After thyroidectomy, 20% of index cases and 61.29% of screening cases were in remission. Mortality from causes directly related to MEN 2A in this series was 5.95%. **Conclusions:** 1) The data we have obtained are similar to those described in the literature regarding the prevalence of the mutations found, the evolution of the disease and the penetrance of medullary thyroid carcinoma and pheochromocytoma. 2) The penetrance of primary hyperparathyroidism we have observed is lower than that previously reported for patientes with these mutations, considering the current average age of patients and an average follow-up o 11 years. Nothing to Disclose: RO-R, RG-V, MdCT, MAM-B, JMM-M, AL-C, EN-G

Pub #	P1-716
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	RET Somatic Mutations Are Not an Early Event in the Tumoral Transformation of Sporadic Medullary Thyroid Cancer
Author String	C Romei, B Cosci, C Ugolini, V Bottici, E Molinaro, L Agate, A Tacito, F Basolo, P Miccoli, A Pinchera, R Elisei University of Pisa, Pisa, Italy; University of Pisa, Pisa, Italy
Body	The reported prevalence of RET somatic mutations in sporadic MTC is about 40-50% and the most frequent somatic RET mutation is Met918Thr in exon 16. MTC harboring a somatic RET mutation have been demonstrated to have both a more advanced stage at diagnosis and a worse outcome. Although RET mutations are believed to be driving events in the MTC tumorigenesis only the finding of somatic mutations in microMTC can confirm this hypothesis. Aim of the present work was to search for RET somatic mutations in sporadic microMTC (<1 cm) and to compare their prevalence between microMTC and MTC of bigger tumor size. We selected a group of 148 MTC cases in which RET exon 16 point mutation was analyzed by direct sequencing. Tumors were classified according to the size of the nodule as follows: group A, < 1 cm; group B, >1 and < 2 cm; group C, > 2 and < 3 cm; group D, >3 cm. We found an overall prevalence of RET mutation of 18.24% (27/148) that was differently distributed in the four groups. In particular it was 4.6% (2/43) in group A, 12.5% (8/64) in group B, 40% (8/20) in group C and 42.8% (9/21) in group D, thus showing an increasing rate according to the increase of the tumor size. Furthermore, when comparing the prevalence of mutations in the four groups we found a high statistically lower prevalence in microMTC ($p<0.0001$). In conclusion these data indicate that: a) the prevalence of RET somatic mutations is lower than expected if particular attention is given in recruiting microMTC; b) the prevalence of RET somatic mutations is very low (4.5%) in microMTC suggesting that they are not an early event in MTC tumorigenesis. As an alternative to this hypothesis we have to suppose that microMTC could be caused by other oncogene(s) with a lower transforming activity. Nothing to Disclose: CR, BC, CU, VB, EM, LA, AT, FB, PM, AP, RE

Pub #	P1-717
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Assessment of the Role of p27Kip1 Gene in MEN2 and MEN2-Related Syndromes
Author String	T Sekiya, RA Toledo, VC Longuini, SPA Toledo University of São Paulo, School of Medicine, São Paulo, Brazil

Body

Context: Germline inactivation of the cdkn1b gene, which encodes the p27Kip1/cyclin-dependent kinase inhibitor 1B, is responsible for the MENX syndrome in rats, a condition characterized by the occurrence of multiple MEN1- and MEN2-related tumors (1). Rare p27Kip1 mutations have been identified in patients with MEN1-like phenotype but carrying no MEN1 mutation (1-5), however the status of p27Kip1 in MEN2 and MEN2-like families is still unknown.
Objective: To gain insight on the role of p27Kip1 in MEN2 and MEN2-like syndromes.
Methods: We undertook a mutation analysis of the coding regions and intron/exon frontiers of p27Kip1 in RET-negative MEN2-like families and in tumor samples from MEN2/RET-mutated patients. Protein expression was assessed by Western Blot.
Patients/Tumors: Four RET-negative families (two with familial MTC and two with nonsyndromic familial PHEO) and 62 tumors from MEN2/RET-mutated patients, including 31 MTCs, 23 PHEOs, 6 MTC metastasis and 2 parathyroid neoplasias, were included in the study.
Results: No germline defect was found in p27Kip1 gene of the four different RET-negative families studied. The heterozygous status of p27Kip1-V109G polymorphism in the tumors from three RET-negative index-cases indicated that LOH has not occurred. The sequencing of p27Kip1 of tumoral samples from patients carrying a germline activating mutation in the RET protooncogene revealed no DNA pathological change.
Conclusions: Our study indicated that the p27Kip1 is likely not responsible for the MEN2-like RET-negative families investigated, as no germline mutation was found, and the patients[acute] tumor presented no LOH and normal p27 protein expression. The normal p27Kip1 status in the MTC metastases indicates involvement of other genes in malignant transformation. Although p27Kip1 analysis of tumor samples from MEN2/RET-mutated patients found no evidence of pathological genetic event, the finding of low protein expression in these tumors suggested that epigenetic downregulation of p27Kip1 may play a role in MEN2 tumorigenesis, as previously suggested by in vitro studies (6).

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Sources of Research Support: FAPESP Ph.D Fellowship.

Nothing to Disclose: TS, RAT, VCL, SPAT

Pub #	P1-718
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Not All New RET Mutations Show Transforming Activity When Analyzed by *In Silico* and *In Vitro* Assays
Author String	R Elisei, A Vivaldi, B Cosci, C Romei, A Tacito, R Ciampi, V Bottici, V Cappagli University of Pisa, Pisa, Italy
Body	Germline RET proto-oncogene point mutations are causative of nearly all hereditary medullary thyroid carcinomas (MTC) and somatic RET point mutations have been described also in about 40% of sporadic MTC. Aim of this study was the analysis of biologic features of 6 new RET mutations (Ala883Thr, Met848Thr, Val648Ile, Ser904Phe, Met918Val, Thr338Ile) found during the routine screening for RET mutations at the Department of Endocrinology of Pisa and of 6 already known RET mutations as control (Cys634Arg, Val804Met, Leu790Phe, Tyr791Phe, Gly691Ser, Met918Thr). The growth rate, focus formation activity and ability to grow in soft agar of transfected cell lines were compared with cells transfected with wild type RET. Besides, in silico analysis to evaluate the ability of mutations to alter RET protein conformation has been performed. The analysis of growth rate showed that cells transfected with the known mutations Cys634Arg and Met918Thr grew sensibly faster than cells transfected with wild type RET, while cells transfected with the other mutations grew similarly or slower than cells transfected with wild type RET. The two known mutations Cys634Arg and Met918Thr, but also the two new mutations Met848Thr and Ser904Phe gave rise to a high number of foci, >100, while cells transfected with the other mutations gave rise to a number of foci variable between 30 and 10 and cells transfected with wild type RET to 9 colonies. Cells transfected by Cys634Arg and Met918Thr gave rise to the highest number of colonies in soft agar, 24 and 20 respectively, the Val804Met to 15 colonies, while the other mutations were not much different from the wild type RET, which gave rise to 7 colonies. In silico analysis demonstrated that Cys634Arg and Met918Thr, but also the 2 new RET mutations Ser904Phe and Met848Thr had the highest score of 65, Ala883Thr had a score of 55, the 2 known Val804Met and Tyr791Phe and the new Met918Val mutations a score of 15 and the 2 known Leu790Phe and Gly691Ser and the 2 new Val648Ile and Thr338Ile had a score of 0. In conclusion, we found that 2 out of the 6 new RET mutations, M848T and S904F, possess a high transforming activity, but probably a low aggressiveness; 2 of them, V648I and T338I, are probably not transforming and the other 2, A883T and M918V, probably have an intermediate transforming activity. Besides, we found that the in silico score showed a positive correlation with the in vitro transforming activity of the RET mutations. Nothing to Disclose: RE, AV, BC, CR, AT, RC, VB, VC

Pub #	P1-719
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Outcome of Cyclophosphamide, Vincristine, and Dacarbazine for the Treatment of Advanced Medullary Thyroid Carcinoma or Malignant Pheochromocytoma: A Single-Center Experience
Author String	T Deutschbein, A Matuszczyk, LC Moeller, N Unger, A Yuece, H Lahner, K Mann, S Petersenn University Hospital of Essen, University of Duisburg-Essen, Essen, Germany
Body	Objective: Experience with chemotherapy in patients with medullary thyroid carcinomas (MTC) is limited. This retrospective study evaluated the outcome of a combination of cyclophosphamide, vincristine, and dacarbazine ('CVD-regimen'), which has previously been suggested for treatment of malignant pheochromocytomas. Methods: 9 patients (5 males; age 55.0 ± 4.0 years) with MTC were enrolled. Prior to chemotherapy, progressive disease was established in all patients by application of the WHO criteria. On day 1 of each cycle, patients started with cyclophosphamide 750 mg/m2, vincristine 1.4 mg/m2, and dacarbazine 600 mg/m2; on day 2, patients received dacarbazine alone (600 mg/m2). Treatment cycles were repeated at 21-day intervals and six cycles were planned for each patient. The standard imaging procedure was computed tomography, and the primary end point was the objective tumor response rate. After chemotherapy, patients were followed up until progression. Results: The 9 patients underwent a total of 57 cycles (mean 6.3 ± 0.3 cycles). Treatment responses were: 0% complete response, 11% partial response, 56% stable disease, and 33% progressive disease. The median progression free survival was 13.6 months (range 5.8 to 24.2 months). The median change (baseline vs. end of treatment) of calcitonin was -19% (range -70% to +174%). Reversible myelosuppression and moderate gastrointestinal symptoms were the most common adverse events. Conclusion: Although objective tumor response rates were low, the CVD regimen had acceptable toxicity and allowed disease stabilization for a substantial period of time. After initial surgery, chemotherapy should therefore be considered as a medical treatment option. In order to improve long-term therapeutic outcome of patients with progressive MTC, however, new therapeutic approaches and larger multi-center studies are needed. Nothing to Disclose: TD, AM, LCM, NU, AY, HL, KM, SP

Pub #	P1-720
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Percutaneous Ethanol Injection for Treatment of Cervical Lymph Node Metastases in Patients with Papillary and Medullary Thyroid Carcinoma
Author String	EG Abate, R Smallridge, T Bozik, R Paz-Fumagalli Mayo Clinic Florida, Jacksonville, FL; Mayo Clinic Florida, Jacksonville, FL
Body	**Introduction**: Percutaneous ethanol injection (PEI) is utilized for recurrent papillary thyroid carcinoma (PTC), but its role for medullary thyroid carcinoma (MTC) is unknown. **Method**: We retrospectively analyzed 17 patients (13- PTC, 4- MTC) with limited nodal metastases confirmed by fine needle aspiration and treated with PEI from 2004-2010. Median follow-up was 22 months (3-50). Patients were followed biochemically and ultrasonography (US) at 3-6 month intervals. PTC recurrences were monitored by thyroglobulin (functional sensitivity = 0.1ng/ml) and MTC cases by calcitonin. Results were reported as % reduction of tumor markers at the end of follow-up period and US measurement changes reported as % volume decrease (%VD) in LN size pre and post PEI at the end of follow-up period. Volume= [Pi]/6 (L x W x H), and % volume decrease calculated as V_{pre}-V_{post}/V_{pre} x 100. Doppler blood flow (BF) reductions were also reported **Results:** Median age of 48 years (33-91), 7 female and 10 males treated with PEI for recurrent disease were reviewed. Median neck surgeries were 1.8 (1-4). All 17 patients were treated with PEI at least once (1-3), 10 patients twice and 2 patients three times due to persistent disease. Median number of LN treated were 2 for first (1-7), 3 LN (1-7) for second and 4 LN (1-7) for third session. Median ethanol dose per session was 1.7cc (0.4-4) for first, 1.0cc (0.4-3.7) for second and 1.0cc (0.25-1.8) for third treatment. PTC patients had median thyroglobulin reduction of 40% (0-100), median % VD=79% (0-100) and BF was either absent (n=5), reduced (n=4), or stable (n=4). At the end of follow-up period, patients with MTC had a median calcitonin reduction of 0% (0-100), median % VD= 67% (10-89), BF absent (n=1), stable (n=3). Although 100% reduction of calcitonin level was seen at 3 months (n=1), and 20-58% at 10 months (n=3), at the end of the follow-up period, calcitonin levels have increased in 75% of MTC cases. **Conclusion**- PEI was a more effective treatment for PTC than MTC in patients with locoregional recurrence. The % VD and thyroglobulin reduction were greatest at the end of the follow-up period for patients with PTC, suggesting a sustained reduction of disease in the treated LNs. In patients with MTC however, despite a significant %VD of LN, calcitonin levels and disease progressed suggesting a discrepancy between the biochemical and US measurements. In MTC, the effectiveness of PEI does not appear to be as long lasting as in PTC. Nothing to Disclose: EGA, RS, TB, RP-F

Pub #	P1-721
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Effect of One Week of Proton Pump Inhibitor Therapy on Serum and Plasma CgA Concentrations
Author String	HH Mosli, A Dennis, W Kocha, LJ Asher, S Van Uum St Joseph's Health Care Centre, London, Canada; London Health Sciences Centre, London, Canada; London Health Sciences Centre, London, Canada; London Health Sciences Centre, London, Canada; St Joseph's Health Care Centre, London, Canada

Body

Background:
Chromogranin A (CgA) is a sensitive marker of neuroendocrine tumours, and is used for diagnosis, prognosis and for monitoring of response to therapy. Use of Proton Pump Inhibitors (PPI's) results in increased production of CgA. Except for some case reports, there is no information on the extent to which PPIs increase CgA, or on the duration of the effect after discontinuation of PPIs.
Methods:
14 healthy volunteers, ages 18-70, were given bedtime lansoprazole 30 mg for 7 days. Fasting serum samples for CgA were obtained at days 0 and 7, and also 1, 2, 4, and 7 days after discontinuation of the PPI. CgA levels were measured by ELISA in both serum and plasma using Alpco and CisBio assays, and in the plasma using the Dako assay.
Results: CgA concentrations (mean±SD) at baseline and at 7 days, specified for assay, were as follows: Alpco Plasma: 71.1±27.6 and 211.7±134.7ng/mL; Alpco Serum 10.5± 6.4 and 33.5±21.0 ng/mL; Cisbio Plasma 52.9±18.0 and131.3±75.5 ng/mL; CISBIO Serum 32.5±18.6 and 93.9±68.8 ng/mL; Dako Plasma 5.4±2.3 and 17.9±13.6 ng/mL $P<0.01$ for all baseline versus day 7 comparisons, paired Student t-test). CgA concentrations returned to baseline at 7 days post PPI discontinuation.
Conclusion: Use of PPIs for at least one week results in a more than 3-fold increase in CgA concentrations, an effect that takes up to a week to disappear. Further, there is major variation in CgA concentrations not only between assays, but also for the plasma versus serum CgA when using the same assay. This information should be kept in mind when interpreting CgA results for diagnosis of and monitoring for neuroendocrine tumors.

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Nothing to Disclose: HHM, AD, WK, LJA, SVU

Pub #	P1-722
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Thyroid Nodules & Medullary Thyroid Cancer (1:30 PM-3:30 PM)
Title	Thyroid Disease in Patients with Type-1 Neurofibromatosis: A New Chapter?
Author String	C Diazzi, A Guidi, A Luberto, E Taliani, B Madeo, V Rochira, C Carani University of Modena & Reggio Emilia, Modena, Italy
Body	**Introduction** In type-1 Neurofibromatosis (NF-1) there is an increased risk of endocrine tumors, especially pheochromocytomas, whereas thyroid carcinomas seem to be extremely rare, with few cases reported in literature. **Subjects and Methods** In order to investigate the frequency of hypercalcitoninaemia and medullary thyroid cancer (MTC) in patients with NF-1, we evaluated the thyroid gland morphology and function in 17 patients with NF-1 by 1) neck US, 2) Fine Needle Aspiration (FNA) biopsy of the nodules detected at US and the measurement of calcitonin (CT) in wash-out fluid from FNA (CT-FNA), 3) serum CT at basal level and after stimulation with pentagastrin (CT-Pg) and 4) thyroid function. **Results** 1) 10/17 (58.8 %) had nodular goiter and 2) on the basis of cytology at FNA, 3/10 underwent total thyroidectomy with histological confirmation of malignancy in 2/10 patients (1 papillary thyroid cancer and 1 infiltrative squamous carcinoma); 3) only 1/17 presented an increase in basal CT with pathologically increased CT-Pg (>100 pg/ml) with an histological finding of C-cell Hyperplasia after thyroidectomy; 4) of the 16 remaining patients, CT-Pg was normal in 10 patients (<50 pg/ml), while 6 patients had a borderline response (50Journal of Clinical Endocrinology and Metabolism 2004); 5) of the 6 patients with 50**Discussion** The finding of goiter in 60% of NF-1 patients, of thyroid cancer in 11%, and 40% of borderline/high CT-Pg suggest that an accurate study of thyroid gland in patients with NF-1 is mandatory and that thyroid diseases may be underestimated in the context of NF-1. Nothing to Disclose: CD, AG, AL, ET, BM, VR, CC

Pub #	P1-723
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Genome-Wide Analysis of Copy Number Variants and Stature
Author String	A Dauber, Y Shen, Y Yu, MC Turchin, B Wu, JN Hirschhorn Children's Hospital Boston, Boston, MA; Broad Institute, Cambridge, MA; Harvard Medical School, Boston, MA; Children's Hospital Boston, Boston, MA; Harvard Medical School, Cambridge, MA; Harvard Medical School, Boston, MA; Children's Hospital Boston, Boston, MA; Massachusetts General Hospital, Boston, MA
Body	Background: Height is a classic polygenic trait with heritability estimates of up to 90%. Recently genome-wide association studies have identified 180 loci associated with height based on SNP analysis (1). There has been no comprehensive assessment of the influence of copy number variation (CNV) on stature. Objectives: 1. Assess for an association between global copy number burden and stature in a clinical population undergoing CNV testing. 2. Assess whether the association is due to rare or common deletions or duplications. Methods: All subjects between the ages of 2-20 years who had a comparative genomic hybridization (CGH) microarray testing performed in the Genetic Diagnostic Laboratory at Children's Hospital Boston were eligible. Subjects with aneuploidy were excluded. Height data was extracted from the medical record. Age-adjusted sex-specific Z-scores were calculated based on CDC growth charts, using the mean of the 3 measurements closest to the date of the CGH array. Short and tall cases were defined as Z<-2 and Z>+2, respectively. All subjects had CNVs assessed with the Agilent 244K genome-wide CGH array. CNVs were called using NEXUS software. Association analysis for global CNV burden was performed using PLINK (2). Results: 4411 subjects had heights and CGH array data that passed quality filters. These subjects included 415 short cases, 196 tall cases, and 3800 controls. The 611 cases (tall and short) had an excess global CNV burden compared to controls, as measured by total kb of CNV, as well as longer average CNV length (p=0.008 and p=0.01, respectively). These associations were absent for common CNVs (>5% population frequency), but present for rare (<5%) (p=0.007, p=0.03) and very rare (<1%) CNVs (p=0.009, p=0.009). When stratified by type of CNV, there were no significant associations between duplications and stature, but associations between rare or very rare deletions and stature remained significant (p values 0.003 to 0.01). Further stratified analysis of only short cases versus controls and only tall cases versus controls revealed that the excess of global CNV burden was limited to cases with short stature (p=0.002), and furthermore was explained by an excess burden of rare and very rare deletions (p=0.002,p=0.005) but not duplications or common deletions. Conclusions: In a clinical population having microarray testing, individuals with short stature have an excess of rare deletions compared to controls. 1. Lango Allen, H., et al., Hundreds of variants clustered in genomic loci and biological pathways affect human height. Nature, 2010. 2. Purcell, S., et al., PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet, 2007. 81(3): p. 559-75. Nothing to Disclose: AD, YS, YY, MCT, BW, JNH

Pub #	P1-724
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Novel Genetic Alterations in Growth-Related Genes Identified by Deep Exonic Sequencing Are Associated with Idiopathic Short Stature in the EPIGROW Study
Author String	M Bonnemaire, A Grupe, A Thiagalingam, E Pham, Z Zhang, L Naudin, J Rih, V Gupta, D Ross, D Wolfson, A Smolgovsky, J Catanese, C Rowland, R Rosenfeld, L Catanzariti, P Dutailly, P Denefle, P Clayton Ipsen Pharma SAS, Boulogne-Billancourt, France; Celera Corporation, Alameda, CA; Ipsen Biomeasure Inc, Milford, MA; Oregon Health and Science University, Portland, OR; Novartis Institutes of Biomedical Research (NIBR), Cambridge, MA; Royal Manchester Children's Hospital, Manchester, UK
Body	**Background** Stature is one of the most heritable human traits, yet many patients with a height SDS <-2 are labelled as idiopathic short stature (ISS). A small number of molecular defects in genes associated with the GH-IGF axis have been identified as the cause of the short stature in ISS. A much broader approach assessing genes impacting on the growth process may lead to the identification of more molecular defects within ISS, providing a diagnosis and better informed therapeutic decisions. **Aim** To identify genetic variants that are associated with ISS. **Methods** This work has been done in the context of the EPIGROW study, which is a prospective epidemiological study conducted in 9 European countries. Short children were included provided they had a normal GH level and no identified cause of short stature (SS). Next-Gen deep re-sequencing was performed on 1,35 Mb of genomic DNA (exons, exon-intron junctions and promoter regions) of 232 candidate genes in 263 Epigrow subjects and 263 matched controls to assess the association of discovered variants with ISS. **Results** After quality filters, 22,052 single nucleotide polymorphism (SNPs) and 4,006 single base insertions or deletions (Indels) were identified on genes belonging to transcriptional or signalling pathways. Twenty-one SNPs from 8 genes were below an allelic p-value threshold $<10^{-4}$ and <20% False Discovery Rate. None of these 21 variants is a missense or nonsense SNP, and thus has no directly predictable functional effect. These 8 genes and the corresponding highest odds ratios are ZBTB38 (OR=1.96), PRKAR1B (OR=17.64, the larges effect size in the study), KRAS (OR=2.13), SOS2 (OR=2.13), PRKCH (OR=2.08), BBS4 (OR=2.72), FANCA (OR=1.82), MAPK1(OR=2.87). Ten of the 12 SNPs in FANCA and the 2 SNPs in MAPK1 share high linkage disequilibrium (LD), and thus are unlikely to represent independently associated SNPs. The 2 most significant SNPs in FANCA share moderate LD. Two SNPs in ZBTB38 (OR=1.96), one Indel in NFKB1 (OR=2.32) and one Indel in IGF1 (OR=0.33) showed significant association with ISS after Bonferroni testing correction for the 22,052 SNPs and 4,006 Indels respectively. **Conclusion**A large number of variants, both known and novel, were identified by resequencing ISS patients and controls. Our results may provide new insight into molecular defects occurring in ISS patients. Replication of these results in other sample sets will be necessary to validate our findings. Disclosures: MB: Employee, Ipsen. AG: Researcher, Celera. AT: Researcher, Ipsen. EP: Researcher, Ipsen. ZZ: Researcher, Ipsen. LN: Researcher, Ipsen. JR: Researcher, Ipsen. VG: Researcher, Ipsen. DR: Researcher Celera. DW: Researcher, Celera. AS: Researcher, Celera. JC: Researcher, Celera. CR: Researcher, Celera. RR: Consultant, Ipsen. PD: Employee, Ipsen. PD: Employee, Ipsen. PC: Principal Investigator, Ipsen; Consultant, Merck & Co. Nothing to Disclose: LC

Pub #	P1-725
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	High Prevalence of Insulin-Like Growth Factor-I Deficiency in Idiopathic Short Stature Children
Author String	P Clayton, P Maisonobe, P Dutailly Academic Health Sciences Centre, Manchester, UK; Ipsen, Boulogne, France

Body

Background

Defining the status of the GH-IGF-1 axis in children with significant short stature (height SDS <-2.5) is important both for diagnosis and for making decisions about possible treatments. For over 40 years, a GH-centric perspective has dominated management using rhGH, irrespective of GH-IGF-1 status. In idiopathic short stature children (ISS), GH levels are by definition not low but IGF-1 levels have been variably reported as low (~ <-2 SDS) in previous studies at a prevalence of 6-25%. It is likely that the molecular pathology is different between those with normal GH-normal IGF-1 and those with normal GH-low IGF-1, and this may impact on management decisions.

Aim

The primary aim was to describe the prevalence of IGF-1 deficiency in a European cohort of ISS children with careful exclusion of identified causes of short stature.

Methods

This interventional prospective cross-sectional study was conducted between 2008 and 2010 in 64 sites representing 9 European countries. Short children (height SDS $\leq$-2.5, age [ge]2yrs, prepubertal) were included in the study providing they had a normal stimulated GH level and no identified causes of short stature. Patient's auxological parameters were collected during their first visit. IGF-1 deficiency was defined as a mean of 2 basal IGF-1 measures $\leq$-2 SDS assessed in a centralized laboratory.

Results

In total, for 924 patients screened, 275 were enrolled in the study (259 were eligible). Included patients were predominantly males (61.0%); the mean age was 8.4 years (SD 3.2). 16% of patients were small for gestational age. Patients' mean height SDS was -2.9 (SD 0.7) and 37% of patients had a standing height SDS $\leq$-3. Patients' target height SDS was -1.3 (SD 0.8), with both the mothers and fathers having a median height SDS of -1.3. Dysmorphic signs were presented by 14% of patients and 28% had a significant medical and/or surgical history. IGF-1 deficiency was found in 53% of patients and the proportion of IGF-1 deficient patients increased with decreasing height, reaching 63% among patients with a standing height SDS $\leq$-3.

Conclusion

This first epidemiological study focusing on IGF-1 levels in ISS children in a large European cohort showed that IGF-1 deficiency was present in more than half of the patients and indicators of possible genetic aetiologies were present in a significant minority (SGA, dysmorphic signs, prior pathologies).

Disclosures: PC: Consultant, Serono; Principal Investigator, Ipsen. PM: Employee, Ipsen. PD: Employee, Ipsen.

Pub #	P1-726
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Association of a Human Growth Hormone Receptor (*HGHR*) Gene Microsatellite Polymorphism with Idiopathic Short Stature (ISS)
Author String	C Dias, CG Goodyer McGill University, Montreal, Canada

Body

Growth hormone (hGH) is known to play an essential role in the growing child through multiple growth promoting as well as metabolic effects by binding to its high affinity cell surface receptor (hGHR) on target cells.The hGHR is encoded by a single gene on chromosome 5p13.1-p12; fourteen different *hGHR* mRNA variants (V1-V5, V7-V9, VA-VE) with different 5'untranslated regions (5'UTR) exist, all of which code for the same protein (1,2). Many polymorphisms have been identified in the coding regions of the *hGHR* gene, certain of which result in growth hormone insensitivity (Laron syndrome) due to functional defects at the receptor protein level (3). Children with Idiopathic Short Stature (ISS) show growth impairment (>2.5SD below mean height) without exhibiting hGH or hGHR defects, suggesting that decreased hGHR expression may be involved (4).

Polymorphic dinucleotide repeats are common throughout the genome and widely used as genetic markers. A highly polymorphic GT microsatellite has been described in the *hGHR* 5'flanking region ~80bp upstream of the transcription start site of the ubiquitously expressed V9 exon in a healthy population (5). To investigate the possible association between length of the $(GT)_n$ repeat and acquisition of the ISS phenotype, we screened the frequencies of alleles with varying numbers of (GT)n repeat in the *hGHR* gene in 39 ISS children and 138 normal stature adults. Microsatellite genotyping was conducted using labeled primers/capillary electrophoresis to discriminate allele size/GT repeat length. We observed a GT mean of 26 repeats and similar frequencies of GT lengths in the two populations. We then clustered the $(GT)_n$ repeats into three allele classes: S (19-23 repeats), M (24-28 repeats) and L (29-35 repeats). None of the individuals screened showed the L/L genotype The proportion of genotypic frequencies with class L alleles showed discrepancies primarily with the L/S genotype frequency which was significantly higher in ISS children than in the adult control population. Investigations have previously shown that GT repeat sequences have the potential to form alternative DNA structures (e.g. Z-DNA), that they tend to be located near transcription start sites and that they can modulate transcriptional activity (6,7). Because of its close proximity to the transcription start site of the *hGHR* V9 mRNA variant, further studies will now be undertaken to examine the biological significance of this polymorphism on hGHR expression.

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2. Y Wei et al J Clin Endocrinol Metab 91 :1901, 2006.
3. MO Savage et al Nature Clin Pract Endocrinol Metab 2 :395, 2006.
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7. A Bhargava and FF Fuentes Mol Biotechnol 44:250, 2010.

Sources of Research Support: Canadian Institutes of Health Research.

Nothing to Disclose: CD, CGG

Pub #	P1-727
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Growth Genetic Consortium Website: A Website Devoted to the GH-IGF Axis
Author String	V Hwa, JM Wit, MJ Walenkamp, MO Savage, AA Jorge, HM Domene, HG Jasper, R Pfaffle, W Kiess, Y Le Bouc, I Netchine, J Warren, SJ DeVries, RG Rosenfeld Oregon Health & Science University, Portland, OR; Leiden University Medical Center, Leiden, Netherlands; VU University Medical Center, Amsterdam, Netherlands; Barts and the London School of Medicine & Dentistry, London, UK; University of São Paulo, São Paulo, Brazil; Hospital de Ni[ntilde]os Ricardo Gutiérrez, Buenos Aires, Argentina; Hospital for Children and Adolescents, University of Leipzig, Leipzig, Germany; UMRS, 938, INSERM/UPMC, Paris, France; Barkani, Inc, New York, NY; Veraxis Health Communications, Inc, Pine Brook, NJ
Body	In mammals, growth hormone (GH) promotes postnatal growth through regulation of insulin-like growth factor (IGF)-I production. The binding of GH to cell surface GH receptors (GHR), induces multiple signaling cascades of which the STAT5b pathway has been shown to be the most critical for regulating IGF-I production. IGF-I is secreted and exerts its biological effects through endocrine as well as autocrine/paracrine mechanisms. Circulating IGF-I, in ternary complex with GH-regulated IGFBP-3 and ALS (acid labile subunit), is delivered to target tissues where it interacts with cell surface IGF-I receptors (IGF1R), resulting in growth promoting activities. Disruption of this complex chain of events can lead to clinical conditions of IGF deficiency (IGFD) or IGF resistance, GH insensitivity (GHI) and significant growth failure. Mutations in the *GHR*, *STAT5B*, and *IGF1* genes result in IGFD and severe growth failure; mutations in the *IGFALS* gene (encoding for ALS) appear to affect only circulating IGF-I levels, resulting in severe IGFD, but only modest growth failure; mutations in the *IGF1R* gene induce resistance to IGF-I, leading to intrauterine growth retardation and poor postnatal growth. Interestingly, abnormalities in IGF-II expression (*IGF2* gene), have involved epigenetic changes and mutation/deletions in the Imprinting Center Region 1. **Goals**: Access to phenotypic, biochemical and molecular data concerning all documented cases, in one centra location, would be invaluable to clinicians and researchers interested in the GH-IGF axis. An international collaborative **Growth Genetics Consortium** was, therefore, formed to collate relevant documented information. The goals of this Consortium are as follows: [bull]To create and curate a publicly available database and website for all documented molecular defects of the GH-IGF axis [bull]To help guide the clinician in the identification, evaluation and management of patients with molecular defects of the GH-IGF axis [bull]To describe phenotypic, biochemical and genotypic characteristics of all known patients with IGF deficiency or IGF resistance [bull]To educate the medical profession and lay public about the causes of IGF deficiency and resistance **Website:** A website, **http://www.growthgeneticsconsortium.org**, has been established to facilitate access to collated information: Six genes are, for the present, included on this website, with 2 curators assigned for eacl gene. Submission of new cases through the website are encouraged. Sources of Research Support: Veraxis Health Communications, Inc. Nothing to Disclose: VH, JMW, MJW, MOS, AAJ, HMD, HGJ, RP, WK, YLB, IN, JW, SJD, RGR

Pub #	P1-728
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Identification of a Novel Heterozygous *IGF1* Splicing Mutation Associated with Familial Short Stature
Author String	JS Fuqua, V Hwa, MA Derr, RG Rosenfeld Indiana University School of Medicine, Indianapolis, IN; Oregon Health & Science University, Portland, OR
Body	Growth hormone (GH) promotes postnatal mammalian growth through regulation of insulin-like growth factor (IGF)-I production. Rare homozygous mutations of the *IGF1* gene severely impair intrauterine growth, intellectual development and postnatal growth. **Case:** A young male, born at 42 weeks with a normal birth weight (3040g) and length (47 cm), presented with postnatal growth retardation; at age 9.1 y, height was 111.1 cm (-4.0 SDS) and weight, 20.6 kg (-2 SDS). His father had normal stature (+1.2 SD), but the mother was significantly short (-4.0 SD). Severe short stature could be traced back several generations on the mother's side. The clinical phenotype of the patient was otherwise unremarkable. A GH stimulation test with arginine/clonidine was normal (baseline 0.82 pmol/L; 15 pmol/L, stimulated). Serum GHBP was normal (642 pMol/L, normal range, NR, 267-1638) as was IGFBP-3 (2.4 mg/L, NR 2.0-4.8) and ALS (13 mg/L, NR 4.2-13). The serum IGF-I level, 115 ng/ml, was just below normal (-2.21 SDS; NR 123-275), and increased modestly (130 ng/ml) with 4 months of GH therapy. **Results:** The possibilities that the proband was IGF resistant or IGF deficient, were considered and the IGF-I receptor (*IGFIR*) and *IGF1* genes consequently analyzed. *IGFIR* was wild-type, but the *IGF1* gene (exons 1, 3, 4 and 6, encoding the major variant 4 mRNA transcript) revealed two novel, heterozygous, SNPs: *c.207G>A*, a silent SNP in exon 3 and *c.402+1G>C* in the donor splice site of intron 4. *IGF1* analysis of the extended family indicated that the mother (-4.0SD), the maternal grandmother (-6.4SD), a half-sibling (-3.8SD) and a cousin (-2.8SD) were all heterozygous for *c.207G>A* and *c.402+1G>A*. Intriguingly, 5 other short statured family members were wild-type for *IGF1*, as were 11 normal statured family members. Evaluation of the *IGF1* variant 4 mRNA (dermal fibroblasts derived from the mother) demonstrated that the *c.402+1G>A* induced splicing out of exon 4. The fusion of exon 3 with exon 6 would predict a truncated IGF-I mature peptide of 33 amino acid residues, due to a frameshift and early protein termination. **Conclusions:** We describe a novel heterozygous *IGF1* splicing variant associated with familial short stature in an extended family. Although all carriers of this heterozygous mutation are short-statured, and though the mutation results in a severely truncated and nonfunctional IGF-I protein, it remains unclear whether this mutation is the cause of the observed growth failure. Sources of Research Support: Grant from Tercica, Inc (RGR). RGR has received payments for consulting or lectures from Tercica. This potential conflict has been reviewed and managed by OHSU. Nothing to Disclose: JSF, VH, MAD, RGR

Pub #	P1-729
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	A Novel Case of Growth Hormone (GH) and Evolving Thyroid-Stimulating Hormone Deficiency Associated with a Splice-Site Mutation in the GH Gene
Author String	JE Shepard, CE Hanna, V Hwa, R Rosenfeld, KA Woods Oregon Health and Science University, Portland, OR; Oregon Health and Science University, Portland, OR
Body	Isolated growth hormone deficiency (IGHD) affects approximately 1 in 200 short children, with up to 30% of cases likely to be genetic in origin. Here we report the identification of a patient with a GH gene mutation and the utility of this diagnosis for patient management. The proband is a 10 year old caucasian male who presented at 2 years of age with severe short stature (height SDS -5.3), undetectable IGF-1, and low IGFBP-3 (0.27mg/L). Peak GH (insulin/arginine stimulation) was 0.5ng/mL. MRI of the pituitary was normal. At 10 years of age he was diagnosed with TSH deficiency based on declining free T4 levels. His father is also short (height SDS -3.6) and was diagnosed with GHD as an adult (peak GH level <1ng/mL to insulin stimulation). The patient's mother was of normal stature (height SDS -1.02). Genomic DNA analysis of the proband's GH gene revealed a heterozygous mutation at the +5 position of intervening sequence (IVS) 3 (IVS 3+5, G>A). This mutation has been described once before in a Japanese family. Mutations within this region have been shown to result in aberrant splicing (exon 3 skipping) of the GH gene, resulting in a misfolded GH protein, lacking amino acids 32-71. They exert a dominant negative effect and are associated with autosomal dominant GHD (also known as Type II IGHD). Patients with Type II IGHD typically respond well to GH therapy. Our patient initially responded to GH, gaining 1.4 SD in height in 2 years of therapy, but his subsequent response was suboptimal, suggesting either acquired GH resistance, or poor compliance. GH antibodies were negative, and administration of GH by a nurse for 1 week led to a prompt rise in IGF levels, suggesting poor compliance. Patients with Type II IGHD with mutations in the first 3bp of IVS 3 have also been shown to be at risk of further pituitary hormone deficiencies. Our subject is the first reported case of a patient with GHD associated with a mutation at IVS3+5 with another pituitary deficit (TSH deficiency). This case highlights the helpfulness of genetic studies in patients with defects in the GH pathway, particularly in those with a positive family history. In addition to the benefits of genetic counseling, knowledge of the genetic defect may clarify appropriate therapy. The clinician may also be alerted to associated disorders, such as the development of further pituitary hormone deficiencies in the case of our patient. Nothing to Disclose: JES, CEH, VH, RR, KAW

Pub #	P1-730
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Identification of Further *CUL7* and *OBSL1* Mutations in 3-M Syndrome and Their Effects on GH-IGF-I Signaling
Author String	D Hanson, PG Murray, T Coulson, E Saunders, A Sud, AJ Whatmore, GC Black, PE Clayton University of Manchester, Manchester, UK; University of Manchester, Manchester, UK
Body	3-M syndrome is an autosomal recessive disorder characterised by pre- and post-natal growth restriction, a characteristic facial appearance, prominent heels, normal intelligence and variable radiological features. We have previously identified that nonsense and missense mutations in the genes encoding Cullin 7 (CUL7) and Obscurin-like 1 (OBSL1) cause 3-M syndrome. CUL7 is the central component of an E3 ubiquitin ligase which is known to target IRS1, a key signalling molecule within the GH/IGF-1 cascade, whereas OBSL1 is postulated to have a role as a cytoskeletal adaptor protein. Now we report a cohort of patients (N=15) with a typical 3-M syndrome phenotype in which genetic analysis of both *CUL7* and *OBSL1* was undertaken. We identified in total 11 *CUL7* mutations in 9 patients and 3 *OBSL1* mutations in 3 patients. 3 patients, although indistinguishable phenotypically to the other patients did not carry mutations in either gene. Of the 11 *CUL7* mutations 9 were novel (3 frameshift, 3 missense, 2 splice site and 1 nonsense mutation) and 2 of the *OBSL1* mutations were novel, 1 nonsense mutation and the other the first splice site mutation described in *OBSL1*. To determine the functional effect of *OBSL1* and *CUL7* mutations on growth signalling, western analysis of patient derived fibroblast cells revealed normal activation of MAPK and STAT5b in response to GH stimulation, but reduced AKT activation in response to IGF-1. Including this cohort there has been genetic confirmation of 3-M syndrome in 88 families, 70% (N=62) have *CUL7* mutations and 30% (N=26) *OBSL1* mutations. *CUL7* mutations are found throughout the whole gene while *OBSL1* mutations are clustered towards the 5' region of the gene thus affecting all known OBSL1 isoforms. The identification of 3 patients without *CUL7* or *OBSL1* mutations indicates there is further genetic heterogeneity of 3-M syndrome. The discovery of novel *CUL7* and *OBSL1* mutations in a cohort of phenotypically identical 3-M syndrome patients enhances the notion that 3-M syndrome is a disorder of a single pathway. Also, the association of altered signal transduction downstream of IGF-1R may be a contributory factor to the growth restriction. The absence of mutations in other clinically identical 3-M syndrome patients suggests that there are further components within this novel growth pathway. Nothing to Disclose: DH, PGM, TC, ES, AS, AJW, GCB, PEC

Pub #	P1-731
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	IGF-II Deficiency in the Primordial Growth Disorder 3-M Syndrome
Author String	P Murray, D Hanson, A Whatmore, G Black, P Clayton Manchester Academic Health Sciences Centre, Manchester, UK; Manchester Academic Health Sciences Centre, Manchester, UK
Body	**Introduction:** 3-M syndrome is an autosomal recessive disorder characterised by pre- and postnatal growth restriction, characteristic facial dysmorphism (full lips, triangular face and a fleshy tipped nose), normal intelligence and radiological features (slender long bones and tall vertebral bodies). It is known to be caused by mutations in the genes encoding Cullin 7 (a component of the ubiquitination system) and Obscurin like-1 (a cytoskeletal protein). The mechanisms through which mutations in these genes impair growth are unclear. **Aims:** The aim of this study was to identify novel pathways involved in the growth impairment in 3-M syndrome. **Methods:** RNA was extracted from fibroblast cell lines derived from four 3-M syndrome patients and 3 control subjects, and hybridised to Affymetrix HU 133 plus 2.0 arrays with quantitative real time PCR used to confirm changes found on microarray. IGF-II protein levels in serum and conditioned cell culture medium were measured by ELISA. CCCTC binding factor (CTCF, which binds to the unmethylated *H19* differentially methylated region and hence silences *IGF2* expresion) was assessed using Western immunoblotting. **Results:** 1926 probesets were differentially regulated between control and 3-M syndrome fibroblasts (defined as fold difference >2 and expression level >50 in one or more cell lines). 779 of these probesets were upregulated and 1147 downregulated. Of the top 10 downregulated probesets 3 represented *IGF2* while *H19* was identified as the 25th and 65th most upregulated probesets. qRT-PCR confirmed upregulation of *H19* ($p<0.001$) and downregualtion of *IGF2* ($p<0.001$). Levels of IGF-II secreted into conditioned cell culture medium were higher for control than for 3-M fibroblasts (10.2 + 8ng/ml v 2.6 + 4.7 ng/ml, $p<0.01$). Serum IGF-II levels did not appear reduced in 3-M syndrome (n=6 1390 + 212 ng/ml). Levels of CTCF protein were found to be increased in 3 out of 4 3-M lines. **Conclusions:** 3-M syndrome is associated with a gene expression profile of reduced *IGF2* expression and increased *H19* expression similar to that found in Silver Russell syndrome. In 3-M sydrome this gene expression pattern may be due to increased levels of CTCF protein. Loss of autocrine IGF-II in the growth plate may contribute to the short stature in 3-M syndrome. Sources of Research Support: MRC Clinical Research Training Fellowship to Dr P Murray. Nothing to Disclose: PM, DH, AW, GB, PC

Pub #	P1-732
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Cockayne Syndrome Presenting Uniquely with Growth Hormone Deficiency Caused by a Novel Splice Site Mutation
Author String	DH Zangen, H Friedman, S Zeligson, P Renbaum, S Perlberg, A Abulibdeh, E Levy-Lahad Hadassah Hebrew University Medical Center, Jerusalem, Israel; Shaare Zedek Medical Center, Jerusalem, Israel; Hebrew University Medical School, Jerusalem, Israel
Body	***Background:*** Cockayne syndrome (CS) is a photosensitive DNA repair progeroid disorder presenting with growth failure, and multisystem progressive degeneration including cutaneous photosensitivity, loss of adipose tissue, mental retardation, and skeletal and neurological abnormalities. Although mice models of CS exhibited suppressed growth hormone(GH) secretion, human CS mutations have not been associated with GH deficiency. Here we describe a novel mutation with unusual presentation of CS. ***Clinical Data:*** Twin boys born to consanguineous parents (having 2 cousins that died from a progeroid syndrome and short stature) presented at 4.8y of age with photosensitive dermatitis, mild learning difficulties, short stature and low growth velocity. GH stimulation tests and basal IGF-1 levels revealed low GH (peak of 4 and 5 ng/ml) and IGF -1 (5, 6.2 nmol/l)serum levels for both children. GH therapy increased the growth rate from 3 to 8 cm/y. Skin derived fibroblasts showed low transcription coupled DNA repair ability in specific (TCR) Transcription Coupled Repair tests. ***Molecular Data:*** DNA extracted from peripheral lymphocytes of the affected sibling and other family members was sequenced for the ERCC6 /CS-B gene responsible for CS type B. A splice site mutation was found at the beginning of intron 18- c.3778+2T>A predicting the addition of 70 nucleic acids from intron 18 into the transcript and a stop codon thereafter. Missing the C-terminal causes the failure of the resultant protein to bind ubiquitin essential for transcription coupled DNA repair. ***Conclusion:*** A novel splice site mutation in the C-terminal of the CSB gene is associated with a mild phenotype of Cockayne Syndrome firstly described in Palestinian kindred. Interestingly the clinical phenotype includes a unique presentation of GH responsive-GH deficiency, a phenotype found so far primarily in the mice model of CS. Further studies on the C terminal motif of this gene may explain its relevance both to the mild phenotype and to the GH deficiency. Nothing to Disclose: DHZ, HF, SZ, PR, SP, AA, EL-L

Pub #	P1-733
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Multiple Children with Short Stature Secondary to a Novel Nonsense Mutation of *SHOX* Gene in a Single Hispanic Family
Author String	Y Yan, K Loechner, D Bidgood, FK Fujimura, N Jain University of North Carolina, Chapel Hill, NC; University of North Carolina, Chapel Hill, NC; Esoterix Laboratory Service, Calabasas Hills, CA
Body	**S**hort stature **ho**meobo**x (***SHOX)* genes, located on the pseudoautosomal regions of the X and Y chromosomes, are the only genes known to be associated with *SHOX*-related haploinsufficiency. *SHOX*-related haploinsufficiency disorders range from Leri-Weill dyschondrosteosis, at the more severe end of the spectrum, to *SHOX*-related short stature, at the mild end of spectrum. Here, we report six out of nine children, three males and three females, in one Hispanic family with a novel heterozygous nonsense *SHOX* gene mutation presenting with growth retardation and short stature. The first patient was initially diagnosed with familial short stature when he was 3 years old and was subsequently suspected to have *SHOX* gene mutation based on his short arm span and deteriorated growth velocity. All of the patients' heights were greater than 3 SD below the mean and BMI was in 5-25th percentile range at initial evaluation. Mid-parental target height for girls: 56.5 inches, 3 SD below the mean and for boys: 61.5 inches, close to 3 SD below the mean. Bone ages were obtained in five out of six patients. Four patients had a normal bone age and one patient had a delayed bone age. One patient has very short 4th and 5th metacarpals, ulna positive variance, flattening and angulation of the ulnar aspect of the distal radial epiphysis, and widening of the distal radio-ulna joint. Three other patients have mild flattening and angulations of the ulnar aspect of the distal radial epiphysis, widening of the distal radio-ulna joint and slight ulna negative variance. All of the patients had a low normal IGFBP-3, low or low normal IGF-I, normal CBC, thyroid, renal, liver functions, and negative celiac screen. Genetic testing detected a sequence variant at nucleotide position c.582 in the SHOX gene. This variant results in a nucleotide change from C to A (c.582C>A) in codon 194 and alters codon 194 from TGC to TGA. This mutation is expected to cause a termination codon resulting in truncation of the *SHOX* protein at residue 582(p.C194X). All six children have the same mutation. Parents' genes have not been tested. Growth hormone treatment resulted in adequate catch-up growth within a few months. *SHOX* gene defects are the most frequent monogenic cause of idiopathic short stature. Genetic testing for *SHOX* gene mutation needs to be considered when a patient presents with significant short stature with short arm span, abnormal skeletal findings, and negative pertinent laboratory analysis. Nothing to Disclose: YY, KL, DB, FKF, NJ

Pub #	P1-734
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Association of Anterior Pituitary Volume by Magnetic Resonance Imaging with Growth Parameters and the Growth Hormone-Insulin-Like Growth Factor-1 Axis
Author String	MO Regelmann, BN Delman, A Tenore, RA Annunziato, ML Klein, E Chacko, L Iazzetti, D Chia, SJ Hyman E Wallach, R Rapaport Mount Sinai School of Medicine, New York, NY; Mount Sinai School of Medicine, New York, NY; University of Udine, Udine, Italy; Fordham University, Bronx, NY

Body

Background: Prior studies have shown total pituitary volume (TPV) correlates with age, height and nocturna plasma growth hormone levels. To our knowledge, there have been no reports evaluating the relationship of anterior pituitary volume (APV) by magnetic resonance imaging (MRI) and indices of growth and the growth hormone-insulin-like growth factor-1 (IGF-1) axis.

Hypothesis: In children with growth failure, MRI APV correlates with growth parameters and markers of growth hormone production.

Design/Methods: We performed a retrospective chart review of patients followed for growth failure who had a pituitary MRI reviewed by the same neuroradiologist (BD), bone age (BA) x-ray read by same group of pediatric endocrinologists, as well as IGF-1 and IGFBP-3 measured by the same laboratory (Esoterix Inc., Calabasas Hills, CA). MRI TPV and posterior pituitary volume (PPV) were calculated using the accepted formula for an ellipsoid, (pi/6)xLxWxH. APV was calculated by subtracting PPV from TPV. Pearson correlation tests were used to evaluate for associations of APV with TPV, age, height standard deviation (SD) BMI SD, IGF-1, IGF-1 SD, insulin-like growth factor binding protein-3 (IGFBP-3) and IGFBP-3 SD. SD for IGF-1 and IGFBP-3 were based on BA, rather than chronological age.

Results: Data on 68 patients were analyzed. There were 53 males and 15 females, with an average age of 11.11±2.75 years; 30 males and 7 females were pubertal. For all of the patients, APV positively correlated with age (r=0.581, p=<0.001), height SD (r=0.362, p=0.002), BMI SD (r=0.265, p= 0.029), IGF-1 (r=0.535, p=<0.001), IGF-1 SD (r=0.392, p=0.001), and IGFBP-3 (r=0.271, p=0.025). When analyzed by sex and pubertal status, APV only correlated with IGF-1 and IGF-1 SD in prepubertal males (r=0.690, p=<0.001 and r=0.434, p=0.039, respectively). Significant correlations for APV with IGF-1 and IGF-1 SD were not noted for pubertal males, pubertal females or prepubertal females. APV did not significantly correlate with IGFBP-3 SD. TPV and APV were strongly correlated (r=0.996, p=<0.001).

Conclusions: APV correlated with age, height SD and BMI SD. APV also correlated with IGF-1 and IGF-1 SD in prepubertal males. Pubertal hormones, including estradiol, may have differential effects on PV and IGF-1. More data, in larger numbers of patients, are needed to help elucidate the relationships between IGF-1 and APV in female and pubertal subjects.

Nothing to Disclose: MOR, BND, AT, RAA, MLK, EC, LI, DC, SJH, EW, RR

Pub #	P1-735
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Comparison of Single Nucleotide Polymorphisms (SNPs) Associated with Growth Response at Years 1 and 2 on Growth Hormone (GH) Therapy in Children with GH Deficiency (GHD) and Turner Syndrome (TS)
Author String	P Chatelain, P Clayton, V Peterkova, A Belgorosky, M Maghnie, F Antoniazzi, B Destenaves, J Raelson, P Croteau, S Larroque, C Olivier Université Claude Bernard, Lyon, France; Royal Manchester Children's Hospital, Manchester, UK; Institute of Pediatric Endocrinology, Moscow, Russian Federation; Hospital Garrahan, Buenos Aires, Argentina; IRCCS, Giannina Gaslini - University of Genova, Genova, Italy; University of Verona, Verona, Italy; Merck Serono SA, Geneva, Switzerland; Genizon BioSciences Inc, St Laurent, Canada

Body

Background

The PREDICT follow-up study investigates the impact of SNPs within growth- and metabolism-associated genes on long-term growth in children with GHD or TS during GH therapy.

Objective

To evaluate prospectively the association of SNPs on growth response (cm/year) in the first and second years of GH therapy.

Methods

Auxological measurements were taken yearly during GH therapy. Year 1 and 2 median GH doses were 35 and 33 [mu]g/kg/day for GHD and 50 and 49 [mu]g/kg/day for TS, respectively. 1451 SNPs contained within 98 candidate genes were genotyped. The association of genotypes at a given locus with growth at years 1 and 2 was assessed in a continuous analysis using Kruskal-Wallis non-parametric tests (corrected p-value [le]0.05).

Results

For GHD, genes found to be significantly associated with height change were *CYP19A1*, *GRB10*, *IGFBP3*, *INPPL1*, *SOS1*, *TGFA* and *TP53* for baseline-year 1 (n=110) and *AKT1*, *CDKN1A*, *GRB2*, *IRS4* (girls), *PIK3CG*, *PIK3R2*, *PIK3R3* and *STAT_cluster* for year 1-year 2 (n=93). For TS, they were *KRAS* and *LHX4* for baseline-year 1 (n=60) and *ARRB1*, *GATA1*, *IGFALS*, *LHX4*, *PTPN1* and *SOS1* for year 1-year 2 (n=42). In GHD, there were no SNPs in common between years 1 and 2, while in TS, *LHX4* was common to both years.

Conclusion

The SNPs associated with growth during years 1 and 2 of therapy were different in GHD compared with TS. In GHD, the SNPs in year 1 were likely to be influencing 'catch-up' growth, and they differed from SNPs in year 2, which probably represent influences on 'maintenance' growth. In TS, a SNP in *LHX4* had a significant influence on growth during both years.

Sources of Research Support: Merck Serono S.A. - Geneva, Switzerland, an affiliate of Merck KGaA, Darmstadt, Germany.

Disclosures: PC: Investigator, Merck Serono S.A.; Consultant, Merck Serono S.A.; Speaker, Merck Serono S.A. PC: Investigator, Merck Serono S.A.; Speaker, Merck Serono S.A. MM: Investigator, Merck Serono S.A.; Speaker, Merck Serono S.A.; Medical Advisory Board Member, Merck Serono S.A. BD: Employee, Merck Serono S.A. JR: Analyst, Merck Serono S.A. PC: Analyst, Merck Serono S.A. SL: Employee, Merck Serono S.A. CO: Employee, Merck Serono S.A. Nothing to Disclose: VP, AB, FA

Pub #	P1-736
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Using Single Nucleotide Polymorphisms (SNPs) To Predict Year 1 and Year 2 Growth Response to Growth Hormone (GH) Therapy in Children with GH Deficiency (GHD) and Turner Syndrome (TS)
Author String	P Clayton, P Chatelain, E Bashnina, J-W Hou, C Deal, S Cabrol, B Destenaves, J Raelson, P Croteau, S Larroque, C Olivier Royal Manchester Children's Hospital, Manchester, UK; H[ocirc]pital M[egrave]re Enfant, Lyon, France; Medical Academy of Postgraduate Education, St Petersburg, Russian Federation; Chang-Gung Children's Hospital, Taoyuan, Taiwan; Centre Hospitalier Universitaire Ste-Justine, Montreal, Canada; Hopital Armand Trousseau (APHP), Paris, France; Merck Serono SA, Geneva, Switzerland; Genizon BioSciences Inc, St Laurent, Canada
Body	**Background** The PREDICT prospective follow-up study is being used to identify SNPs that could predict annual growth responses to GH therapy in prepubertal children with GHD or TS at treatment initiation. **Objective** To find SNPs that could identify those children with high or low growth responses to GH in years 1 and 2 of therapy. **Methods** All subjects (year 1: 110 GHD, 60 TS; year 2: 93 GHD, 42 TS) were in Tanner stages 1 and 2. Year 1 and 2 median GH doses were 35 and 33 [mu]g/kg/day for GHD and 50 and 49 [mu]g/kg/day for TS, respectively. Height (cm) was measured yearly during therapy and change in height was calculated over two time periods: baseline to year 1 (Y1) and year 1 to year 2 (Y2). 1451 SNPs contained within 98 candidate genes were genotyped. SNPs identified as having a significant effect on growth response by the Kruskal-Wallis test were used in further analyses. Growth responses were placed into 3 categories by age group based upon quartiles: High ([ge]Q3), Intermediate (>Q1 and **Results** In GHD, in Y1, 3 SNPs in 3 genes predicted those unlikely to have a high response to GH ([le]Q3), e.g. of the 36% of children carrying the C allele for rs933360 (*GRB10*) only 8% (25% expected) had a high response and 92% (75% expected) had a lower change in height (PIK3CG); of these, 40% (25% expected) had a low response. In TS, in Y1, rs3845395 (*LHX4*) was associated with growth response; only 7% (25% expected) with the GG genotype were Low responders ([le]Q1), while 48% (25% expected) with either CC or CG genotypes were High responders ([ge]Q3). In Y2, 4 SNPs in 4 genes could predict growth response, e.g. 58% (expected 25%) of those with GG or TG genotypes for rs3787335 (*PTPN1*), carried by 29% of girls, were low responders. **Conclusions** SNPs associated with high or low responses to GH differ between GHD and TS. These SNPs differ between the first ('catch-up') and second ('maintenance') years of therapy. For SNPs associated with high responses, GH therapy at standard doses should be effective, while for those carrying SNPs associated with low responses, modifying the starting GH dose may be considered. Sources of Research Support: Merck Serono S.A. - Geneva, Switzerland, an affiliate of Merck KGaA, Darmstadt, Germany. Disclosures: PC: Investigator, Merck Serono S.A.; Speaker, Merck Serono S.A. PC: Investigator, Merck Serono S.A.; Consultant, Merck Serono S.A.; Speaker, Merck Serono S.A. CD: Speaker, Merck Serono S.A.; Clinical Researcher, Merck Serono S.A.; Ad Hoc Consultant, Multiple companies. BD: Employee, Merck Serono S.A. JR: Analyst, Merck Serono S.A. PC: Analyst, Merck Serono S.A. SL: Employee, Merck Serono S.A. CO: Employee, Merck Serono S.A. Nothing to Disclose: EB, J-WH, SC

Pub #	P1-737
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Growth Hormone (GH) Pharmacogenetics in Patients with Turner Syndrome: The Interactive Effect of a Polymorphism in the IGF-Binding Protein-3 (IGFBP-3) Promoter Region with the GHR-Exon3 Variants on Treatment Outcomes
Author String	AF Braz, EF Costalonga, LR Montenegro, SR Antonini, CE Martinelli, ES Ramos, BB Mendonca, IP Arnhold, AA Jorge Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, Ribeirao Preto, Brazil
Body	**Introduction:** Previous studies demonstrated that patients with *GHR*-exon 3 deleted allele (d3) (1,2) and -202 A allele at *IGFBP3* promoter region (3) had better growth response during rhGH therapy. **Objective:** To assess the combined influence of GHR-exon3 and -202 A/C *IGFBP3* (rs2854744) polymorphisms on the short and long term outcomes to rhGH therapy in a large group of patients with Turner syndrome. **Patients and Methods:** 112 patients with Turner syndrome were genotyped for the presence (fl) or absence (d3) of GHR-exon 3 and for -202 A/C *IGFBP3* polymorphism. Clinical and laboratory parameters were assessed before and during rhGH therapy (mean dose of 48 [micro]g/kg.d). Multiple linear regressions were performed to estimate the proper effect of genotype on 1st year growth velocity (GV) (n = 104) and final height SDS with or without adjustment for target height SDS (n=62). **Results**: Basal clinical and laboratory data were similar among patients with different -202 A/C *IGFBP3* and *GHR* exon 3 genotypes. Despite similar rhGH doses and period of treatment, TS patients presenting the combination of the two favorable genotypes (GHR-exon 3 d3/* + -202 A/* *IGBP3*) had better 1st year growth velocity and final height SDS (with or without adjustment for target height) than patients carrying the most unfavorable genotype (GHR-exon 3 fl/fl ±202 C/C *IGFBP3*), whereas patients with intermediate genotypes (fl/fl + A/* or d3* + C/C) had in-between values. The observed 1st year growth velocity were 8.1 ± 1.3 for d3/*+A/* (n=43); 7.6 ± 1.6 for d3/*+C/C (n=7); 6.5 ± 2.0 for fl/fl+A/* (n=28) and 5.9 ± 2.0 for fl/f+C/C (n=27); p = 0.001. Data regarding to final height SDS adjusted for target height was -0.6 ± 0.8 for d3/*+A/* (n=27), -1.1 ± 0.1 for d3/*+C/C (n=2), -1.3 ± 1.0 for fl/fl+A/* (n=16) and -1.6 ± 1.5 (n=17), p = 0.012. Forward stepwise regression showed that age (p<0.001) and combined GHR-exon 3 and -202 *IGFBP3* genotypes (p=0.009) explain 40% of 1st year growth velocity variability. Regarding to final height SDS, height SDS at start the therapy (p<0.001), age at the start of puberty (p<0.001) and genotype (p<0.001) explained 63% of its variability. **Conclusions**: We concluded that homozygosity for the *GHR*-exon3 full length (fl) allele and -202 C *IGFBP3* allele are associated with less favorable short and long term growth outcomes after rhGH treatment in patients with Turner syndrome. Furthermore, these polymorphisms exhibit a nonadditive interaction in rhGH outcomes. (1) Jorge et al.,JCEM 2006;91:1076 (2) Wassenaar et al.,JCEM 2009;94:3721 (3) Costalonga et al.,JCEM 2009;94:588 Nothing to Disclose: AFB, EFC, LRM, SRA, CEM, ESR, BBM, IPA, AAJ

Pub #	P1-738
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Sequential Application of Annual Prediction Models Permits Accurate Simulation of Height Development during Long-Term Growth Hormone (GH) Treatment in Prepubertal Children with Growth Hormone Deficiency (GHD) and Turner Syndrome (TS)
Author String	MB Ranke, A Lindberg, M Brosz, S Kaspers, J Loftus, H Wollmann, M Koltowska-Haggstrom, M Roelants University Children's Hospital, Tuebingen, Germany; Pfizer Health AB, Sollentuna, Sweden; Gesellschaft für Klinische und Versorgungsforschung mbH, Magdeburg, Germany; Pfizer Ltd, Tadworth, UK; Free University Brussels, Brussels, Belgium
Body	Context: Treatment with GH during the pre-pubertal years is essential for improvement of the height outcome of short children. Optimizing and individualizing GH therapy requires the accurate simulation of height development based on empirical growth prediction models early during the course of treatment. Methods: Pre-pubertal children with idiopathic GHD or TS documented within KIGS (Pfizer International Growth Database) were analysed. In a first step, cohorts which had previously been used to develop models for the prediction of height velocity (HV) during the first four pre-pubertal years of GH treatment were analyzed and a prediction algorithm for the annual gain in weight standard deviation scores (dWt SDS) for an observed gain in height (dHt SDS) was developed using multiple regression analysis. In a second step, the height simulations were validated in a separate population (validation cohort: 664 GHD and 607 TS patients from GH start up to 4 years). The most likely height development was simulated prospectively by sequential application of the newly developed algorithms for gain in weight and the existing yearly prediction algorithms for HV. Results: In both disorders, dWt SDS could be described in each treatment year as a function of Wt SDS and dHt SDS (R[sup2] > 0.89). When height was simulated from GH start in GHD, the predicted mean (and standard deviation, SD) gain after 4 years was 30.4 (3.4) cm when the first year model included GHmax, and 30.5 (2.9) cm when not, while the observed gain in height was 30.0 (5.0) cm. In TS the corresponding predicted and observed mean gains were 27.2 (2.2) cm and 26.5 (3.8) cm respectively. The simulation model was predictive in all but 22 (3.3%) of the 664 cases of the GHD validation cohort from GH start. This proportion was below 2% for all of the TS cohort or when simulation started after the first year of treatment (GHD and TS), using 98% confidence intervals. Conclusion: Sequential application of annual prediction models permits accurate simulation of height development during the first four years of GH treatment in GHD and TS. The system is applicable for groups from GH start and for individuals after experiencing the 1st year growth response. Sources of Research Support: Pfizer Inc. Disclosures: MBR: Consultant, Pfizer, Inc. AL: Employee, Pfizer, Inc. MB: Consultant, Pfizer, Inc. SK: Employee, Pfizer, Inc. JL: Employee, Pfizer, Inc. HW: Employee, Pfizer, Inc. MK-H: Employee, Pfizer, Inc. MR: Consultant, Pfizer, Inc.

Pub #	P1-739
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Long-Term Effects of Oxandrolone in Growth Hormone-Treated Females with Turner Syndrome: The Dutch Experience
Author String	K Freriks, TCJ Sas, MAF Traas, RT Netea-Maier, ARMM Hermus, JM Wit, BJ Otten, SMPF de Muinck Keizer-Schrama, M Gotthardt, PH Dejonckere, GRJ Zandwijken, LA Menke, HJLM Timmers Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; Albert Schweitzer Hospital, Dordrecht, Netherlands; Erasmus Medical Centre, Rotterdam, Netherlands; Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; Leiden University Medical Centre, Leiden, Netherlands; Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; Radboud University Nijmegen Medical Centre Nijmegen, Netherlands; University Medical Centre Utrecht, Utrecht, Netherlands; Dutch Growth Research Foundation, Rotterdam, Netherlands; Haga Hospital, The Hague, Netherlands
Body	INTRODUCTION Short stature is a prominent feature of Turner syndrome (TS), which can be partially overcome by growth hormone (GH) treatment. We have recently reported the results of a trial on the effect of the addition of the weak androgen oxandrolone (Ox) to standard GH and estrogen treatment in girls with TS.[1] Compared with GH+placebo (Pl), GH+Ox in a dose of 0.03 mg/kg/day (Ox 0.03) significantly increased adult height gain (9.5 vs. 7.2cm in Pl) at the cost of mild deceleration of breast development. At a higher dose of 0.06 mg/kg/day (Ox 0.06), no significant increase in height gain was found and significantly more girls reported virilization. In the Ox groups a decrease in fat mass, an increase in muscle mass and lowering of the voice was found. In the current follow-up study of adult participants of the pediatric trial we investigated the long term effects of Ox. METHODS During the original multi-center randomized, placebo-controlled, double-blind trial (1), 133 girls were treated with GH (1.33 mg/m^2/day) from baseline combined with Pl, Ox 0.03, or Ox 0.06 from the age of eight and estrogen from the age of twelve. Sixty-eight women (Pl n=23, Ox 0.03 n=27, Ox 0.06 n=18) participated in the double-blind follow-up study (mean age 24.0 y, mean time since end of GH 8.7 y). We assessed height, breast size, virilization (Ferriman & Gallwey score, gynecological examination, voice analysis) and body composition using DEXA. RESULTS We confirm that adult height gain (adult height minus predicted adult height) is higher with Ox 0.03 (10.2 cm compared to Pl (8.0 cm) (p 0.094). Objective breast size and Tanner stage were not different between groups, nor were body mass index and % fat mass. In the Ox groups women still reported more subjective virilizing effects than in the Pl group (Ox 0.03 48.1%, Ox 0.06 83.3% vs. Pl 34.8%), but without overt objective virilization. The mean voice frequency, however, was lower in Ox 0.06 compared to Pl (210.1 vs. 222.7Hz, p 0.064). CONCLUSION The beneficial effect of Ox 0.03 in addition to GH and estrogen treatment in TS patients on adult height gain is maintained during long-term follow-up. Deceleration of breast development during Ox 0.03 treatment is transient, since final breast size and Tanner stage were similar in Ox and Pl groups. Although Ox treated women subjectively reported more virilization, objective virilization was observed in a minority of the cases. (1) L.A. Menke et al, 'Efficacy and safety of oxandrolone in growth hormone-treated girls with Turner syndrome' JCEM 2010, 95:1151-60 Sources of Research Support: Pfizer. Nothing to Disclose: KF, TJS, MAFT, RTN-M, ARMMH, JMW, BJO, SMPFd-MK-S, MG, PHD, GRJZ, LAM, HJLMT

Pub #	P1-740
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Turner Syndrome Patients Treated with Growth Hormone: Personal Experience
Author String	C Galesanu, N Lisnic, A Loghin, A Florescu, V Gorduza, V Mocanu University of Medicine and Pharmacy Gr T Popa, Iasi, Romania; University of Medicine and Pharmacy Gr T Popa, Iasi, Romania
Body	Introduction: Growth Hormone(GH)treatment during childhood can lead to a higher adult height in girls with Turner Syndrome (TS). The optimal ages to start GH therapy and introduce estrogens for pubertal induction have not been defined. Factors contributing to wide variability in the estimated effects of GH supplementatior on adult height include age of initiation of GH therapy, dosing regime and adjuvant therapies. Aim: To assess the effect of GH supplementation on the growth of girls with Turner Syndrome. Subjects and methods: The girls to be included in the treatment, their diagnosis had to be confirmed by karyotype, bone age less than 12.5 years and slow growth rate. The girls included in this study (n=12) were patients using GH for at least one year and followed over 4 years. The patients were treated with daily s.c. GH injection of 0.2 mg/kg/week. The estrogen replacement was used for pubertal induction. During the follow-up period height was measured every six months. The initial height SDS of each girl was then compared to the mean height of normal girls. Target height (TH) was defined as the mean parental height -6.5 cm. The final height (FH) was compared with the predicted final height. Height deficit was also calculated (TH-FH). The mean chronological age of 12 girls at the initiation of GH therapy was 11.5±3.8 years and the mean height SDS was - 4.2±2.8. Results: Growth velocity (GV) increased in all girls after GH therapy. GV pre-treatment was under 4 cm/yr and increased to 7.7 cm/yr during the first year (between 5-11 cm/yr). In the subsequent years the GV declined but remaining above pre-treatment GV during the 4 years of following. In our group two girls reached FH. One with karyotype 45X/46XX with FH = 162 cm after 4 years of treatment and spontaneous puberty. The TH was 163.5 cm in this case. In another one FH was 150 cm and TH was 162 cm with a height deficit of 12 cm. In this case we used estrogen replacement for induced puberty. Ten girls are under GH therapy yet. Conclusion: In our group the increase in GV was significant only in the first year due to the decline in the response to GH. The increase in FH in girls with TS depends on age at start treatment and duration of GH therapy before estrogens. The number of years of GH therapy is probably the most important factor for predic the response to treatment. Nothing to Disclose: CG, NL, AL, AF, VG, VM

Pub #	P1-741
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Analysis of 3-Year Height Outcomes by Gender and Pubertal Status in Children Treated with Growth Hormone for Growth Hormone Deficiency and Other Growth Hormone-Related Disorders: Data from the ANSWER Program[reg]
Author String	JL Ross, PA Lee, RZ Gut, JA Germak Thomas Jefferson University duPont Hospital for Children, Philadelphia, PA; Penn State College of Medicine The Milton S Hershey Medical Center, Hershey, PA; Novo Nordisk Inc, Princeton, NJ

Body

Background: Long-term safety and efficacy data on pediatric patients treated with Norditropin[reg] (somatropin rDNA origin) are being collected through the American Norditropin Studies: Web-Enabled Research (ANSWER) Program[reg], a US-based registry.
Methods: Data were collected by October 2010 from 8359 treatment-na[iuml]ve pediatric patients with isolated/idiopathic growth hormone deficiency (GHD; n=4454), multiple pituitary hormone deficiency (MPHD; n=387), small for gestational age (SGA; n=461), idiopathic short stature (ISS; n=758) and Turner syndrome (TS; n=435). Change from baseline in height standard deviation scores ([Delta]HSDS) were analyzed at years 1, 2, and 3.
Results: Patients with MPHD (mean age, 7.4±5.44 years), SGA (mean age, 8.5±3.84 years), and TS (mean age, 8.6±4.08 years) were generally younger than patients within other diagnostic categories (mean age, 10.8±3.51 years for GHD and 11.2±3.07 years for ISS) at the start of GH treatment. The lowest mean peak GH levels at baseline were observed in patients with MPHD (3.1±3.03 ng/mL) and GHD (5.3±2.76 ng/mL) vs other diagnostic categories (ISS, 17.0±20.03 ng/mL; SGA, 13.8±12.63 ng/mL). Limited gender differences in DHSDS were found across diagnostic categories with a significant effect observed in GHD and SGA patients at year 1 (male > female, p=0.022). When examined by pubertal status for patients with GHD, the best DHSDS at year 3 was observed in patients who remained in a prepubertal stage throughout the treatment period (1.51±0.86) as compared to patients who were already pubertal at treatment start (1.26±0.68) or who transitioned into puberty during the study (1.24±0.65). When stratified by age at treatment start, younger patients showed greater DHSDS than older patients in all diagnostic categories for both genders; the overall DHSDS at year 3 for males <11 years was 1.39±0.83 vs 1.13±0.66 for males [ge]11 years (p< 0.0001); for females <10 years, the overall DHSDS at year 3 was 1.26±0.88 vs 1.10±0.78 for females [ge] 10 years (p=0.0089). Similar results were observed for years 1 and 2.
Conclusions: These results show that at 3 years after starting GH treatment, a better growth response is observed in younger and prepubertal children. The data also emphasize the importance of starting GH treatment at a younger age.

Disclosures: RZG: Employee, Novo Nordisk. JAG: Employee, Novo Nordisk. Nothing to Disclose: JLR, PAI

Pub #	P1-742
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Analysis of Height Outcomes in Pediatric Subjects Who Achieved Adult Height in Response to Growth Hormone Therapy: Data from the ANSWER Program[reg]
Author String	J Ross, PA Lee, R Gut, J Germak Thomas Jefferson University duPont Hospital for Children, Philadelphia, PA; Penn State College of Medicine Hershey, PA; Novo Nordisk Inc, Princeton, NJ

Body

The American Norditropin Studies: Web-enabled Research (ANSWER) Program[reg], a US-based registry, has collected long term efficacy and safety information on patients treated with Norditropin[reg] (somatropin rDNA origin, Novo Nordisk A/S, Bagsv[aelig]rd, Denmark) at the discretion of participating physicians. The purpose of this analysis was to assess the height standard deviation score (HSDS) at baseline, year 1, year 2, and at adult height for all growth hormone (GH) na[iuml]ve pediatric subjects in the ANSWER Program[reg] who achieved adult height as defined by the physicians.

As of October 2010, 417 GH na[iuml]ve pediatric subjects (262 boys and 155 girls) with different diagnostic conditions including isolated/idiopathic growth hormone deficiency / multiple pituitary hormone deficiency (GHD/MPHD, N=284), idiopathic short stature (ISS, N=27), and Turner syndrome (TS, N=26) from the ANSWER Program[reg] who had reached adult height were included in this analysis. For all subjects at baseline, mean (SD) age was 12.9 (2.1) years (ranging from 12.4 years for MPHD to 13.2 years for TS). The mean baseline age of this population was older than the mean age (10.3 years) of all subjects enrolled in the ANSWER Program[reg]. Overall, mean (SD) HSDS increased from -2.1 (0.9) at baseline to -0.7 (0.9) at the last visit. The adult HSDS for each indication were as follows: GHD/MPHD, -0.6 (0.64); ISS, -0.9 (0.66); and TS, -1.6 (0.89). The mean duration of GHT before subjects reached adult height was 3.8, 3.9, 3.5, and 3.8 years for the overall, GHD/MPHD, ISS, and TS subjects. For the overall population, there was a positive correlation between the year 1 change in HSDS ([Delta]HSDS) and the adult HSDS (correlation coefficient = 0.22; $p<0.001$), and between year 1 [Delta]HSDS and the adult [Delta]HSDS (correlation coefficient = 0.52; $p<0.001$). This positive correlation was also observed in subjects with GHD/MPHD, and ISS. For subjects with TS, a positive correlation with year 1 [Delta]HSDS was observed for adult [Delta]HSDS, but not for adult HSDS.

In conclusion, GH na[iuml]ve subjects who achieved adult height in response to growth hormone therapy experienced increased HSDS from baseline to final visit. The adult height achieved was well within the normal reference range (> -2 SDS). The year 1 [Delta]HSDS was positively correlated with the adult [Delta] HSDS and HSDS in the overall population and in subjects with GHD/MPHD and ISS, consistent with early treatment response as a potential predictor of longer term growth.

Disclosures: PAL: Ad Hoc Consultant, Abbott Laboratories; Study Investigator, Novo Nordisk; Lilly USA, LLC; Abbott Laboratories; Pfizer, Inc. RG: Employee, Novo Nordisk. JG: Employee, Novo Nordisk. Nothing to Disclose: JR

Pub #	P1-743
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Compliance among Pediatric Patients Treated with Growth Hormone: Results from the ANSWER Program [reg]
Author String	D Rotenstein, BS Miller, LC Deeb, T Wisniewski, N Khutoryansky, J Germak Pediatric Alliance, Pittsburgh, PA; University of Minnesota Amplatz Children's Hospital, Minneapolis, MN; Tallahassee Memorial Healthcare, Tallahassee, FL; Novo Nordisk, Inc, Princeton, NJ
Body	Trends associated with Norditropin[reg] (somatropin [rDNA origin]) treatment compliance over 3 years were analyzed in 8359 (5672 M/2687 F) GH treatment-na[iuml]ve pediatric patients enrolled in the American Norditropin Studies: Web-Enabled Research (ANSWER) Program[reg] at the discretion of participating physicians as of October 2010. Change in height standard deviation scores from baseline ([Delta]HSDS) in this cohort was also assessed. Compliance data were recorded by physicians based on patient report at each visit. Data were categorized as missing 0, 1-3, 4-6, or > 6 doses/month. Data were converted into continuous noncompliance scores of 0, 1, 2 or 3, respectively. Longitudinal mixed effects models which account for missing data and repeated measures over time were used for analysis. Models accounted for different covariates including age, gender, and diagnostic indications. Estimated means for noncompliance scores were reported at 4 months, 1, 2, and 3 years with higher means corresponding to greater noncompliance. Mean change in [Delta]HSDS was also calculated using time as a factor and age as a covariate. Diagnostic indications included growth hormone deficiency (GHD, N=4454), multiple pituitary hormone deficiency (MPHD, N=387), Turner syndrome (TS, N=435), Noonan syndrome (NS, N=99), small for gestational age (SGA, N=461), idiopathic short stature (ISS, N=758), and other indications (N=1765). Mean overall compliance was good (<1) across all timepoints. Estimated mean noncompliance scores increased slightly over time from 0.25 at 4 months to 0.48 at Year 3. Gender did not have a significant effect on noncompliance scores. Baseline age showed a small but significant positive correlation (younger children were more compliant) with noncompliance score ($p < 0.05$ at years 1, 2, and 3). Mean noncompliance scores for the various indications over 3 years were: NS (0.32); GHD (0.35); TS (0.39); MPHD (0.40); SGA (0.43); ISS (0.50). Among patients in this cohort, [Delta]HSDS increased with treatment duration at 4 months (0.19), 1 year (0.54), 2 years (0.94), and 3 years (1.23). Overall compliance with Norditropin[reg] therapy was good, regardless of which covariates were used. Older patients were less compliant, but the effect was minimal. Slight variation in compliance was observed within the different indications but poor compliance was not demonstrated among any indication. Consistent with good compliance, [Delta]HSDS continued to increase over 3 years of Norditropin[reg] therapy in this cohort. Disclosures: BSM: Consultant, Novo Nordisk. LCD: Speaker, Novo Nordisk; Pfizer, Inc.; Clinical Researcher, Eli Lilly & Company; Ipsen. TW: Employee, Novo Nordisk. NK: Employee, Novo Nordisk. JG: Employee, Novo Nordisk. Nothing to Disclose: DR

Pub #	P1-744
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Growth Hormone Na[iuml]ve Subjects with Noonan Syndrome Experienced Continued Increasing HSDS after 3 Years of Treatment with Growth Hormone: Data from the ANSWER Program[reg]
Author String	PA Lee, J Ross, J Germak, R Gut Penn State College of Medicine, Hershey, PA; Thomas Jefferson University duPont Hospital for Children, Philadelphia, PA; Novo Nordisk Inc, Princeton, NJ
Body	Noonan syndrome (NS) is a genetic disorder characterized by phenotypic features, including facial dysmorphology, cardiovascular anomalies, and short stature. In 2007, the US Food and Drug Administration (FDA) approved the use of growth hormone for short stature in children with NS. The American Norditropin Studies: Web-enabled Research (ANSWER) Program[reg], a US-based registry, has collected long term efficacy and safety information on patients treated with Norditropin[reg] (somatropin rDNA origin, Novo Nordisk A/S, Bagsv[aelig]rd, Denmark) at the discretion of participating physicians. The purpose of this analysis was to assess the height standard deviation score (HSDS) and change in HSDS ([Delta]HSDS) at baseline, year 1, year 2, and year 3 for subjects with NS in the ANSWER Program[reg]. As of October 2010, there were 99 subjects (75 boys and 24 girls) diagnosed with NS enrolled in the ANSWER Program[reg]. The mean (SD) age of all subjects with NS was 9.5 (3.8) years and the mean (SD) HSDS was -2.7 (0.72) at baseline. The number of subjects who completed 1, 2, and 3 years of treatment was 53, 34, and 15, respectively. The mean (SD) dose of GH at baseline, 1, 2, and 3 years was 47 (10), 51 (12), 48 (12), and 56 (19) mcg/kg/day, respectively. Mean (SD) HSDS increased to -2.3 (0.75) in year 1, -2.1 (0.95) in year 2, and -1.7 (1.36) in year 3. Both male and female subjects showed continued increase in HSDS from baseline over the 3-year period ([Delta]HSDS for males at Year 1, 2, and 3 = 0.38 (0.40), 0.69 (0.70), and 0.9 (0.77), respectively; [Delta]HSDS for females at Year 1, 2, and 3 = 0.32 (0.42), 0.54 (0.59), and 0.89, respectively). No significant differences were found between male and female subjects. There was a negative correlation between subject age at baseline and year 1 [Delta]HSDS (correlation coefficient = -0.3102; p=0.0238) and year 2 [Delta]HSDS (correlation coefficient = -0.4551; p=0.0068). However, there was no significant correlation between baseline age and year 3 [Delta]HSDS. In conclusion, growth hormone na[iuml]ve subjects with NS from the ANSWER Program[reg] showed continued increase in HSDS after treatment with growth hormone. There were no significant differences between male and female subjects. Baseline age was negatively correlated with [Delta]HSDS in years 1 and 2 but not in year 3. Whether longer-term therapy increased adult height in NS remains to be investigated with longer GHT duration and a larger patient population. Disclosures: PAL: Ad Hoc Consultant, Abbott Laboratories; Study Investigator, Novo Nordisk; Lilly USA, LLC; Abbott Laboratories; Pfizer, Inc. JG: Employee, Novo Nordisk. RG: Employee, Novo Nordisk. Nothing to Disclose: JR

Pub #	P1-745
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Validation of Growth Charts for Patients with Noonan Syndrome and Noonan-Like Syndrome Based on Current Molecular Knowledge
Author String	AC Malaquias, AS Brasil, AC Pereira, DR Bertola, AAL Jorge Universidade de São Paulo, São Paulo, Brazil; Universidade de São Paulo, São Paulo, Brazil; Universidade de São Paulo, São Paulo, Brazil; Universidade de São Paulo, São Paulo, Brazil

Body

The Noonan syndrome (NS) and Noonan-like syndrome (NLS) are autosomal dominant disorders characterized by dysmorphic face, congenital heart disease and short stature. Heterozygous mutations in the RAS/MAPK genes cause NS and NLS. The natural history of growth deficit associated with NS and NLS was previously described regardless the molecular diagnosis. Growth curves for height constructed for developmental syndromes allow identifying individuals with severe growth deficit that need additional medical concerns.

Objectives: To validate growth pattern for NS and NLS patients with mutations identified in *PTPN11*, *KRAS*, *SOS1* and *RAF1* genes.

Patients and Methods: One-hundred NS and 6 NLS patients with mutations identified in the *PTPN11* (n = 76), *SOS1* (n = 13), *RAF1* (n = 10) and *KRAS* (n = 7) genes were selected. Auxological measurements were obtained at birth and during childhood to adulthood. Height values are tabulated at the exact age in 6-month intervals and each child contributed only one single set of data for each age group. Height and weight were collected in a mixed longitudinal and cross-sectional mode. None of these patients were treated with growth hormone. The analyzed variables were gestacional age, birth weight and lenght, chronological age and height. Height was expressed as SDS for age and sex for normal population and NS-specific standards (1). The differences between genotypes were analyzed by t-test and ANOVA.

Results: Information on weight and length at birth was available in 90 and 65 patients, respectively. Patients had birth weight and length within normal ranges: birth weight SDS = 0.0 ± 1.5 and length SDS = -1.2 ± 1.3. Ninety-eight out of 106 patients were evaluated during growth period, resulting in 379 observations (mean of 4 measurements per patient). As a group, mean height SDS was -2.5 ± 1.2 and -0.5 ± 1.2 for normal population and NS-specific standards, respectively. Concerning the genotype influences on postnatal growth, patients with *SOS1* mutations were taller (mean height SDS for NS-standard = 0.0 ± 1.0, p = 0.008), whereas patients with *KRAS* (-1.2 ± 1.1, p = 0.034) and *RAF1* (-1.1 ± 0.9, p = 0.002) mutations were shorter than other genotypes.

Conclusions: The small negative height SDS for NS-specific standards observed in our population could be explained by ethnic population backgrounds. Patients with *KRAS* and *RAF1* mutations had more severe height deficit, whereas *SOS1* mutated patients had less severe growth impairment.

(1) Ranke MB et al., Eur J Pediatr 1988; 148:220.

Sources of Research Support: FAPESP 07/59555-0.

Nothing to Disclose: ACM, ASB, ACP, DRB, AALJ

Pub #	P1-746
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Cellular Metabolomics in Children Born Small for Gestational Age: Biomarkers of Growth Failure and Altered Metabolites Involved in Metabolic Disease
Author String	P Murray, I Butcher, A Stevens, W Dunn, M Westwood, P Clayton University of Manchester, Manchester, UK; Manchester Interdisciplinary Biocentre, Manchester, UK
Body	**Background:** Being born small for gestational age (SGA) with failure of postnatal catch up growth is a common indication for treatment with recombinant human growth hormone. Currently the cause of growth failure in the majority of these children is unknown. Specific biomarkers of this poor growth have not been identified. We have used metabolomics in this pilot study to identify novel cellular biomarkers and their associated functional pathways in fibroblast cell lines derived from children with post-natal growth failure. **Aim:** To compare the metabolome in fibroblast cell lines derived from children born SGA with failure of post-natal catch up growth to that in control fibroblasts. **Methods:** Skin fibroblast cell lines were derived from three healthy control children and eight children born SGA (2 no cause identified, 2 Russell Silver Syndrome and 4 3-M Syndrome). Confluent cells were incubated in serum free media, which was removed after 24 hours (to generate the metabolomic *footprint*) and the cells lysed to generate the metabolomic *fingerprint*. Samples were analysed by gas-chromatography mass spectrometry and Ingenuity Pathway Analysis software (IPA) used to assess the biological function of identified metabolites. **Results:** 15 metabolites in the fingerprint and 18 in the footprint were significantly different between SGA and controls. In the fingerprint there were alterations in amino acid metabolism (alanine, fold change SGA:control (FC) 0.3, aspartic acid FC 4.0) ($p<0.05$), carbohydrate metabolism (glucose-6-phosphate FC 0.5, lactic acid FC 0.6) ($p<0.05$) and the signalling molecule myo-inositol (0.2 fold, $p<0.0001$). In the footprint there was also a significant association with carbohydrate metabolism (lactic acid FC 0.6 and hexanoic acid FC 0.3, $p<0.05$) and amino acid metabolism ($p<0.05$). The largest changes were in the amino acids alanine and cysteine (FC 0.1 and 0.2, $p<0.01$). In both the fingerprint and footprint, the metabolite profile was associated with metabolic disease pathways (e.g. type 2 diabetes; $p<0.05$) and HIF1 α signalling ($p<0.01$). **Conclusions:** These significant differences in the metabolome profile of control and SGA fibroblasts represent potential biomarkers of post-natal growth failure. Future studies using urine or serum from SGA patients are required to determine clinical relevance of these metabolites. Nothing to Disclose: PM, IB, AS, WD, MW, PC

Pub #	P1-747
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Final Height of Idiopathic Short Stature Children Treated with Recombinant Human Growth Hormone (rhGH
Author String	TC Martins, CN Lauretti, IJP Arnhold, BB Mendonca, AAL Jorge Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

Body

Background: The use of recombinant human growth hormone (rhGH) therapy to promote growth in children with idiopathic short stature is controversial. A fundamental issue underlying this controversy is the paucity o studies that reports achieved final height(1).
Objective: To retrospectively assess the height gain after rhGH treatment in children with short stature without an identified cause.
Patients: We studied seventy two patients (28 females) with idiopathic short stature (ISS, n = 55) or born small for gestational age (SGA, n = 17). Exclusion criteria were growth hormone deficiency, chromosome disorders, malformation syndrome, skeletal disorders, chronic diseases, malnutrition and steroid therapy.
Intervention: Thirty-eight patients (16 females) were treated with rhGH in a dose ranging from 33-50 g/kg/d for 4.7 ± 2 years. Thirty-four patients were followed without any specific intervention. Final height was determined when the growth rate was < 1 cm/y observed during a minimum 12 months of follow-up.
Results: There were no difference between treated and not-treated patients related to gender distribution, number of patients born SGA, target height, chronological age (11 ± 2.5 y), bone age (8.5 ± 2.5 y) and body mass index SDS at the first evaluation. ISS and SGA children had similar basal parameters. Treated patients were significantly shorter (height SDS -3.7±0.9 vs. -3.0 ± 0.8, p = 0.001) and had lower predicted final height SDS by Bayley-Pinneau method (-2.3 ± 1.0 vs -1.3 ± 1.3, p = 0.002) than non-treated patients. Both groups of patients treated and non-treated reached similar final height SDS (-2.3 ± 1.0 and -2.3 ± 0.9, respectively) although total height SDS gain was higher in treated patients (1.3 ± 1.2) than non-treated ones (0.6 ± 1.0, p = 0.005). The estimated effect of rhGH therapy on final height was 4.8 cm (95%CI 1.5 to 8.0 cm). Patients born SGA reached a shorter adult height SDS corrected to target height SDS (-1.4 ± 0.8) than ISS children (-0.6 ± 1.0, p = 0.019) after long term rhGH treatment.
Conclusion: Growth hormone treatment to children with ISS or born SGA increased adult height to a level above the predicted adult height and improved height SDS gain more than with untreated children. Although the mean height gain (4.8 cm) was modest and highly variable (1.5-8.0 cm), rhGH treatment provided significantly better adult height outcomes for the majority of treated children.

(1) Beth S. Finkelstain et al., Anch Pediatr Adolesc Med 2002; 156:230-240
(2) John G. Buchlis et al., J Clin Endocrinol Metab 1998; 83:1075-1079
(3) Raymond L. Hintz et al., Endocr J 1999; 340:502-507

Nothing to Disclose: TCM, CNL, IJPA, BBM, AALJ

Pub #	P1-748
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	The Safety of Two Different GH Dosages Administered for Five Years in Short Japanese Children Born Small for Gestational Age
Author String	S Kanzaki, T Tanaka, S Yokoya, Y Seino, H Togari, J Mishina, A-M Kappelgaard Tottori University Faculty of Medicine, Tottori, Japan; Tanaka Growth Clinic, Tokyo, Japan; National Center for Child Health and Development, Tokyo, Japan; Osaka Kosei Nenkin Hospital, Osaka, Japan; Nagoya City University, Nagoya, Japan; Nakanoshima Clinic, Tokyo, Japan; Novo Nordisk A/S, Virum, Denmark
Body	**Objective:** Few long-term studies of growth hormone (GH) therapy have been conducted in children born small for gestational age (SGA). This trial investigated the safety of 2 GH dose levels in a Japanese population over 260 weeks. **Study Design and Methods:** 77 children born SGA with height [le] 2 SDS for their age (3-<8 years) were randomised in the initial 104-week to 1 of 2 GH (Norditropin[reg], Novo Nordisk A/S, Denmark) doses, 0.033 mg/kg/day (Group A; n=39) or 0.067 mg/kg/day (Group B; n=38). 66 of these patients enrolled in the 156-week study extension and continued the same dose (n=33 in both groups). Total exposure was 260 weeks. Levels of insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), and antibodies were measured week 2, week 4, then every 13 weeks, while HbA_{1c} was measured every 52 weeks. Adverse events were recorded. **Results:** 65 patients were included in the safety analysis (31 in Group A, 34 in Group B) for the entire 260 week period due to patient withdrawals or non-exposure. Only 1 withdrawal was due to a serious adverse event possibly related to the trial product: a moderately severe case of IgA nephropathy at week 151 in Group A. During 260 weeks of treatment, the majority of AEs were mild or moderate childhood infections unlikely to be related to the trial product. In both dose groups, IGF-1 SDS and IGFBP-3 SDS levels were below normal at baseline (IGF-1 SDS, A/B: -0.7/-0.6; IGFBP-3 SDS, A/B: -0.1/0.1). During the first 8 weeks of treatment, IGF-1 and IGFBP-3 rose steeply in both groups before reaching a plateau (IGF-1, A: from 121.6 to 465.4 ng/mL; B: from 120.1 to 665.9 ng/mL; IGFBP-3, A: from 2.1 to 3.1 [mu]g/mL; B: from 2.2 to 3.4 [mu]g/mL). At week 260, values for IGF-1 SDS and IGFBP-3 SDS were below upper normal levels (IGF-1 SDS, A/B: 0.8/1.7; IGFBP-3 SDS, A/B: 0.7/1.2). Although HbA_{1c} increased during the first 104 weeks before a plateau was reached (A: from 4.8 to 4.9%; B: from 4.7 to 5.0%), no patient had an HbA_{1c} above the reference range (4.3-5.8%) while in the study. During treatment years 4 and 5, 14 patients tested positive for GH antibodies. However, antibody formation did not appear to affect height gain which was similar between patients with and without antibodies. **Conclusion:** Long-term safety of GH therapy was demonstrated during 5 years of treatment in Japanese children with short stature born SGA. Both dose levels - 0.033 mg/kg/day and 0.067 mg/kg/day - were well tolerated. Disclosures: A-MK: Employee, Novo Nordisk. Nothing to Disclose: SK, TT, SY, YS, HT, JM

Pub #	P1-749
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Changes in Bone Age and Patient Satisfaction in Short Japanese Children Born Small for Gestational Age during Growth Hormone Treatment for Five Years
Author String	E Ogawa, T Tanaka, S Yokoya, Y Seino, H Togari, J Mishina, A-M Kappelgaard Teikyo University School of Medicine, Tokyo, Japan; Tanaka Growth Clinic, Tokyo, Japan; National Center for Child Health and Development, Tokyo, Japan; Osaka Kosei Nenkin Hospital, Osaka, Japan; Nagoya City University, Nagoya, Japan; Nakanoshima Clinic, Tokyo, Japan; Novo Nordisk A/S, Virum, Denmark
Body	**Objective:** A potential concern with growth hormone (GH) treatment is acceleration of bone maturation relative to chronological age (CA). To determine the effects of GH therapy on bone maturation and patient satisfaction with treatment, this study monitored bone age (BA), body weight, BMI, and quality of life (QoL) in short children born small for gestational age (SGA) treated with GH therapy for 5 years. **Study Design and Methods:** After completing a 104-week randomised trial of 2 GH (Norditropin[reg], Novo Nordisk A/S, Denmark) doses, 0.033 mg/kg/day and 0.067 mg/kg/day, 66 children born SGA with height [le] 2 SDS for their age (3-<8 years) were enrolled in a 156-week extension trial, continuing the initial dose. Group A received the smaller dose (n=33), Group B the larger (n=33). BA was measured by the Tanner-Whitehouse II (TW2) radius, ulna and short finger bones (RUS) method. Bodyweight and BMI were assessed week 2, week 4, then every 13 weeks, and BA every 52 weeks. Parents or legal guardians were instructed at baseline and every 52 weeks to complete a 43-question QoL survey. The score range for each question was 1-3, with 3 the most positive. The questionnaire covered: physical discomforts (5 questions), physical health (7), contact with other children (6), reactions from adults (5), physical appearance (6), and behaviour (14). **Results:** Mean BA at baseline (Group A/B: 4.3/4.1 years), which was delayed compared to CA (BA/CA ratio A/B: 0.9/0.8), tended to be normalised, approaching CA after 5 years of treatment (BA/CA ratio, A/B: 1.0/1.1). The mean increase in BA at study end was 5.8 and 7.2 years for Group A/B, respectively. Gradual increases over time in body weight (A: from 13.4 to 24.0 kg; B: from 12.8 to 26.2 kg) and BMI (A: from 14.4 to 15.2 kg/m^2; B: from 14.2 to 15.5 kg/m^2) were reported as expected. Overall survey scores increased (A: from 2.3 to 2.5; B: from 2.4 to 2.6). The higher mean scores at study end for 'physical discomforts' (A: from 1.8 to 2.4; B: from 2.2 to 2.7) and 'reactions from adults' (A: from 1.8 to 2.4; B: from 2.0 to 2.8) indicated that patients experienced less difficulty related to short stature after treatment and that their size was regarded as more age appropriate by adults. **Conclusions:** In this study, final height was not jeopardised by a treatment-induced acceleration in BA. Patient QoL, especially perceived physical discomfort and reaction from adults, improved with either GH treatment dose. Disclosures: A-MK: Employee, Novo Nordisk. Nothing to Disclose: EO, TT, SY, YS, HT, JM

Pub #	P1-750
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Long-Term GH Treatment Response in Short Children Born SGA Is Comparable to That in GHD Children: Data from the NordiNet[reg] IOS
Author String	O Blankenstein, I Oliver, M Snajderova, HT Christesen, PA Lee, V Rakov, BT Pedersen, L Saevendahl Charité-Universit[auml]tsmedizin, Berlin, Germany; H[ocirc]pital des Enfants, Toulouse, France; University Hospital - Motol, Prague, Czech Republic; Universitetshospital, Odense, Denmark; Indiana University School of Medicine and Penn State College of Medicine, Indianapolis and Hershey, IN; Novo Nordisk Health Care AG, Zurich, Switzerland; Novo Nordisk AS, Soeborg, Denmark; Karolinska Hospital, Stockholm, Sweden

Body

Introduction

Clinical studies conducted with children born small for gestational age (SGA) demonstrates that long term treatment with growth hormone (GH) results in a normalization of height during childhood[1]. There is still a concern whether GH treatment in non-GHD patients is effective at the same extend as in GHD children.

Objectives

The aim was to analyse 4-year treatment outcomes in SGA children in comparison with that in GHD children.

Methods

Short children born SGA and children with GHD treated with Norditropin[reg]for at least 4 years and enrolled into NordiNet[reg] International Outcome Study (IOS) were included in this analysis. Descriptive statistics and simple t-tests were used for the analysis of the baseline values and HtSDS levels. The yearly changes of htSDS have been estimated in a repeated measurements model including year as a fixed effect and patient as a random effect for each diagnosis separately.

Results

268 short children born SGA (152 males) and 489 GHD children (344 males) were identified in the NordiNet [reg]IOS database for this analysis. SGA children were significantly younger, mean±SD 7.2±2.7 vs 8.1±3.4 yrs ($p<0.001$) and more severely growth retarded at baseline, mean Ht SDS±SD -3.3±0.7 vs. -2.8±1.1 ($p<0.001$), they received in average a higher relative GH dose during the treatment course compared to GHD (39.4 ug/kg/d in SGA vs. 31.5 ug/kg/d respectively ($p<0.001$)). We observed a continuous significant improvement of HtSDS in both patient populations which was similar in both indications. After 4 years cumulative mean increase in HtSDS was 1.60±0.71 in SGA and 1.55±0.97 in GHD (difference 0.05 (-0.06; 0.15), $p=0.412$); 68% of SGA children and 79% of GHD children achieved a height within the normal range after 4 years of GH treatment.

Conclusions

Long term GH treatment results in a similar height improvement in SGA and GHD children. These results support the GH treatment benefit in non-GHD children such as short children who were born SGA. However, a lower mean age and HtSDS at treatment start, as well as a higher GH dose in SGA children may contribute to a better treatment response in this patient population.

van Pareren et al. JCEM 2003, 88(8):3584-3590

Disclosures: VR: Employee, Novo Nordisk. BTP: Employee, Novo Nordisk. Nothing to Disclose: OB, IO, MS, HTC, PAL, LS

Pub #	P1-751
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Temporal Trends in Growth Hormone Treatment of Children with GHD, Born SGA and with Turner Syndrome: German Data from the Longitudinal NordiNet[reg] International Outcome Study
Author String	O Hiort, T Rohrer, M Wabitsch, J Wolfle, C Brack, V Rakov, BT Pedersen, D Schnabel University of Lübeck, Lübeck, Germany; Saarland University Hospital, Homburg/Saar, Germany; University of Ulm, Ulm, Germany; Center for Pediatric Medicine, Bonn, Germany; Gemeinschaftspraxis, Celle, Germany; Novo Nordisk Health Care AG, Zurich, Switzerland; Novo Nordisk AS, Soeborg, Denmark; Otto-Heubner-Centrum für Kinder- und Jugendmedizin, Charite, Berlin, Germany

Body

Introduction

Treatment of short stature of varying etiology with recombinant human growth hormone (GH) has been available for over two decades. However, data are sparse on trends and changes in treatment practices which may have affected treatment outcomes in recent years.

Objective

To investigate whether patient characteristics (gender distribution (except Turner Syndrome (TS)), age, height (HtSDS), BMI and relative GH dose) showed any discernible trends depending on calendar year of GH treatment start.

Methods

Patients included were GH deficient (GHD) children, short children born small for gestational age (SGA) and children with TS treated with Norditropin[reg] and enrolled in the German subcohort of the NordiNet[reg] International Outcome Study (IOS). Baseline data were collected per year from 2002 (study initiation in Germany) through 2009. Trends were analyzed per indication using simple mixed linear models including random variation between both individual patients and annual mean levels.

Results

Our cohort comprised 1089 GHD, 690 SGA and 138 TS patients who started GH treatment during 2002-2009 In GHD and SGA, the sex ratio showed a male excess (69% and 58% boys on average), which did not vary substantially. During 2002-2009, mean age at treatment start showed no significant change in GHD children (9.6±3.8 yrs) but decreased in SGA (9.7±4.6 [rarr] 7.5±2.8 yrs, p=0.026) and TS patients (9.3±3.5 [rarr] 7.6±4.7, n.s.). Baseline HtSDS showed no significant changes for all three indications; the respective mean values for GHD, SGA and TS were [minus]2.82±1.01, [minus]3.38±0.73 and [minus]3.16±0.91. BMI was without significant changes during 2002-2009 except in SGA patients, who showed a nonsignificant decrease in BMI (19.08±8.39 [rarr] 14.96±2.72). No significant changes were observed for relative GH dose, with GHD, SGA and TS patients receiving mean daily doses of 29.5±6.8 [mu]g/kg, 35.1±6.0 [mu]g/kg, 45.5±12.2 [mu]g/kg, respectively.

Conclusions

During 2002-2009, baseline characteristics revealed a tendency towards earlier treatment of SGA and TS patients. In contrast, GHD was associated with a relatively late start of GH treatment. This likely reflects the persistent delay that children with GHD experience between diagnosis in primary care and appropriate treatment by a pediatric endocrinologist. Further studies are needed to investigate whether the observed trends affect treatment outcomes.

Disclosures: VR: Employee, Novo Nordisk. BTP: Employee, Novo Nordisk. Nothing to Disclose: OH, TR, MW, JW, CB, DS

Pub #	P1-752
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Growth Hormone Treatment in Australia for Patients with Growth Hormone Deficiency and Short Stature and Slow Growth: Evaluation of the First Three Years
Author String	IP Hughes, C Choong, PSW Davies, A Cotterill, M Harris University of Queensland, Herston, Australia; Princess Margaret Hospital for Children, Subiaco, Australia; Mater Children's Hospital, South Brisbane, Australia

Body

Introduction

Australia uses auxological criteria in determining eligibility for Growth Hormone (GH) treatment in both GH Deficiency (GHD; peak GH<10mU/L) and Short Stature and Slow Growth (SSSG), comprising primarily Idiopathic Short Stature patients (ISS). GH treatment is similar for GHD and SSSG and follows national guidelines. We evaluated response in the 1st, 2nd and 3rd years of treatment in a large clinical cohort of GHD and SSSG patients in Australia.

Patients and Methods

GHD and SSSG patients who completed 1y (n=186 GHD, 524 SSSG), 2y (n=172, 422), or 3y (n=123, 341) o treatment and were currently receiving GH. Demographic variables were recorded. Response, change in height standard deviation score ([Delta]SDS), was measured for each year of treatment. Stepwise multiple regression analyses (LR) were used to identify factors that significantly influenced response.

Results

Median values of demographic variables for GHD and SSSG: -Age at GH start, (5.11, 6.60); Height SDS at GH start, (-2.78, -3.01); BMI-Z at GH start, (0.46, -0.33). Mean GH dose mg/m^2/wk (GHD, SSSG): Year 1 (4.27, 4.58), Year 2 (4.07, 4.76), Year 3 (4.29, 5.10). Median response [Delta]SDS (GHD, SSSG): Year 1 (0.92, 0.57), Year 2 (0.32, 0.31), Year 3 (0.30, 0.21). A benchmark response of [Delta]SDS>0.5 has been suggested for ISS in the first year. Percentage failing to reach this for SSSG was 39.9% and 26.9% for GHD. Variables identified by LR to have a + or - influence on response; GHD Year 1: -Height SDS start, -Age Start +BMI-Z Start, +Mean Parent Height SDS. Year 2: -Dose Y2, -SDS start, Gender (+girls). Year 3: -SDS Start, +[Delta]SDS Y2. SSSG: -Age Start, +GHD (some receiving GH as SSSG patients were diagnosed as GHD), -SDS Start, -Dose Y1. Year 2: + [Delta]SDS Y1, -Age Start, -SDS start Y2, +GHD, +SDS start GH, -Dose Y2, +BMI-Z Y2. Year 3: +Mean Parent Height SDS.

Conclusions

GHD and SSSG are initially treated similarly but differ in demographics and response. Response is greatest for both indications in the first year but declines thereafter for both. Dose is increased in each year only for SSSG. Variables known to influence GH response such as height, age, and BMI-Z were identified in these cohorts. GHD girls were seen to respond better in the 2nd year of therapy than boys. Within the heterogenous subgroup of SSSG patients those with diagnosis of GHD had the best response to GH therapy over the 3 year interval.

Sources of Research Support: Australasian Paediatric Endocrine Group.

Nothing to Disclose: IPH, CC, PSWD, AC, MH

Pub #	P1-753
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Analysis of Growth Response to Treatment of Different Diagnostic Entities Comprising the [ldquo]Short Stature and Slow Growth[rdquo] Indication for GH Treatment in Australia
Author String	IP Hughes, C Choong, PSW Davies, A Cotterill, M Harris University of Queensland, Herston, Australia; Princess Margaret Hospital for Children, Subiaco, Australia; Mater Children's Hospital, South Brisbane, Australia

Body

Introduction and Background

[ldquo]Short stature and slow growth[rdquo] (SSSG) is an indication for GH therapy in Australia and is defined by height less than the first centile (CDC Growth Charts) and growth velocity less than 25th centile fo skeletal age and sex. SSSG is a heterogeneous diagnostic group comprising mostly patients with idiopathic short stature (ISS, n=186) but also Prader Willi (PWS, n=20), Noonan (n=10), and Russell (n=19) syndromes, small for gestational age (SGA, n=31), familial short stature (FSS, n=56), and GH deficiency (GHD, n=117). PWS and GHD patients may also be categorised under their own specific indication.

Treatment for SSSG is standardised with a starting dose of 4.5mg/m^2/wk. We examined, by retrospective analysis of the national APEG database, OZGROW, differences between the diagnostic groups within the SSSG cohort in terms of base demographics, treatment, and response.

Results

A younger age, a smaller height-SDS, and a higher BMI-Z at the start of GH treatment are associated with a better response to GH therapy. PWS, Russell, and SGA children were found to be younger than the SSSG mean of 7.2y (PWS=3.7, P=1x10^{-6}; Russell=5.8, P=0.04; SGA=5.7, P=0.004) while ISS children were older (8.2, P=8x10^{-5}). SGA children were shorter (SDS=-3.52, P=0.031) than the SSSG mean (-3.21) but FSS were taller (-2.91, P=0.00015). PWS, FSS, and GHD had higher BMI-Z values than the SSSG mean of -0.76 (PWS=0.383, P=0.018; FSS=-0.411, P=0.043; GHD=-0.208; P=1.9x10^{-5}) while Russell syndrome children were smaller (-2.52, P=6.9x10^{-5}).

The mean dose in the first year of treatment for SSSG was 4.80mg/m^2/wk. Only GHD patients varied significantly from this (4.54, P=9.5x10^{-5}).

During the first year of therapy, the mean change in height SDS for the v cohort was 0.55. PWS, SGA, and GHD children achieved better responses than other patients (PWS=1.06, P=6x10^{-6}; SGA=0.77, P=0.00026; GHD=0.86, P=1.4x10^{-7}). Dividing response into tertiles (low L, middle M, high H), the nature of these differences became evident: PWS L, M, H (0,4,16), SGA (2,17,12), GHD (31,27,58). Additionally, the distributions of ISS (64,55,35 P=0.011) and FSS (25,20,11, P=0.06) were noteworthy.

ConclusionsResponse varied between diagnoses; however, this may be attributed to age, height and BMI-Z differences at the start of treatment.

Sources of Research Support: Australasian Paediatric Endocrine Group.

Nothing to Disclose: IPH, CC, PSWD, AC, MH

Pub #	P1-754
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Does Co-Administration of Growth Hormone (rhGH) and a Gonadotropin-Releasing Hormone Analog (GnRHa) Improve Growth Outcomes in Early Pubertal Adolescents with Idiopathic Short Stature (ISS)?
Author String	A Colmenares, P Gunczler, R Lanes Centro Clinico San Cristobal, San Cristobal, Venezuela; Hospital de Clinicas Caracas, Caracas, Venezuela
Body	Objective: To evaluate the effect of rhGH treatment associated to a GnRHa on height velocity and near adult height in healthy short children in early puberty. Patients and methods: We treated twenty adolescents with ISS (12 females) with a combination of rhGH (mean dose of 0.04±0.02 mg/kg/day) and a GnRHa (triptorelin, mean dose of 87.9±58 ug /kg every 28 days) for 24 months (group 1). Our patients had a CA of 11.6±1.4 years, BA of 11±1 years, height SDS of -2.3 ±1.1 and were in early puberty (Tanner II-III). All subjects had normal thyroid function tests, IGF1 and peak GH levels following stimulation. Eleven children with ISS (6 females) in early puberty with a CA age of 11.1±2.2 years treated only with rhGH for 2 years (group 2) and ten early pubertal GHD children (6 females) with a CA of 11.5±1.3 years treated with rhGH and GnRHa for 24 months (group 3), served as controls. Results: HSDS of group 1 patients increased from -2.3 ±1.1 to -1.7 ±1.5 ($p<0.05$), their height velocity decreased from 7.5±1.6 cm/yr at baseline to 7±2.4 and 5.7±2 cm/yr at 12 and 24 months of combined treatment and BA increased from 11.2 ± 0.9 to 12.9±1.1 years over this period ($p<0.002$). PAH increased from 154.8±13.6 cm before therapy to 157.9±9.3 cm at the end of 2 years of treatment ($p<0.05$), which was below their target height of 163.8±6.4 cm. In group 2, HSDS improved from -2.6±1.3 to -1.1±1.6 ($p<0.01$), height velocity was 9.7±3.7 and 7.7±2.4 cm/yr at 12 and 24 months and bone age advanced from 10.7±2.2 years at baseline to 12.4±1.5 years after 2 years of treatment ($p<0.03$). Adult height prediction increased from 161.6±10.7 to 164.5±12.1 cm ($p<0.028$), above their target height of 162±9.1 cm. Height velocity in group 3 subjects changed from 8±2.2 cm/yr at baseline to 8.1±1.7 and 5.2±2.1 cm/yr at 12 and 24 months of combined treatment ($p<0.01$), bone age advanced from 10.7±1.9 to 12.4±1.4 years ($p<0.02$) and PAH increased from 151.8±8.1 to 158.4±9.9 cm ($p<0.01$), slightly below their target height of 159.6±6.6 cm. There was a clear tendency towards a greater change in PAH during treatment in group 3 when compared to that seen groups 1 and 2. BMI remained unchanged during therapy in all three groups. Conclusions: While 2 years of combined rhGH and GnRHa treatment improved the adult height prediction of adolescents with ISS in early puberty, this increase was not larger than that seen in short healthy subjects of a similar age and degree of puberty treated with rhGH alone. Nothing to Disclose: AC, PG, RL

Pub #	P1-755
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Anastrozole Increases Predicted Adult Height in Adolescent Males with Idiopathic Short Stature
Author String	A De Rosa, GF Fiore, S De Rosa, G De Rosa Catholic University, Rome, Italy; Catholic University, Rome, Italy
Body	**BACKGROUND:** Estrogens have an essential role in the regulation of bone maturation and in epiphyseal fusion during puberty in both sexes. The inhibition of estrogen synthesis delays bone maturation and results in increased adult height. Aromatase inhibitors that block estrogen biosynthesis have therefore emerged as a new potential treatment option for children with short stature. **OBJECTIVE:** Our objective was to investigate whether inhibition of estrogen biosynthesis with the aromatase inhibitor, anastrozole, during adolescence, delays bone age acceleration and increase predicted adult height in boys. **PATIENTS AND METHODS:** Twelve boys with idiopathic short stature were randomized to treatment with aromatase inhibitor anastrozole 25 mg/day orally, or placebo for 2 years. Informed consent was obtained from the patients and their parents. The diagnosis of constitutional short stature was made by exclusion of systemic diseases, GH deficiency, psychosocial deprivation, or syndromatic causes. Pubertal stage was III according to Tanner, confirmed by testosterone plasma levels. **RESULTS:** Linear growth was similar between two groups; however, there was a slower increase in bone age advancement from baseline in the anastrozole group *vs.* placebo group after 2 yr. Estradiol concentrations in the boys treated with anastrozole remained at the pretreatment level, whereas the concentrations increased during spontaneous progression of puberty in placebo group. Testosterone concentrations increased in both groups, but during the anastrozole treatment the level was higher than in the control group. The bone maturation was delayed in the boys treated with anastrozole despite higher androgen concentrations and, an increase of 4.3 ± 1.1 cm in predicted adult height was observed in the boys who received anastrozole, but no change was seen in the untreated boys. All boys had normal virilization. **CONCLUSIONS:** Our findings indicate that in adolescent boys an increase in adult height can be obtained by use of aromatase blockade with anastrozole caused by a slowing of the bone age maturation, resulting in a gain in predicted adult height compared with placebo. Nothing to Disclose: ADR, GFF, SDR, GDR

Pub #	P1-756
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Final Adult Height in Children with Congenital Adrenal Hyperplasia Treated with Growth Hormone
Author String	K Lin-Su, MD Harbison, O Lekarev, MG Vogiatzi, MI New Weill Medical College of Cornell University, New York, NY; Mount Sinai School of Medicine, New York, NY

Body

Context: Patients with congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency typically reach a final adult height well below their mid-parental target height.
Objective: The objective of this study was to examine whether growth hormone (GH) alone or in combination with a luteinizing hormone releasing hormone analogue (LHRHa) improved the final adult heigh in patients with CAH.
Design: The study was a non-randomized study.
Setting: The study was conducted at two university hospitals in New York City, NY.
Participants: Thirty-four patients with CAH treated with GH participated in this study. Nineteen males and 15 females who were predicted to be more than 1 standard deviation (SD) below their mid-parental target height received GH until reaching final adult height. In addition to GH, 27 patients (16 males, 11 females) were also treated with an LHRHa.
Intervention: The mean duration of GH treatment was 5.6 $\pm$ 1.8 years in males and 4.5 $\pm$ 1.6 years in females. The mean duration of LHRHa therapy was 3.7 $\pm$ 1.7 years for both sexes.
Main Outcome Measures: The primary endpoint variables were final adult height, final height discrepancy, and gain in height.
Results: Males reached a significantly higher final adult height (172.0 $\pm$ 4.8 cm) than their initial predicted height (162.8 $\pm$ 7.7 cm) (p <0.00001). Females also reached a significantly higher final adult height (162.2 $\pm$ 5.3 cm) than initially predicted (151.7 $\pm$ 5.2 cm) (p <0.0000001). Mean gain in height was 9.2 $\pm$ 6.7 cm in males and 10.5 $\pm$ 3.7 cm in females.
Conclusion: Our results indicate that GH alone or in combination with LHRHa improves final adult height in patients with CAH.

Sources of Research Support: In part by USPHS Grant HD-00072, General Clinical Research Center Grant 06020, and Eli Lilly & Co.

Nothing to Disclose: KL-S, MDH, OL, MGV, MIN

Pub #	P1-757
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Intracranial Hypertension (IH) in Pediatric Patients Treated with Recombinant Human Growth Hormone (rhGH): Data from 25 Years of the Genentech National Cooperative Growth Study (NCGS)
Author String	RA Noto, J Frane, TJ Maneatis, BM Lippe, A Davis New York Medical College, Sleepy Hollow, NY; Genentech, Inc, South San Francisco, CA; Genentech, Inc, South San Francisco, CA
Body	Context: With the introduction of rhGH for pediatric growth disorders, IH was reported as a potential consequence of treatment. An estimate from a tertiary referral center of the annual incidence of IH in the general pediatric population was 0.9/100,000 children (1). Quantification of the IH risk associated with rhGH treatment as well as other predisposing factors for IH in these children need to be more clearly defined. Methods: Reports of IH were extracted from the Genentech safety database, linked to NCGS patients, and considered for analysis if no other cause was reported. Incidence rates (IR) were calculated within each growth failure etiology as well as an "Other" group. A standardized incidence rate (SIR) was calculated in the Idiopathic Short Stature (ISS) subcohort to index the risk of IH relative to the general pediatric population since these groups are similar except for the use of rhGH. Analyses of patient characteristics that may influence IH risk were performed. Results: IH was reported in 71 of 65,204 NCGS patients for an overall IR of 29.7/100,000 patient-years (42 males and 29 females - mean enrollment age of 10.8 and 9.4 years, respectively). The ISS cohort had the lowest incidence while the highest occurred in patients with Chronic Renal Insufficiency (CRI). The SIR estimate for the ISS group revealed a risk 10.7X higher than the general pediatric population. Other significan risk factors were: BMI > 85%ile and stimulated GH levels < 5 ng/mL. Age, pubertal status, rhGH dose at initiation, and gender (when females with Turner Syndrome were excluded) were not significant risk factors. Two distinct groups emerged - patients with IH events early (78% - mean onset of 97 days/median of 57 days and late in their rhGH course (22% - mean onset of 45 months/median of 36 months). Of the late onset group, 60% had additional risk factors for IH other than rhGH therapy. Conclusion: The risk spectrum of IH associated with rhGH varies by growth failure etiology and may be influenced by a higher baseline risk due to their underlying disease (2). Most patients experience their event within the first three months after initiation, but our data suggest that patients with IH risk factors other than rhGH use may present later in their treatment course. Although our data confirm the increased IH risk associated with rhGH therapy, the SIR results should be interpreted with caution since the available background rate may underestimate the true incidence. (1) Gordon K. Can J Neurol Sci 1997; 24: 219-21 (2) Fine RN et al. J Peds 2003; 142: 539-45. Sources of Research Support: The NCGS is a post-marketing registry supported by Genentech, Inc. Disclosures: JF: Consultant, Genentech, Inc.; Tercica. TJM: Employee, Genentech, Inc. BML: Consultant, Genentech, Inc. AD: Employee, Genentech, Inc. Nothing to Disclose: RAN

Pub #	P1-758
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Children with Untreated Growth Hormone Deficiency (GHD) Have Cognitive Abnormalities When Compared to Short Non-GHD Controls
Author String	V Viswanathan, SJ Pongonis, LA Clift, JM Katzenstein, BC McDonald, EC Walvoord Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, IN; Indiana University School of Medicine, Indianapolis, IN; Indiana University School of Medicine, Indianapolis, IN
Body	Introduction: Adults with untreated growth hormone deficiency (GHD) have cognitive abnormalities that improve with growth hormone (GH) treatment. However, studies of cognitive functioning in children with GHD are limited. The goal of our study was to assess differences in cognitive functioning between children with GHD and stature and age matched non-GHD children. Methods: Subjects were children with heights [le] 3rd percentile for age who underwent GH stimulation testing. Subjects were excluded if they had other medical or psychiatric conditions known to be related to cognitive changes. Subjects were considered GHD if their peak GH level was <5 ng/mL. All subjects underwent a targeted neuropsychological test battery to assess cognitive functions of interest. Testing was conducted blind to group status. Independent samples t-tests and chi square tests were used for between-group comparisons (SPSS18). Results: Twenty-two children were included: GHD: 9 subjects (5 boys), non-GHD:13 subjects (9 boys). Mean age (11.56±2.40 years vs 11.31±2.18 years, p=0.8) and height SDS (-2.31±0.24 vs -2.69±0.18, p=0.5) did not differ between groups. Peak GH levels were lower in GHD (2.63±0.30) vs non-GHD children (24.6±7.78, p<0.01). Across all cognitive domains GHD children tended to perform worse than non-GHD children. GHD children were significantly slower than non-GHD children on speeded response tasks including the Stroop Color-Word Interference Test-Color Naming Trial (p=0.05) and CPT-II Hit Rate SE Block Change score (p=0.03). They also tended to be slower on the Stroop Interference Trial (p=0.08), and showed lower scores on the WASI Vocabulary subtest (p=0.09) than non-GHD children. The parents of GHD children also reported more somatization symptoms on the BASC-2 than the parents of non-GHD children (p=0.03), with their symptoms falling in the [ldquo]at-risk[rdquo] range for clinical concern. Conclusions: Children with GHD showed poorer performance on measures of processing and reaction speed and vocabulary skills. They also tended to have overall lower scores in every cognitive domain measured when compared to their short, non-GHD counterparts. Whether these deficits improve with GH treatment remains unknown, but this pilot data suggests that GHD children have significant processing abnormalities. Nothing to Disclose: VV, SJP, LAC, JMK, BCM, ECW

Pub #	P1-759
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Body Mass Index Does Not Affect Spontaneous Nocturnal GH Secretion in Children with Short Stature
Author String	C Guzzetti, S Pilia, MR Casini, M Benassai, A Rollo, S Zucchini, G Radetti, S Loche Ospedale Microcitemico, ASL Cagliari, Cagliari, Italy; Ospedale Regionale di Bolzano, Bolzano, Italy; Universit[agrave] di Bologna, Bologna, Italy

Body

Background. Obesity is characterized by reduced spontaneous as well as stimulated GH secretion. An inverse relationship has been shown between body mass index (BMI) and the peak growth hormone (GH) response to stimulation in adults and in children with short stature. This relation is observed even within a normal range o BMI.

Objective. The aim of this study was to investigate the effect of BMI on spontaneous nocturnal GH secretion in children with short stature.

Subjects and Methods. This was a retrospective study in 164 short children (age 2.3-17.9; bone age 1.6-15; 91 M and 73 F; 92 prep and 51 pub; mean±SD height-SDS -2.27±0.7) who underwent nocturnal GH secretion studies in the last 18 years. Spontaneous nocturnal GH secretion was assessed with use of blood samples take with a short intravenous catheter every 30 min for 12 h (from 20[middot]00 to 08[middot]00 h). Children were admitted to the hospital before the overnight sampling and had a balanced dinner at 18[middot]00-18 [middot]30 h. Mean GH concentrations (MGHC) were based on measurements taken over 12 h for each child IGF-I was also determined in all children at baseline. GH and IGF-I were measured by conventional immunoassays.

Results. Mean±SD BMI-SDS in the entire cohort was -0.84±0.82 (range -2-1.75). 150 patients had a nocturnal GH peak >10 [micro]g/L and 14 patients had a GH peak <10 [micro]g/L. Only one patient had MGHC <3 [mu]g/L (2.94 [mu]g/L)) but a GH peak of 28.3. All children were ultimately found to be normal based on their GH secretion and on long-term follow-up. In univariate regression analysis nor GH peak (r=-0.16, P=0.051) or MGHC (r=0.13, P=0.12) were correlated with BMI-SDS. Peak GH was associated only with age (r=0.17, P=0.03) and bone age (r=0.23, P=0.01). GH peak and MGHC were also not correlated with BMI-SDS in prepubertal or pubertal children. GH peak and MGHC were correlated with baseline IGF-I. Mean GH peak and MGHC (20.3±8.6 and 5.3±2.5 [micro]g/L, respectively) were similar between subjects with BMI-SDS -2 to 0, and those with BMI-SDS 0 to +2 (18.0±9.9 and 6.2±4.6 [micro]g/L).

Conclusions. In this cohort of short children with normal BMI-SDS, BMI has no significant impact on nocturnal spontaneous GH secretion. These data contrast with previously reported observations indicating tha BMI markedly influences the GH response to provocative stimuli. These findings suggest that evaluation of the spontaneous nocturnal GH peak might be more accurate in the diagnostic work-up of children with suspected GHD.

Nothing to Disclose: CG, SP, MRC, MB, AR, SZ, GR, SL

Pub #	P1-760
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Insulin-Like Growth Factor Deficiency (IGFD) in Clinical Practice: Analysis of a Retrospective Cohort of Patients with Short Stature and IGFD
Author String	JE Shepard, V Hwa, R Rosenfeld, KA Woods Oregon Health and Science University, Portland, OR; Oregon Health and Science University, Portland, OR
Body	Insulin like growth factor (IGF) measurement has become a mainstay in the evaluation of children with poor growth. Once other causes of growth failure have been excluded, low IGF levels are often an indication for growth hormone (GH) provocation testing. Low GH levels indicate GH deficiency (GHD) or 'secondary' IGF deficiency [IGFD], and lead to institution of GH therapy. The management of short patients with low IGF levels yet normal GH levels ('primary' IGFD) is less clear. Options include no therapy, GH therapy, or recombinant human (rh) IGF-I, approved for patients with severe primary IGFD (height SDS and IGF-I SDS both <3). We analyzed clinical and biochemical data and GH response in a cohort of patients in our clinic referred for GH provocation testing over the past 2 years, comparing subjects diagnosed with either primary o secondary IGFD. 115 patients were referred for GH provocation testing over the past 2 years. Of these, 73 had IGF-I and IGFBP-3 levels measured by Esoterix labs, allowing calculation of IGF-I and IGFBP-3 SDS. Of these, 68/73 (93%) patients were IGFD (defined as IGF-I SDS <-2), and 43/68 (63%) also short (height SDS <-2). These 43 short IGFD patients formed our primary cohort. 82.5% (35/43) of short IGFD subjects had primary IGFD (peak GH>7 ng/dl). When compared to subjects with GHD/secondary IGFD there was no difference with respect to age (pIGFD: 9.6 yrs vs 9.8 yrs, p=0.44), height SDS (pIGFD: -3.0 vs -3.1, p= 0.34), target height deficit (pIGFD: -2.2SD vs -2.8SD, p=0.11) or height velocity (pIGFD: 5.8cm/yr vs 6.5 cm/yr, p=0.28). Bone age delay was significantly greater in subjects with primary IGFD (pIGFD -1.94yrs vs 1.0yrs, p= 0.005). Both IGF-I SDS (pIGFD: -2.41 vs -3.53, p= <0.001) and IGFBP-3 SDS (pIGFD: -1.65 vs -2.74) were significantly lower in subjects with GHD/secondary IGFD. All 8 subjects with GHD/ secondary IGFD and 8/35 subjects with primary IGFD were treated with GH. Despite similar GH dosing in both groups (mean 0.04mcg/kg/day), gain in height SDS over 6 months of therapy was similar in both groups (pIGFD: +0.54 SD vs+ 0.47, p=NS). In summary, short children with primary IGFD are similar in many respects to short children with secondary IGFD/GHD. In general, they are less severely IGF deficient, and have a greater degree of bone age delay. Preliminary data suggests they also may not differ in their response to GH therapy. Nothing to Disclose: JES, VH, RR, KAW

Pub #	P1-761
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Growth Hormone Is More Effective Than IGF-I in Promoting Changes in Body Proportions
Author String	A Silbergeld, R Kauli, Z Laron Schneider Children's Medical Center, Petah Tikva, Israel; Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
Body	**Background**: Linear height (Ht) is composed of the head, spine (measured as sitting Ht) and lower limbs. Untreated GH and insulin-like growth factor-I (IGF-I) deficient children have an above normal upper/lower (U/L) body ratio denoting retarded limb growth and assymetrical body proportions. **Objective**: To compare the differential growth effects of hGH and IGF-I on the upper/lower body segment ratio in relation to body height (measured as length). **Subjects**: 3 groups of patients were studied: a) Congenital IGHD (7M, 8F) aged 0.8-10.5 y (m±SD 5.0±3.2 y) treated with hGH (33 [micro]g/kg/day/kg/d for a period of 10.0±3.6 y; b) Congenital MPHD (14M, 7F) aged 10.0±3.8 y, treated with hGH for 6.1±2.3 y; c) Laron Syndrome (LS - primary GH insensitivity) (4M, 5F) aged 6.9±5.6 y treated with recombinant IGF-I (Fujisawa, Japan; 200 [micro]g/kg/d) for 9.0±5.8 y. **Methods**: Body height (as length) is expressed as SDS according to Tanner. The upper/lower body segment ration (U/L) was calculated by subtracting the sitting height from the length and expressed as the number of SD from the mean for age. The statistical evaluation of the difference between the means before and after therapy was calculated by the paired t test. **Results**: The findings in the 3 groups of patients show that the m±SD U/L ratio in IGHD patients decreased during treatment from 2.3± 0.7 to 1.1± 0.7 ($p<0.001$) compared with the HT SDS increase from -4.9± 1.3 to -2.3± 1 ($p<0.001$). In MPHD patients the U/L ratio decreased from 1.1± 1.2 to -0.6± 1.0 ($p<0.001$), and the height SDS increased from -3.3± 1.4 to -2.5± 1.0 ($p<0.009$). In the LS group there was little change in the U/I ratio, the basal level was 2.7± 1.3 and post IGF-I treatment 2.4± 1.1; there was however a significant improvement in height from -6.1± 1.3 to -4.6± 1.2 ($p<0.001$). **Conclusions:** hGH treatment of IGHD and MPHD children stimulated both lower limb and spine growth more effectively than IGF-I treatment of LS children, resulting in an improvement in the U/L body ratio. IGF-I treatment caused little change in the U/L ratio. Nothing to Disclose: AS, RK, ZL

Pub #	P1-762
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Increlex Growth Forum Database Registry, 2006-2010: Evaluation of Targeted Adverse Events in Children and Adolescents
Author String	OV Fofanova-Gambetti, BS Miller, B Reiner, M Hutchison, S Chang, B Bakker Ipsen US, Inc, Brisbane, CA; University of Minnesota Amplatz Children's Hospital, Minneapolis, MN; Private Practice, Baltimore, MD; UT Southwestern Medical Center, Dallas, TX; Ipsen US, Inc, Brisbane, CA
Body	**Introduction**: The Increlex Growth Forum Database (IGFD) Registry has enrolled 1137 patients as of December 2010 and monitors long-term safety of rhIGF-1 (Increlex). **Objective:** Evaluate clinical and demographic factors associated with the Registry- *a priori* specified 14 targeted adverse events (TAEs). **Methods**: Patients with at least one follow-up visit comprised the safety cohort. Clinical and demographic characteristics, serious adverse events and discontinuations were examined for these TAEs. Subgroup difference was tested (Fisher exact test) for relevant clinical and demographic parameters. **Results:** The safety cohort consisted of 1010 patients (73% Primary IGF-1 Deficiency [PIGFD], including severe) with 1506 patient-years of follow-up. Baseline characteristics: 76% males, median age of 11.5 years (range 1.3 - 18.6), median BMI SDS of -0.6 (-4.5 - 2.9). Concomitant medication usage included 17% on attention deficit disorder (ADD) medications and 3% on medications for diabetes. Increlex was dosed bid in 73% of patients, and 92% were dosed at $\leq$ 120 mcg/kg bid. 235 (23.3%) patients reported one or more of the 14 targeted TAEs. The most commonly reported targeted TAEs ($\geq$2%), hypoglycemia, headache, and hypersensitivity (including injection site reactions and generalized), were reported by 79 (7.8%), 55 (5.4%) and 24 (2.4%) patients, respectively. For most clinical/demographic parameters, there were no differences between the subgroups in reporting those 3 targeted TAEs. Consistent with population-based data (1), incidence of headache was significantly higher in patients with ADD than in non-ADD (15/170; 9% vs 40/840; 4.8%; p=0.041). Also seen were significant differences in hypoglycemia between diabetic and non-diabetic patients (7/27; 25.9% vs 72/983; 7.3% reporting; p=0.003), and between diagnosis of PIGFD and other (66/736; 9.0% vs 13/274; 4.7%; p=0.025). **Conclusions:** Analysis of clinical and demographic features associated with targeted TAEs in the IGFD registry demonstrates that concomitant medications and primary diagnosis may play a role. The results from the analysis of all 14 targeted TAEs will be presented in the poster. (1) Arruda MA, et al. Migraine, tension-type headache, and attention-deficit/hyperactivity disorder in childhood: a population-based study. Postgrad Med. 2010 Sep;122(5):18-26. Sources of Research Support: Scientific Strategy Partners and was funded by Ipsen US, Inc. Disclosures: OVF-G: Employee, Ipsen. BSM: Consultant, Genentech, Inc.; Pfizer, Inc.; Novo Nordisk; Eli Lilly & Company; Ipsen; Sandoz; Research Funding, Genentech, Inc.; Pfizer, Inc.; Novo Nordisk; Eli Lilly & Company; Ipsen; Abbott Laboratories. BR: Advisory Group Member, Novo Nordisk; Principal Investigator, Pfizer, Inc.; Registries, Genentech, Inc.; Registries, Lilly USA, LLC; Registries, Serono; Principal Investigator, Macrogenics. MH: Advisory Group Member, Ipsen. SC: Employee, Ipsen. BB: Employee, Ipsen

Pub #	P1-763
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Aleppo Algorithm for GHRH/GH/IGF-I Axis Work-up
Author String	S Sakkal MCC, Aleppo, Syrian Arab Republic
Body	**Objective**:There is a need for an algorithm for the workup of the growth failure. We have used with good results the following algorithm to determine the potential benefit of GH Rx after tests in 350 child. **GH/IGF1 Axis Workup Algorithm:** **Start with IGF1**:- *if High*: No Rx usually will succeed. - *if Low*: proceed with :+ TSH Glucose SGPT: if High: No GHRx .Treat primary Disorder + Cortisol : if High: proceed with adrenal Dx/Rx. +If above are all Normal: **proceed with GH**:- *if High*: No Rx with GH will succeed (GH/IGF1 resistance) - *if Low*: proceed with dynamic test: Clonidine /L Dopa /Exercise/Insulin Stim. * if response of GH is high : No Rx with GH proceed withOther subclinical Disorder: Ca, P, CBC, D Xylose, Kariotype * if Low: treat with GH (Gene deletion, Pituitary, Hypothalamic) To differentiate **proceed with GHRH**:- if response+ is High: Hypothalamic +If Low: Pituitary, Gene deletion **(**:# IGF1 is done first for Dx and Rx,if nl or high look for other 1ry disorders # IGFBP parallel IGF1 direction not needed for Dx or Rx. #Growth Hormone Rx is useful when both: IGF1 and GH are low,Turners, CKF) **Conclusion**: for growth hormone axis workup start with IGF1 ,if low proceed with GH ,if low proceed with GHRH or consider Rx with GH therapy for GH/IGF1 deficiency or Turner's or CKF. Nothing to Disclose: SS

Pub #	P1-764
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Insulin Glargine Enhancement of Growth Velocity in Prepubertal Girls with Diabetes Mellitus Type 1
Author String	GW Moll, Jr, MY Torchinsky University of Mississippi Medical Center, Jackson, MS

Body

Insulin glargine has been associated with activation of the human insulin-like growth factor-1 receptor to a greater extent than human insulin when present in high doses and concerns for its potential influence upon abnormal cellular growth have been debated. The use of 24-hour insulin regimens for diabetes mellitus type 1 (IDDM)control have proven highly successful. We hypothesized insulin glargine as a FDA approved 24-hour insulin for 6 years of age on up can enhance physical growth of prepubertal girls to a greater extent than the other FDA approved 24-hour insulin detemir.

Methods: We performed a retrospective IRB approved review of our diabetes mellitus type 1 patients that maintained patient confidentiality while identifying 330 who employed insulin glargine and 178 who employed insulin detemir within their IDDM multiple subcutaneous insulin control regimens of at least 1 year duration. In order to reduce variability in growth patterns we chose prepubertal girls of 6 to 10 years of age who achieved glycohemoglobins (HgbA1c meeting national glycohemoglobin standard program criteria) less than or equal to 8.5% for the 6 to 4 year assessment of their 5 to 8 month interval growth rates. We arrived at 11 girls using insulin glargine and 11 girls using insulin detemir as our study groups. Growth velocity data were expressed as cm/year from 5-8 month interval data and t-statistic comparisons of means for groups of unequal variance were performed.

Results: Our patient groups consisted of similar age ranges and HgbA1c levels. Their growth rates indicated a statistically significant increase for the insulin glargine group versus the insulin detemir group.

Insulin Detemir versus Insulin Glargine

Average Age 7.6 ± 0.2 years versus 8.2 ± 0.2 years

Average HgbA1c 7.7 ± 0.1 % versus 7.4 ± 0.1 %

Average Growth Height 5.9 ± 0.4 cm/y versus 6.7 ± 0.4 cm/y *

Average Growth Weight 3.3 ± 0.4 kg/y versus 4.2 ± 0.6 kg/y

* $p<0.5$

Discussion: We conclude insulin glargine can be associated with a growth velocity enhancement within the prepubertal female population relative to insulin detemir 24-hour insulin regimens when similar groups of IDDM control are evaluated. The insulin glargine group did not have a significantly larger gain in weight over our retrospective study period, and the weight distributions for our study groups were similar. Longer term assessment through puberty with larger groups of both girls and boys should assist efficacy with on-going favorable safety profiles.

Nothing to Disclose: GWM, MYT

Pub #	P1-765
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Disorders (1:30 PM-3:30 PM)
Title	Inadequate [ldquo]Catch Up[rdquo] Growth during Gluten-Free Diet in Children with Celiac Disease
Author String	C Bouvattier, L Henry, F Campeotto Paris V University, Paris, France; Paris V University, Paris, France
Body	Introduction: Growth failure during celiac disease (CD) is related to a decrease in growth velocity. Restoratio of a normal height during the gluten free diet depends on the [ldquo]catch up[rdquo] growth. Aim: Evaluation of the [ldquo]catch up[rdquo] growth during the gluten-free diet in CD patients. Population and methods: We retrospectively studied the quality of the [ldquo]catch up[rdquo] growth, using z-score, and influencing factors in 21 infants (15 girls/6 boys, median age at diagnosis 6.2 years) with CD during the gluten-free diet. Results: Following gluten elimination, the increase in growth velocity always remained below the normal value: - 1.8SD at baseline, -0.3 SD after 1 year, -0.1 after 2 years, preventing any restoration of the normal height. This increase in z-score of growth velocity was higher in males than in females (+5 SD vs +1.4 SD, p< 0.05). The [ldquo]catch up[rdquo] growth velocity was correlated with the adherence to the gluten-free diet, the short stature at diagnosis and the target height, but not with the degree of villous atrophy, gastrointestinal symptoms and age at diagnosis. Four children were started on growth hormone therapy. Conclusion: In CD children, the [ldquo]catch up[rdquo] growth is inadequate to allow the achievement of normal height, suggesting the persistence of adverse mechanisms leading to a reduced final height. The follow-up of these children should focus on an appropriate increase in growth velocity. Nothing to Disclose: CB, LH, FC

Pub #	P1-769
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)
Title	Ligand-Dependent Regulation of the Activity of the Orphan Nuclear Receptor, Small Heterodimer Partner (SHP), in the Repression of the Hepatic Bile Acid Biosynthetic CYP7A1 and CYP8B1 Genes
Author String	S-E Choi, J Miao, SM Seok, L Yang, JK Kemper University of Illinois, Urbana-Champaign, Urbana, IL
Body	Small Heterodimer Partner (SHP) plays important roles in diverse biological processes including metabolism, tumorigenesis, and reproduction by directly interacting with transcription factors and inhibiting their activities. SHP has been designated as an orphan nuclear receptor but whether its activity can be modulated by ligands has been a long-standing question. Recently, retinoid-related molecules including 4-[3-(1-adamantyl) -4-hydroxyphenyl]-3-chlorocinnamic acid (3Cl-AHPC) were shown to bind to SHP and enhance apoptosis. We have examined whether 3Cl-AHPC acts as an agonist and increases SHP activity in the regulation of bile acid biosynthetic CYP7A1 and CYP8B1 genes and delineated the underlying mechanisms. Nanomolar concentrations of 3Cl-AHPC repressed CYP7A1 expression in HepG2 cells but little repression was observed when SHP was downregulated by shRNA. Surprisingly, micromolar concentrations of 3Cl-AHPC increased CYP7A1 expression via p38 kinase signaling. Mechanistic studies revealed that 3Cl-AHPC bound to SHP, increased interaction of SHP with LRH-1, a hepatic activator for CYP7A1 and CYP8B1 genes, and with repressive cofactors, Brm, mSin3A, and HDAC1, and, subsequently, increased the promoter occupancy of SHP and these cofactors. Mutation of Leu-100, predicted to contact 3Cl-AHPC within the SHP ligand binding pocket by molecular modeling, severely impaired increased interaction with LRH-1 and repression of LRH-1 activity mediated by 3Cl-AHPC. Treatment with 3Cl-AHPC repressed SHP target genes in metabolic pathways in a gene-specific manner in human heptocytes and HepG2 cells. These data suggest that SHP can act as a ligand-regulated nuclear receptor in metabolic pathways. Modulation of SHP activity by synthetic ligands may be a useful therapeutic strategy. Nothing to Disclose: S-EC, JM, SMS, LY, JKK

Pub #	P1-770
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)
Title	A Novel Treatment Strategy for Ovarian Cancer Based on Immunization Against Zona Pellucida Protein (ZP) 3
Author String	NA Rahman, HJT Coelingh Bennink, M Chrusciel, V Sharp, Y Zimmermann, R Dina, X Li, A Ellonen, A Rivero-Muller, S Dilworth, S Ghaem-Maghami, O Vainio, I Huhtaniemi University of Turku, Turku, Finland; Florida International University College of Medicine, Miami, FL; Pantarhei Bioscience B.V., Zeist, Netherlands; Imperial College London, London, United Kingdom; Imperial College London, London, United Kingdom; China Agriculture University, Beijing, China; University of Oulu Oulu, Finland
Body	Ovarian cancer is the most common cause of death from a gynecologic malignancy, and among them ovarian granulosa cell tumors pose a therapeutic challenge with high rate of recurrence and mortality. We tested hereby, the principle of treating ovarian malignant tumors by vaccination against an ectopically tumor-expressed protein zona pellucida glycoprotein (ZP) 3, using as the experimental model the granulosa cell tumors of transgenic mice expressing the Simian Virus 40 T-antigen under the inhibin-α promoter (inhα/Tag). We found high ectopic ZP3 expression in granulosa cell tumors of the transgenic mice, and also in human granulosa cell tumors and their metastases. Early preventive immunization (between 2-5.5 months of age) of transgenic mice with recombinant human (rh) ZP3 prevented ovarian tumorigenesis, and delayed therapeutic immunization (between 4.5-7 months), reduced weights of existing tumors, by 86 and 75%, respectively ($p<0.001$), compared to vehicle-treated control mice. No objective side effects of the immunizations were observed. Liver metastases were found in non-treated/vehicle-treated controls (n=7/39), but none following active rhZP3 immunizations (n=0/36) ($p < 0.01$). The immunization boosting with rhZP3 was highly effective as demonstrated by anti-ZP3 titers, and cellular responses. These results prove the principle of rhZP3 immunization as a novel lead into the immunotherapy of ovarian granulosa cell tumors with ectopic ZP3-expression. HJTCB: Yes, I am the inventor of the concept and the CEO and one of the major shareholders of Pantarhei Bioscience, the company developing the treatment of ovarian cancer by active ZP3 immunization, Pantarhei Bioscience. YZ: Yes, I am employed by Pantarhei Bioscience, the company developing the treatment of ovarian cancer by active ZP3 immunization, Pantarhei Bioscience. Nothing to Disclose: NAR, MC, VS, RD, XL, AE, AR-M, SD, SG-M, OV, IH

Pub #	P1-771
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)
Title	Altered Hypothalamic Development Associated with Delayed Puberty as a Result of Postpartum Anxiety in Mothers
Author String	CM Larsen, DR Grattan University of Otago, Dunedin, New Zealand
Body	Low prolactin levels in early pregnancy increases maternal anxiety and impairs maternal behavior postpartum in mice. In this model of postpartum anxiety we observed that daughters of anxious mothers have delayed onset of puberty. The aim of this study was to investigate the mechanism underlying the delay in puberty. Correct levels of neuronal apoptosis [ldquo]neuronal pruning[rdquo] during late development is essential for subsequent normal reproductive behaviors, and is at least partially regulated by paternally expressed genes that are sensitive to epigenetic regulation. Hence, we hypothesized that maternal anxiety would alter neuronal pruning in offspring during late development, and/or alter levels of neurons essential for puberty onset. To investigate neuronal pruning during late development, levels of activated caspase 3, a marker of the final stage of apoptosis, were assessed on postnatal day 4 in first generation daughters of anxious mothers. To determine whether any changes seen were [ldquo]epigenetically[rdquo] transmitted, levels of activated caspase 3 were also assessed on postnatal day 4 in third generation daughters of anxious mothers. Kisspeptin expression in the hypothalamus, an essential prerequisite for puberty onset, was also assessed in adult daughters of anxious and control mothers at the time of expected puberty. The delay in puberty was associated with an increase in level of activated caspase-3 on postnatal day 4, in both first and third generation daughters of anxious mothers, in the nucleus accumbens. Third generation daughters also had a significant increase in activated caspase 3 levels in the bed nucleus of the stria terminalis and the preoptic area, indicating a cumulative effect of maternal anxiety during the plastic period of late development in successive generations. The delay in puberty was further characterised by reduced kisspeptin expression in the hypothalamus of adult female offspring of anxious mothers at the time of expected puberty. These results offer insight into how maternal anxiety, induced by perturbations in the hormones of early pregnancy, may program mechanisms in the fetal brain that trigger the onset of puberty. Nothing to Disclose: CML, DRG

Pub #	P1-772
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)
Title	Testosterone Treatment Is Associated with Reduced Mortality in Men with Low Serum Testosterone Levels
Author String	MM Shores, NL Smith, CW Forsberg, BD Anawalt, AM Matsumoto VA Puget Sound Health Care System, Seattle, WA; University of Washington, Seattle, WA; University of Washington, Seattle, WA; University of Washington, Seattle, WA; VA Puget Sound, Seattle, WA; VA Puget Sound Health Care System, Seattle, WA

Body

Low serum testosterone levels have been associated with increased mortality in older men (1-8). However, the influence of testosterone treatment on mortality in men with low testosterone is unknown.
Objective: To examine the association between testosterone-treatment and mortality in men with low testosterone and to investigate whether prevalent diabetes, coronary heart disease, or age modify this relationship.
Design: An observational, retrospective cohort study using VA electronic medical records, from Jan 2001 through Dec 2005, of 1031 male veterans, 40 years or older, with low total testosterone levels ($\leq$250 ng/dL), excluding those with a history of prostate cancer and baseline anti-androgen or testosterone treatment.
Outcome: Total mortality in men who initiated testosterone treatment compared to untreated men.
Methods: We assessed total mortality in testosterone-treated vs. untreated men with Cox proportional hazard models using time dependent treatment-initiation, and adjusting for age, medical morbidity, baseline testosterone level, coronary heart disease, diabetes, hospitalization, and the propensity for treatment. Mortality data was obtained from VA, Social Security, and Washington State death records.
Results: The testosterone-treated men comprised 36% of the cohort (n=372) and were treated for an average of 13.1$\pm$12.5 (SD) months. In unadjusted analyses, treated men had a cumulative mortality of 10% compared to 21% in the untreated men ($p<.001$). In an unadjusted Cox regression analysis, testosterone treated men had decreased mortality with a hazards ratio (HR) of 0.67 (95% CI, 0.46-0.97). In a fully adjusted model, testosterone-treatment was associated with a HR of 0.69 (95% CI, 0.47-1.01). In a fully adjusted sensitivity analysis, in which men who died within the first year were removed from the sample to minimize the effects of acute illness, the HR for treatment was 0.55 (95% CI, 0.34-0.91). Effect modification was noted with age, diabetes, and coronary heart disease ($p<.05$ for all) with a greater mortality-reduction in men ages 40-65, with diabetes, and without cardiac disease.
Conclusions: Our data suggest that testosterone-treatment in men with low testosterone levels is associated with decreased mortality, particularly in men ages 40-65 years old and in diabetic men. Given the observational study design, residual confounding may be an issue and these results must be interpreted appropriately.

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Sources of Research Support: 1. VA Office of Research and Development, Seattle, WA
2. VA Geriatric Research, Education and Clinical Care (GRECC), Seattle, WA
3. VA Seattle Epidemiologic Research and Information Center (ERIC), Seattle, WA
4. University of Washington, Royalty Research Fund, Seattle, WA.

AMM: Advisory Group Member, Abbott Laboratories; Researcher, GlaxoSmithKline, Ascend/Besins; Advisory Group Member, Endo Phramaceuticals, Ligand Advisory Board, Trimmel Advisory Board. Nothing to Disclose: MMS, NLS, CWF, BDA

Pub # P1-773

Session Information POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)

Title Reproductive Deficits in Mice Lacking *Tacr3*: Closing the Gap between Mice and Men

Author String JJ Yang, SB Seminara
Massachusetts General Hospital, Boston, MA

Body

Background: Patients bearing mutations in *TAC3* and *TACR3* (which encode for neurokinin B and its receptor respectively) have abnormal pubertal development, GnRH deficiency, and hypogonadotropic hypogonadism. In contrast, *Tacr3*$^{-/-}$ mice have been reported to be fertile. Because of the phenotypic discordance underlying disabling mutations in *TACR3* and *Tacr3* in mice and men, we hypothesized that *Tacr3*$^{-/-}$ mice have subtle reproductive defects that might have been previously overlooked.

Methods: *Tacr3*$^{+/-}$ mice (Deltagen) were bred to generate *Tacr3*$^{-/-}$ mice. Reproductive phenotypes were characterized in *Tacr3*$^{-/-}$ and *Tacr3*$^{+/+}$ (WT) littermates.

Results: *Tacr3*$^{-/-}$ mice appear to have unperturbed sexual maturation, as evidenced by normal timing of preputial separation and vaginal opening. However, *Tacr3*$^{-/-}$ animals have significantly smaller testes and lower uterine weights at P60 compared to WT. *Tacr3*$^{-/-}$ males have significantly lower serum FSH compared to WT counterparts, but LH and testosterone were not different. *Tacr3*$^{-/-}$ females do not demonstrate any differences in gonadotropin or estradiol levels compared to WT animals matched for the phase of the estrous cycle. However, null females have abnormal estrous cyclicity with 97% of their time spent in diestrus compared to 49% in controls. While *Tacr3*$^{-/-}$ males appear to have normal testicular histology, 2/5 *Tacr3*$^{-/-}$ females had no detectable corpora lutea. Although some male and female *Tacr3*$^{-/-}$ mice have demonstrated fertility, the number of litters and pups per litter are being catalogued to compare their overall fertility to WT animals.

	Male Testicular Weight (mg)	Female FSH (ng/ml)	Diestrus Uterine Weight (mg)	% of Time Spent in Diestrus
Tacr3 $^{+/+}$	208 ± 11	28.9 ± 2.0	71 ± 7	49 ± 3
Tacr3 $^{-/-}$	159 ± 11*	9.9 ± 0.7*	34 ± 5*	97 ± 3*

* $p<0.01$; t-test comparing *Tacr3* $^{+/+}$ vs *Tacr3*$^{-/-}$

Conclusion: Although originally reported as fertile, *Tacr3* $^{-/-}$ male mice have smaller testes and lower FSH levels compared to WT, and *Tacr3* $^{-/-}$ female mice have smaller uteri, abnormal cycling and a possible ovulatory defect. The reproductive defects observed in *Tacr3* $^{-/-}$ mice suggest greater similarities between mouse and human models than previously appreciated.

Sources of Research Support: NIH U54 HD028138
NIH T32 HD07396.

Nothing to Disclose: JJY, SBS

Pub #	P1-774
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)
Title	Mediobasal Hypothalamic Crtc1 Regulates Energy Balance in Mice
Author String	C Blouet, G Schwartz Albert Einstein College of Medicine, New York, NY
Body	Cyclic AMP response element-binding protein (CREB)-regulated transcription coactivators (Crtcs) function as calcium- and cyclic AMP-sensitive detectors and shuttle to the nucleus in response to these signals of fuel availability. Crtc1 is primarily expressed in the brain and the metabolic phenotype of Crtc1 knock-out mice indicates that Crtc1 contributes to the regulation of energy homeostasis. The mediobasal hypothalamus (MBH) has been importantly implicated in the regulation of energy metabolism and is considered as a cornerstone of central detection and integration of nutrient-related signals. However, the specific role of MBH Crtc1 in the regulation of various determinants of energy balance remains unexplored. Using a lentivector expressing a Crtc1 shRNA, we determined the behavioral and metabolic consequences of MBH-specific Crtc1 knock-down in adult, normally developed mice. Stereotaxic Crtc1 shRNA lentiviral injections into the MBH led to a 2.3 fold decrease in Crtc1 protein expression in this region, with no change in Crtc1 expression in adjacent areas. Crtc1 knock-down increased food intake, body weight gain and adiposity in animals maintained on a high-fat diet. Oxygen consumption was not significantly affected but respiratory quotient was significantly lower in MBH Crtc1 knock-down mice during both day and night phases of the light/dark cycle, and locomotor activity was significantly decreased during the night in that group compared to controls. MBH-specific Crtc1 knock-down also attenuated the metabolic response to beta-adrenergic stimulation, indicating an impaired response to sympathetic stimuli. Together, these results indicate that Crtc1 is an important mediator of hypothalamic nutrient sensing pathways regulating energy homeostasis. Sources of Research Support: New York Obesity research Center pilot and Feasibility Program. Nothing to Disclose: CB, GS

Pub #	P1-775
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)
Title	Genetic Variation within the Dopamine Receptor 2 (DRD2) Gene Is Associated with Differences in Behavior and Biochemical Measures Related to Obesity in Indian Rhesus Macaques (Macaca mulatta)
Author String	ZP Johnson, V Michopoulos, ME Wilson Yerkes National Primate Research Center, Atlanta, GA
Body	The public health burden imposed by excess food intake and obesity is enormous, with some estimates suggesting that obesity related conditions account for 9% of all the medical expenses in the US, costing our healthcare system 96.2 billion dollars annually. Beyond the economic impact, the cost to individual health is high as well, increasing the risk for type II diabetes, cardiovascular disease, cancer, and cognitive decline. While there may be several proximate causes for the emergence of such a phenotype, including cost of available foods, exposure to psychosocial stressors and consumption of foods that provide comfort, evidence also suggests that genetics factors have significant influence. Because food is inherently rewarding, we have begun a series of studies to better understand the relation in candidate genes and a number of phenotypes related to food intake, behavior, and metabolic profiles in a rhesus monkey model. Here we report the association of recently identified polymorphisms in the gene encoding the dopamine 2 receptor (DRD2) with total caloric intake, meal size, meal frequency, as well as numerous behavioral, anthropomorphic, and biochemical variables in 50 socially housed adult female monkeys. Given that critical role of DRD2 in addiction and feeding behavior in mice and the lack of data concerning this gene within the rhesus macaque species, we set out to assay the genetic variation in coding regions of this gene within this population. Through this analysis we have identified two single nucleotide polymorphisms (SNPs) that result in nonsynonymous predicted protein sequences, both within exon 3 of the gene. Subsequently we conducted association studies to determine if any relationships existed between the newly identified genetic variants and the numerous phenotypic measures within this population. We have identified preliminary relationships between the first SNP and levels of adiponectin within whole blood (p=027), 3,4-Dihydroxyphenylacetic acid (DOPAC) a major metabolite of dopamine within the cerebrospinal fluid (CSF) (p=.016), 5-Hydroxyindoleacetic acid (5-HIAA) the primary metabolite of serotonin within the CSF (p=.016), and aggressive behavior (p=.01). The second SNP shows association with levels of affiliative behavior within these animals (p=.007). These preliminary data on a small number of animals suggest that polymorphisms in DRD2 could be important in a number of phenotypes related to behavior and metabolism. Nothing to Disclose: ZPJ, VM, MEW

Pub #	P1-776
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)
Title	Magmas, a Novel Over-Expressed Gene in Pituitary Rat Cell Lines and in Human Pituitary Adenomas: A New Pituitary Biomarker?
Author String	F Taglati, T Gagliano, R Rossi, S Borsetto, E degli Uberti, MC Zatelli University of Ferrara, Ferrara, Italy
Body	Pituitary tumors are mostly benign, being locally invasive in 5-35% of cases. Deregulation of several genes has been suggested as a possible alteration underlying the development and progression of pituitary tumors. Recently we have demonstrated that Magmas is over-expressed in a mouse ACTH-secreting pituitary adenoma cell line. Here we investigate by RT-QPCR the expression of Magmas in 4 rat pituitary adenoma cel lines: three growth hormone (GH) and prolactin (PRL)-secreting cells lines (GH1, GH3, and GH4C1) and one PRL-secreting cell line (MMQ). We found that Magmas is over-expressed in GH3 (2.2 fold) and MMQ (3.6 fold) cell lines, while GH1 and GH4C1 display Magmas expression levels similar to those detected in a pool of normal rat pituitaries. These results were confirmed by Western blot analysis that showed the same expression pattern found by RT-QPCR. Magmas mRNA expression was also assessed in 29 human pituitary tissues, including 19 nonfunctioning, 8 GH-secreting, 2 PRL-secreting, and one TSH-secreting pituitary adenoma, as well as in a pool of human normal pituitary mRNAs. We found that 24 out of 29 pituitary adenomas (82.7%) displayed a Magmas mRNA expression level >2-fold than that detected in a pool of human normal pituitaries (from 2 to 30-fold), in all types of pituitary adenomas, except for the TSH-secreting pituitary adenoma. Our results suggest that Magmas could be a novel tumor selective over-expressed gene and could be a novel molecular target to be studied in order to understand pituitary adenomas pathophysiology. EdU: Principal Investigator, Pfizer, Inc. Nothing to Disclose: FT, TG, RR, SB, MCZ

Pub #	P1-777
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)
Title	New Phenotype in the Familial *DICER1* Tumor Syndrome: Pituitary Blastoma Presenting at Age 9 Months
Author String	S Wildi-Runge, A Bahubeshi, A-S Carret, L Crevier, Y Robitaille, K Kovacs, E Horvath, BW Scheithauer, WD Foulkes, C Deal CHU Sainte-Justine/University of Montreal, Montreal, Canada; Jewish General Hospital/McGill University, Montreal, Canada; CHU Sainte-Justine/University of Montreal, Montreal, Canada; CHU Sainte-Justine/University of Montreal, Montreal, Canada; CHU Sainte-Justine/University of Montreal, Montreal, Canada; St. Michaels Hospital/University of Toronto, Toronto, Canada; Mayo Clinic, Rochester, MN
Body	**Background.** *DICER1* is an RNase endonuclease important for production of microRNAs which regulate multiple protein-coding genes. It has been linked to several tumors particularly pleuropulmonary blastoma (PPB), cystic nephroma and ovarian Sertoli-Ledig cell tumors as well as to familial multinodular goiters (1-4) We enlarge the endocrine phenotype to include pituitary blastoma in an infant with a positive family history suggestive of the *DICER1* Syndrome, an autosomal dominant, incompletely penetrant cancer predisposing syndrome. **Case.** This 9 m-old male French Canadian boy was first seen after an ophthalmology consult for strabismus and right eye proptosis led to the diagnosis of a 16 x 30 x 23 mm sellar and suprasellar tumor. His growth was normal. Family history revealed a PPB and cystic nephroma in a 2.7 y male second cousin once removed, whose grandmother was treated for renal cysts and an ovarian tumor. Physical examination of this well-looking baby was significant for R proptosis. Bone age was 6-9 m. Baseline endocrine evaluation detected elevated serum AFP (174 ug/L) and central hypothyroidism. Following partial tumor resection, the patient developed signs of Cushing Syndrome, confirmed by endocrine testing. Tumor pathology was consistent with a pituitary blastoma, revealing primitive Rathke-type epithelium, brisk mitotic activity, small folliculo-stellate cells and larger secretory cells immunoreactive for ACTH, beta-endorphin and O-6-methylguanine-DNA-methyltransferase. Polychemotherapy with vincristine, cyclophosphamide, VP-16 and cisplatin was started and the child, now 22 months, is doing well on treatment. The residual tumor is stable and signs of Cushing Syndrome have receded, although subclinical hypercortisolemia persists. Genetic analyses in peripheral blood leukocytes detected a microduplication of 516 kb involving 1q21.3 and containing at least 6 potential oncogenes as well as a novel germline heterozygous *DICER1* nonsense mutation (*c.*2379T>G; Y793X) which was also present in his second cousin once removed. Extended genetic analyses on the family are ongoing. **Conclusion.** This is the second reported case of pituitary blastoma in infancy (5); we suspect that other cases previously labelled as pituitary ACTH-producing adenoma in very young infants may be part of a larger *DICER1* familial cancer syndrome (6). Incomplete penetrance of the various tumors are likely due to modifying loci, such as that described in our patient. (1)Slade I et al. J Med Genet, 2011;48(4):273-8. (2)Rio Frio T et al. JAMA, 2011;305(1):68-77. (3)Bahubeshi A et al. J Med Genet. 2010 Dec;47(12):863-6. (4)Hill DA et al. 2009 Aug 21;325(5943):965. (5)Scheithauer BW et al. Acta Neuropathol, 2008;116:657-666. (6)Pullins DI et al, Histopathology, 1984;8:157-163. Nothing to Disclose: SW-R, AB, A-SC, LC, YR, KK, EH, BWS, WDF, CD

Pub #	P1-778
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)
Title	Impact of Early Parenteral Nutrition To Complete Failing Enteral Nutrition in Adult Critically Ill Patients: A Randomized Controlled Trial
Author String	MP Casaer, D Mesotten, G Hermans, PJ Wouters, M Schetz, G Meyfroidt, S Van Cromphaut, C Ingels, P Meersseman, J Muller, D Vlasselaers, Y Debaveye, L Desmet, J Dubois, A Van Assche, A Wilmer, G Van den Berghe Catholic University of Leuven, Leuven, Belgium; Catholic University of Leuven, Leuven, Belgium; Jessa Hospitals, Hasselt, Belgium
Body	Abstract Embargoed.

(1) Trial registration at ClinicalTrials.gov: NCT 00512122
(2) Casaer MP, Hermans G, Wilmer A, Van den Berghe G. Impact of early parenteral nutrition completing enteral nutrition in adult critically ill patients (EPaNIC trial): a study protocol and statistical analysis plan for a randomized controlled trial. Trials 2011;12:21.

Sources of Research Support: Methusalem program of the Flemish government; Research Fund of the K.U.Leuven (GOA-2007/14); Fund for Scientific Research Flanders, Belgium; Clinical Research Fund of the UZ Leuven, Belgium; a partial (<30%) unconditional and non-restrictive research grant from Baxter Healthcare (Maurepas, France).

Nothing to Disclose: MPC, DM, GH, PJW, MS, GM, SVC, CI, PM, JM, DV, YD, LD, JD, AVA, AW, GV

Pub #	P1-779
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)
Title	Diet-Induced Visceral Obesity in Female Mice Depends on Autocrine Retinoic Acid Production
Author String	R Yasmeen, A Lynch, B Reichert, F Yang, K Lee, G Duester, O Ziouzenkova The Ohio State University, Columbus, OH; The Ohio State University, Columbus, OH; Sanford-Burnham Medical Research Institute, La Jolla, CA
Body	Effector mechanisms mediating visceral obesity remain poorly understood. Endogenously produced retinoic acid (RA) plays an essential role in adipocyte differentiation where it regulates expression of key transcriptior factors ZFP423 and PPARgamma in vitro (1). In vivo, 70% of PPARgamma mRNA expression in mouse visceral fat is under the control of RA that is produced by aldehyde dehydrogenase-1a1 (Aldh1a1), a member of the Aldh1 family of enzymes (1). Here we investigated the role of autocrine RA generation in high-fat (HF diet-induced fat formation in transgenic mice expressing a RA receptor response element (RARElacZ) LacZ reporter. Quantitative Taqman analysis of beta-galactosidase expression indicating intrinsic RA production revealed that formation of subcutaneous fat (261% and 221% in RARElacZ males and females, respectively) was accompanied by increased endogenous RA production in both male (363%) and female (495%) RARElacZ mice receiving a HF diet (45% kcal from fat) vs. regular chow for 5 months. Strikingly, visceral fat formation (478%) in RARElacZ male mice was not associated with RA production, whereas in RARElacZ female mice, visceral fat formation (354%) was accompanied by a 860% increase in RA generation. The causative role of autocrine RA production in sex-specific fat formation on a HF diet was demonstrated in mic deficient in Aldh1a1, a major RA-producing enzyme in white adipose tissue. After 300 days on the same HF diet, Aldh1a1KO mice showed decreased subcutaneous fat formation compared to WT mice that was similar between Aldh1a1KO males and females. Visceral fat accumulation was not influenced in Aldh1a1KO males, as compared to WT male mice. In contrast, Aldh1a1KO females were able to resist visceral obesity, e.g. Aldh1a1KO females had 9.5 times less visceral fat mass than WT female mice on same HF diet. Visceral fat in Aldh1a1KO females lacked all RA-generating Aldh1 enzymes in this depot, while Aldh1a1KO male mice expressed Aldh1a2 and a3 enzymes in this depot compensating for RA production. Our data suggest that HF diet effects in adipose tissue depend on autocrine RA production and reveal the pivotal role of this autocrine pathway in the formation of visceral fat in females. (1) Reichert B et al.,Mol Endo 2011;E pub ahed of print Sources of Research Support: SEED grant College of Education and Human Ecology,Ohio State University (OSU), Pilot grant Food Innovation center, OSU (OSU), Personal funds (OZ). Nothing to Disclose: RY, AL, BR, FY, KL, GD, OZ

Pub #	P1-780
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)
Title	Short-Chain Fatty Acids Regulate Incretin Secretion and Protect Against Diet-Induced Obesity Via GPR41-Independent Mechanisms
Author String	A Frassetto, AR Nawrocki, EJ Kowalik, J Kosinski, JA Hubert, M Qatanani, Y Xiong, JM Cox, HV Lin Merck Sharp & Dohme Corp., Rahway, NJ
Body	Short-chain fatty acids (SCFAs) have been implicated in the regulation of body weight and glucose metabolism, but the mechanisms remain unclear. Here we show that acute oral administration of sodium butyrate in mice led to two- to five-fold increases in plasma GLP-1 and GIP, coinciding with elevated plasma insulin levels. Sodium propionate acutely induced GIP, but not GLP-1. In contrast, sodium acetate had no acute effect on plasma incretins. Chronic dietary supplementation of sodium butyrate or sodium propionate in mice dramatically inhibited high fat diet-induced weight gain, associated with improved glucose tolerance, while sodium acetate had a much more modest effect. Analyses of food intake and locomotor activity indicate the primary cause of SCFA-dependent resistance to weight gain is likely reduced nutrient absorption and/or increased resting energy expenditure, and suggest distinct mechanisms of action for different SCFAs. Interestingly, chronic dietary exposure to all three SCFAs led to significant decreases, rather than increases, in plasma GLP-1 and GIP, likely reflecting reduced adiposity. Since Gpr41 and Gpr43, the endogenous SCFA receptors, are expressed in intestinal L cells, we examined their roles in incretin secretion using immortalized L cell lines. Two Gpr43 agonists increased GLP-1 secretion, while a Gpr41 agonist had no effect, suggesting a predominant role for Gpr43 in SCFA-induced GLP-1 secretion. Consistent with the in vitro data, studies in Gpr41 knockout mice suggest that butyrate and propionate regulate incretin secretion and body weight independently of Gpr41. In summary, our data show that acute and chronic SCFAs differentially regulate incretin secretion and protect against diet induced obesity and implicate Gpr43 in their mechanism of action. This might explain the beneficial effects of these nutrients on body weight regulation and glucose homeostasis. AF: Employee, Merck & Co. ARN: Employee, Merck & Co. EJK: Employee, Merck & Co. JK: Employee, Merck & Co. JAH: Employee, Merck & Co. MQ: Employee, Merck & Co. YX: Employee, Merck & Co. JMC: Employee, Merck & Co. HVL: Employee, Merck & Co.

Pub #	P1-781
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)
Title	NPY Signaling in Osteoblasts Controls Glucose Metabolism in Mice
Author String	N Lee, D Sousa, A Sainsbury, P Baldock, H Herzog Garvan Institute of Medical Research, Sydney, Australia
Body	The skeleton has recently emerged as a potential player in the control of whole-body glucose metabolism, however, the mechanism behind this is not clear. We demonstrated that mice lacking Y1 receptors solely in cells of the osteoblastic lineage not only display a high bone mass phenotype due to significantly increased bone formation associated with reduced bone resorption, but also display altered whole-body glucose metabolism. This is due to significantly decreased pancreatic insulin content, pancreas weight, and insulin secretion leading to elevated glucose levels and reduced glucose tolerance, but with no effect on insulin induced glucose clearance. Furthermore, increased activity of Y1 signalling induced by adult onset over-expression of its ligand PYY, solely in osteoblastic cells also led to impaired glucose tolerance, elevated insulin secretion and impaired insulin sensitivity in mice. Together these data reveal a novel mechanism by which NPY signalling in bone tissue is involved in the control of glucose and energy homeostasis. Nothing to Disclose: NL, DS, AS, PB, HH

Pub #	P1-782
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)
Title	Characterizing the Compensatory Islet Proteome Response to Insulin Resistance by Quantitative Proteomics
Author String	J-Y Zhou, CW Liew, CD Nicora, TRW Clauss, RJ Moore, DG Camp II, RD Smith, RN Kulkarni, W-J Qian Pacific Northwest National Laboratory, Richland, WA; Havard Medical School, Boston, MA
Body	Compensatory islet hyperplasia is a distinct feature of the pre-diabetic insulin resistant state in both rodents and humans. In this study, we aim to identify specific protein alterations that characterize this compensatory response using liquid chromatography coupled with mass spectrometry (LC-MS) based quantitative proteomics using two well-established insulin-resistant mouse models without overt diabetes, namely, (1) the high-fat diet (HFD) fed mouse, and (2) the obese *ob/ob* mouse, We used freshly isolated islets from individua six-month old male mice (n=6 for each condition). The LC-MS quantitative proteome profiling revealed ~1,700 islet proteins that were identified and quantified by at least two unique peptides with a false discovery rate <1%. Statistically significant alterations in protein abundances between control and insulin resistant conditions were observed for ~500 proteins with good consistency in the two different models, suggesting common functional changes occurring as a response to the insulin resistance. Bioinformatics analyses using Ingenuity Pathway Analysis (IPA) revealed significant alterations in a number of pathways or functions. For example, proteins involved in protein biosynthesis, protein folding, transportation, and endocytosis were significantly up-regulated while proteins involved in glucose metabolism, oxidative stress response, and mitochondrial dysfunction were down regulated in both models. Moreover, the abundances for islet secretory hormones were markedly decreased. The results indicated enhanced islet activity in terms of protein synthesis transport, and secretion; however, the data also indicated potential defects in islet function based on the down-regulation of sugar metabolism, oxidative stress response, and mitochondrial function. These defects could eventually lead to beta-cell failure. This study represents the first quantitative study of the islet proteome that is focused on the compensatory response to insulin-resistance in the absence of overt diabetes. The extensive dataset provides a resource of islet proteins that could be novel candidates in studies aimed at islet cell growth or apoptosis. Currently, we are validating a selected list of potential candidates related to islet cell regeneration pathways. Sources of Research Support: National Institutes of Health grant R01 DK074795 and RO1 DK067536. Nothing to Disclose: J-YZ, CWL, CDN, TRWC, RJM, DGC, RDS, RNK, W-JQ

Pub #	P1-783
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)
Title	Half of an Unselected Series of Aldosterone-Secreting Adenomas Have Somatic Mutation of the KCNJ5 Channel, and Have a Zona Fasciculata Profile
Author String	EA Azizan, MJ Brown University of Cambridge, Cambridge, United Kingdom
Body	**Background**: Two somatic mutations in the selectivity filter region of the K^+ channel KCNJ5 were recently reported in large (>2 cm) aldosterone-secreting adenomas (APA) (1). Either G151R or L168R mutations allowed Na^+ entry through the channel, causing complete membrane depolarisation. Our aims were to measure frequency of somatic KCNJ5 mutations in unselected APAs, and determine any genotype-phenotype relationship. **Methods:** 31 consecutive APAs and NA were dissected within 30 minutes of adrenalectomy, and snap-frozen for storage at -80[deg]C. cDNA from 22 of the APAs, and gDNA (exons only) from the remaining 9 APAs, were sequenced. The proportion of zona glomerulosa (ZG): zona fasciculata (ZF) cells in each APA was assessed by an adrenal pathologist, and the biochemical profile assessed by qPCR measurement of cDNA encoding the ZG-specific enzyme, CYP11B2, and ZF-specific enzyme, CYP17A1. Immunohistochemistry (IHC) was performed of both APA and NA. **Results**: 26 of the APAs were < 2 cms. Astonishingly, 15 out of the 31 APAs had a non-synonymous SNP in KCNJ5. Seven APAs each had the G151R or L168R mutations, and one a novel isoleucine deletion (I157DEL). The L168R, G151R and wild-type APAs were, respectively, 18, 13, and 11 mm diameter ($p=0.01$). The CYP11B2 cDNA of the mutants were 1.2% and 6.4% of wild-type APAs, whilst reversely CYP17A1 cDNA was 9.0- and 4.2-fold more abundant ($p<0.05$). The blindly assessed histology showed L168R, G151R and wild-type APAs to have, respectively, low, variable and high proportions of ZG:ZF-like cells. IHC showed staining of normal ZG and outer ZF, and patchy staining of APAs. **Conclusion:** APAs appear to join the list of benign endocrine tumors commonly due to somatic mutation in a single gene. The APAs with somatic mutation in KCNJ have a different phenotype from other APAs, being larger and more ZF-like than ZG-like. Why many APAs resemble ZF rather than ZG cells has long been a mystery. We suggest that mutation of KCNJ5, allowing Na^+-influx and membrane depolarisation, changes the appearance and biochemical profile of the ZG cells. Choi M et al. Science 2011;331:768-772 Nothing to Disclose: EAA, MJB

Pub #	P1-784
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)
Title	Subtype Analysis of Primary Aldosteronism According to 18-Oxocortisol Peripheral Blood Concentration by the High Sensitive Ms/ms Measurement
Author String	F Satoh, R Morimoto, M Kudo, K Takase, CE Gomez-Sanchez, WE Rainey, H Sasano, Y Nakamura, S Ito Tohoku University Hospital, Sendai, Japan; Tohoku University Hospital, Sendai, Japan; Tohoku University Hospital, Sendai, Japan; G.V. Montgomery VA Medical Center, and the University of Mississippi Medical Center, Jackson, MS; Georgia Health Sciences University, Augusta, GA
Body	18-oxocortisol (18-oxoF) is a derivative of cortisol (F) that is produced by aldosterone synthase (CYP11B2). The potential usefulness for this steroid, as a biomarker for differentiating patients with aldosterone-producing adenoma (APA) from those with idiopathic hyperaldosteronism (IHA), has not been clarified. **Objectives:** We measured 18-oxoF, aldosterone and F in peripheral plasma of patients with primary aldosteronism. We compared 18-oxoF levels and 18-oxoF/F ratios for their potential to differentiate APA from IHA. **Design, Setting, and Subjects:** This study measured 18-oxoF, F and aldosterone in peripheral plasma obtained from 51 patients with surgically proven APAs or 26 patients with bilateral IHA (including 7 surgically proven IHA cases), using highly sensitive liquid chromatography-tandem mass spectrometry (*LC/MS/MS*) and radioimmunoassay (RIA) analyses. Adrenal venous sampling (AVS) was successfully performed in all of 77 patients. **Results:** The levels of 18-oxoF and the ratios of 18-oxoF /F, before and after ACTH stimulation, were significantly higher in the peripheral plasma from the patients with APAs than those in the peripheral plasma from the patients with IHA. The cutoff value of 18-oxoF levels was calculated by ROC curve analysis with high sensitivity of 0.92 and specificity of 0.92. **Conclusions:** The 18-oxoF levels and ratios of 18-oxoF/F in peripheral plasma samples can be a clinically useful biomarker for differentiating APA from IHA. Nothing to Disclose: FS, RM, MK, KT, CEG-S, WER, HS, YN, SI

Pub #	P1-785
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)
Title	Cardiac Induction of the Thyroid Hormone Degrading Enzyme Deiodinase Type III in Human Ischemic Cardiomyopathy
Author String	CJ Pol, A Muller, R Janssen, MJ Zuidwijk, CG dos Remedios, TJ Visser, WJ Paulus, WS Simonides VU University Medical Center, Amsterdam, Netherlands; The University of Sydney, Sydney, Australia; Erasmus University Medical Center, Rotterdam, Netherlands
Body	Deiodinase type III (D3) is a thyroid hormone (TH) degrading enzyme and its activity is markedly and stably induced in the left ventricle (LV) following myocardial infarction in mice. The subsequent remodeling of the LV and the development of cardiac dysfunction is associated with decreased LV tissue TH content and decreased TH-dependent transcription activity in cardiomyocytes. This local hypothyroid condition is suggested to contribute to the development of heart failure, given the known regulation by TH of several key cardiac genes implicated in contractile dysfunction in ischemic cardiomyopathy (ISHD). Here, we investigated whether D3 is also induced in myocardium of heart-failure patients suffering from ISHD. Tissue samples from ISHD patients (n=23) were obtained during heart transplantation surgery. Non-failing heart tissue was obtained from donor hearts without evidence of impaired heart function (n=55). Tissue micro arrays comprising up to 120 individual 4 [micro]m thick paraffin sections of duplicate LV cores of 1 mm diameter of patients and donors were stained with the validated, affinity-purified anti-human-D3-antibody (#718). Staining intensity of the blinded samples was scored by three investigators using a visual grading score of 1 to 4 (no staining to strong staining) and the scores were averaged. Little or no D3 staining was found in most donor samples with 46 out of 55 scoring 1 to 2.5. In contrast, D3 staining in the ISHD group ranged from 1.7 to 4, with 14 out of 23 scoring 3.5 to 4. In most of these all cardiomyocytes showed high D3 expression, but in some samples both D3-positive and non-stained cardiomyocytes were intermixed, similar to what is found in remodeled mouse myocardium following myocardial infarction. Interstitial cells did not show D3 expression in any of the samples. This study demonstrates for the first time that cardiac D3 activity is induced in human ischemic cardiomyopathy. This suggests that the D3-dependent local hypothyroid condition of the heart found in mice following myocardial infarction, may also apply to the human situation and contribute to the development of heart failure. Sources of Research Support: Netherlands Heart Foundation grant 2006B240. Nothing to Disclose: CJP, AM, RJ, MJZ, CGdR, TJV, WJP, WSS

Pub #	P1-786
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30 PM-3:30 PM)
Title	The PAX8/PPARγ Fusion Gene Modulates Follicular Thyroid Tumorigenesis by Regulating the Expression o Two Known Tumor Suppressors, PTEN and microRNA-122
Author String	HV Reddi, P Madde, D Milosevic, B McIver, A Algeciras-Schimnich, SKG Grebe, NL Eberhardt Mayo Clinic, Rochester, MN; Mayo Clinic, Rochester, MN
Body	The PAX8/PPARγ fusion protein (PPFP) present in 36% of follicular thyroid carcinomas (FTC) is considered a putative oncogene due to its oncogenic potential in vitro. However, its precise role in FTC tumorigenesis is unknown. Meta-analysis of extant studies of disease progression in human FTC with and without PPFP indicate that 68% (c2 = 0.008) of PPFP-positive FTC are associated with minimally invasive disease (1). Constitutive expression of PPFP in PTEN-intact FTC-derived WRO-82-1 cells inhibited xenograft tumor progression (6-fold, $p<0.0001$) and was associated with up-regulation of the tumor suppressor miR-122 (3- and 40-fold in cells and xenografts, respectively, $p<0.05$), suggesting PPFP functions as a tumor suppressor in vivo (1). To further elucidate mechanisms of PPFP's tumor suppressor activity, we expressed PPFP in a PTEN-null FTC-derived FTC-133 cell line. In contrast to our observations with WRO cells, FTC-133-PPFP cells demonstrated only 1.5-fold ($p<0.0079$) inhibition of tumor progression and showed no change in miR-122 expression. Ectopic expression of PTEN in FTC-133 cells restored PPFP-mediated up-regulation of miR-122 (4-fold, $p<0.05$), suggesting a role for PTEN in PPFP function. Therefore, we evaluated PTEN expression in the WRO cell model and FTC tumors. Western Blotting demonstrated that PPFP expression is associated with increased PTEN levels in WRO cells. This observation is mirrored in human PPFP-positive FTC as demonstrated by immunohistochemical staining for PTEN. While 100% (8/8) PPFP-expressing FTC were strongly positive for PTEN, only 40% (4/10) of PPFP-negative FTC exhibited weak to modest PTEN expression. Protein turnover studies showed that PPFP modulates PTEN expression in part by stabilization and inhibition of PTEN degradation, based on the effects of cycloheximide and MG-132 treatment. These data demonstrate that PPFP exhibits tumor suppressor activity in vivo that is mediated in part through PTEN and miR-122. Given our observations that PTEN expression is absent in 60% ($p<0.005$) of PPFP-negative FTC, the data suggest that PPFP modulates PTEN expression during tumor progression, thereby contributing to less aggressive disease observed in most FTC expressing PPFP. Our data provides the first evidence for the role of PTEN in PPFP-mediated tumor suppressor function and emphasizes PPFP's potential as a prognostic marker for follicular thyroid cancer. (1) Reddi et al., Genes & Cancer, In Press, 2011. DOI: 10.1177/1947601911405045 Sources of Research Support: NIH R01 CA80117. Nothing to Disclose: HVR, PM, DM, BM, AA-S, SKGG, NLE

Pub #	P1-787
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (1:30:00 PM-3:30:00 PM)
Title	Potential Roles of Corticotropin-Releasing Hormone (CRH) Receptors in the Differentiation of White Adipocytes
Author String	B Lu, D Markovic, J Pessin, H Lehnert, DK Grammatopoulos University of Warwick, Coventry, UK; Albert Einstein College of Medicine, Bronx, NY; University of Lübeck Medical School, Lübeck, Germany
Body	In addition to roles in mammalian adaptive responses to stressful stimuli, [ldquo]stress[rdquo] peptides such as corticotropin releasing hormone (CRH) and urocortins (Ucns) are emerging as important regulators of the homeostatic mechanisms regulating energy balance and metabolism. CRH and Ucns acting through CRH-R1 and R2 receptors can target multiple peripheral tissues such as skeletal muscle and adipose tissue to influence important metabolic pathways. Functional CRH-Rs as well as CRH, Ucn1 and Ucn2 are expressed in both brown and white adipose tissue, however their biological roles are not well understood. We investigated the potential actions of CRH-R in the differentiation of white pre-adipocytes, using the 3T3L1 fibroblast cell line that can differentiate into white adipocytes, and express both type 1 and 2 CRH-Rs. Exposure of 3T3L1 to Ucn2, to specifically activate CRH-R2, during cell differentiation led to increased expression by 2-3 fold of uncoupling protein 1 (UCP-1), which is uniquely expressed in brown adipocytes. Real-time RT-PCR studies also showed that Ucn2 up-regulated expression of essential determinants of brown adipocyte differentiation such as PRDM16, and its associated coregulators PGC1-α and CtBP1/2. Moreover, Ucn2-treated 3T3L1 cells were characterized by repression of the white fat genes Wdnm1, resistin and chemerin. This observation raised the possibility that CRH-R2 may play an important role in phenotypic trans-differentiation of white adipocytes. Similar results were obtained when CRH-R1 activity was specifically blocked by NBI 27914, during the differentiation process. Exposure of 3T3L1 to NBI 27914 upregulated UCP-1 mRNA expression by 3-4 fold. Also the expression level of PRDM16, PGC1-α and CtBP1/2 were upregulated by 2-3 fold, whereas Wdnm1, resistin and chemerin mRNAs were significantly downregulated. Immunofluorescent microscopy showed that COX IV and UCP-1 protein expression was significantly increased in 3T3L1 differentiated in the presence of NBI 27914. Thus, the differentiation of white adipocytes appears to be under the intricate control of CRH-R and their cognate agonists, which exert contrasting actions. Inhibition of CRH-R1 or activation of CRH-R2 in 3T3L1 white pre-adipocytes drives cells towards a pro-brown-like adipocyte phenotype. This data identifying the CRH/CRH-R system as a potential candidate for strategies to enhance the brown phenotype in white adipocytes and combat obesity-associated disorders. Nothing to Disclose: BL, DM, JP, HL, DKG

Pub #	M7
Session Information	MEET-THE-PROFESSOR: CLINICAL - Evaluation of the Transition Patient for Growth Hormone Replacement (2:45 PM - 3:30 PM)
Title	Evaluation of the Transition Patient for Growth Hormone Replacement
Author String	G Johannsson Sahlgrenska University Hospital, Gothenburg, Sweden
Body	Disclosures: GJ: Speaker, Eli Lilly & Company, MerckSerono; Clinical Researcher, Novo Nordisk, Pfizer, Inc.; Advisory Group Member, Otsuka; Chief Scientific Officer, DuoCort Pharma.

Pub #	M9
Session Information	MEET-THE-PROFESSOR: CLINICAL - Metabolic Management of Polycystic Ovary Syndrome (2:45 PM - 3:30 PM)
Title	Metabolic Management of Polycystic Ovary Syndrome
Author String	DA Ehrmann University of Chicago, Chicago, IL
Body	Nothing to Disclose: DAE

Pub #	CMF1-1
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Inpatient Management of Blood Glucose Levels (2:45 PM - 3:30 PM)
Title	Inpatient Management of Blood Glucose Levels
Author String	GE Umpierrez Emory University School of Medicine, Atlanta, GA
Body	Session supported by: Lilly USA, LLC Disclosures: GEU: Researcher, Sanofi-Aventis, Takeda.

Pub #	CMF1-2
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Inpatient Management of Blood Glucose Levels (2:45 PM - 3:30 PM)
Title	Inpatient Management of Blood Glucose Levels
Author String	JL Leahy University of Vermont College of Medicine, Colchester, VT
Body	Session supported by: Lilly USA, LLC Disclosures: JLL: Advisory Group Member, Bristol-Myers Squibb, Merck & Co., Novo Nordisk, Sanofi-Aventis; Research Funding, Takeda.

Pub #	CMF2-1
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Management of Apparent Secondary Hypogonadism due to Obesity, Opiates & Anabolic Steroids (2:45 PM - 3:30 PM)
Title	Management of Apparent Secondary Hypogonadism Due to Obesity, Opiates & Anabolic Steroids
Author String	FC Wu Manchester Royal Infirmary, Manchester, UK
Body	Disclosures: FCW: Consultant, Bayer Schering Pharma, Eli Lilly & Company.

Pub #	CMF2-2
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Management of Apparent Secondary Hypogonadism due to Obesity, Opiates & Anabolic Steroids (2:45 PM - 3:30 PM)
Title	Management of Apparent Secondary Hypogonadism Due to Obesity, Opiates & Anabolic Steroids
Author String	RA Bebb University of British Columbia, Vancouver, Canada
Body	Nothing to Disclose: RAB

Pub #	M6
Session Information	MEET-THE-PROFESSOR: CLINICAL - Disorders of Sexual Development in Adults (2:45 PM - 3:30 PM)
Title	Disorders of Sexual Development in Adults
Author String	GS Conway University College of London Hospital, London, UK
Body	Session supported by: Novo Nordisk Inc. & Pfizer, Inc. Nothing to Disclose: GSC

Pub #	M8
Session Information	MEET-THE-PROFESSOR: CLINICAL - Investigating Mineralocorticoid Hypertension (2:45 PM - 3:30 PM)
Title	Investigating Mineralocorticoid Hypertension
Author String	CE Kater Federal University of Sao Paulo, Sao Paulo, Brazil
Body	Nothing to Disclose: CEK

Pub #	M10
Session Information	MEET-THE-PROFESSOR: CLINICAL - New Options in the Investigation & Management of (Neuro-) Endocrine Tumors of the Digestive Tract & Pancreas (2:45 PM - 3:30 PM)
Title	New Options in the Investigation & Management of (Neuro) Endocrine Tumors of the Digestive Tract & Pancreas
Author String	WW de Herder Erasmus Medical Center, Rotterdam, Netherlands
Body	The majority of neuroendocrine tumors (NETs) of the gastrointestinal (GI) tract are non-functioning and their presentation is non-specific. Functioning NETs of the GI tract may present with the typical 'carcinoid syndrome', which is clinically manifested as cutaneous flushing and diarrhea. Most pancreatic NETs are non-functioning and are diagnosed either incidentally or by local mass effects. Insulinomas are the commonest functioning pancreatic NETs, mostly benign (95%), small sized tumors, characterized by insulin hypersecretion, neuroglycopenia and catecholamine responsive symptoms. Hypergastrinemia in gastrinomas causes acid hypersecretion with peptic ulceration of the upper GI tract and diarrhea. Glucagonomas are large tumors that present with weight loss, diabetes, cheilosis, diarrhea and necrolytic migratory erythema. VIPomas are rare, usually large metastatic tumors and present with severe secretory diarrhea. Surgery is essential in many phases of the management of NETs. Somatostatin analogs remain the mainstay of symptomatic therapy for GEP NETs and are usually well tolerated, safe drugs, with mild side effects. Their effects on tumor growth have been recently demonstrated in NETs of the gastrointestinal tract. Interferon alpha has high symptomatic and biological response rates, but has substantial adverse effects. Etoposide plus cisplatin is effective for poorly differentiated GEP NETs, whereas streptozotocin + 5-FU + doxorubicin has some benefit in poorly differentiated pancreatic NETs. Traditional cytotoxic agents are of limited efficacy in the treatment of well differentiated GEP-NETs. However, their high expression of several pro-angiogenic molecules including VEGF render them potentially susceptible to antiangiogenic strategies. Other therapeutic targets include epithelial grown factor receptor (EGFR), insulin-like growth factor receptor (IGFR), and dowr signaling pathway components PI3K/AKT/MTOR which are overexpressed, with loss of the related tumor suppressor protein PTEN. In this spectrum, Everolimus and Sunitinib have shown promising results in phase III trials in GEP-NETs. GEP-NETs over-express somatostatin receptors and are, therefore, attractive targets for radiolabelled somatostatins. Peptide radioreceptor therapy has utilized high dosages of [^{90}Y-DOTA^{0},Tyr^{3} octreotide, [^{90}Y-DOTA]lanreotide, [^{90}Y-DOTA^{0},Tyr^{3}]octreotate and [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Very promising results have also been achieved using these targeted therapies. Disclosures: WWdH: Advisory Group Member, Novartis Pharmaceuticals, Ipsen.

Pub #	M11
Session Information	MEET-THE-PROFESSOR: CLINICAL - Phosphate Disorders in Children & Adolescents (2:45 PM - 3:30 PM)
Title	Phosphate Disorders in Children & Adolescents
Author String	TO Carpenter Yale University School of Medicine, New Haven, CT
Body	Disclosures: TOC: Consultant, Kyowa Hakko Kirin Pharma.

Pub #	M12
Session Information	MEET-THE-PROFESSOR: CLINICAL - Surgical Management of Thyroid Cancer (2:45 PM - 3:30 PM)
Title	Surgical Management of Thyroid Cancer
Author String	E Kebebew National Cancer Institute/National Institutes of Health, Bethesda, MD
Body	Nothing to Disclose: EK

Pub #	M13
Session Information	MEET-THE-PROFESSOR: CLINICAL - When & How To Use Vitamin D (2:45 PM - 3:30 PM)
Title	When & How To Use Vitamin D
Author String	DD Bikle Veterans Affairs Medical Center 111N-University of California, San Francisco, CA
Body	Nothing to Disclose: DDB

Pub #	S11-1
Session Information	SYMPOSIUM SESSION: BASIC - Pituitary Stem/Progenitor Cells (3:45 PM - 5:15 PM)
Title	Pituitary Stem/Progenitor Cells & Tissue Regeneration after Injury
Author String	HEJ Vankelecom Katholieke Universiteit Leuven, Leuven, Belgium
Body	The pituitary gland represents the endocrine core of the organism and is well-known for its cellular plasticity to meet the body's fluctuating hormonal demands. In the past, it has repeatedly been postulated that the pituitary harbors tissue-specific stem cells that participate in the generation of new hormonal cells. Stem/progenitor cells in the pituitary remained elusive for a long time and were only very recently identified. Our group tracked down the multipotent stem/progenitor cells in a specific subset of the anterior pituitary 'side population' (SP). The stem/progenitor cells were found to express Sox2, a well-known gatekeeper of pluripotency in many other stem cells. $Sox2^{+}$ cells are located in multiple putative niches, including the cleft-lining marginal zone and clusters scattered over the gland's parenchyma. Now that pituitary stem/progenitor cells are identified and can be assessed by Sox2 and SP phenotype, their function in pituitary cell remodeling can be explored. In many adult organs, stem cells play an important role in tissue repair after injury. Whether the mature pituitary is also capable of cell regeneration after loss by damage is at present not well studied and controversial. To profoundly investigate the pituitary's regenerative capacity and the possible involvement of stem/progenitor cells, we set up a transgenic injury model in which anterior pituitary cells can be destroyed in a conditional manner using the 'inducible diphtheria toxin receptor (iDTR)' system. In this model, we observed a prompt and impressive reaction of the stem/progenitor cell compartment to the infliction as well as a regenerative response that restores the ablated cells to a considerable extent. Our study demonstrates that the adult pituitary gland is capable of repairing cell-ablation injury, and that stem/progenitor cells appear to be involved in this regenerative process. Nothing to Disclose: HEJV

Pub #	S11-2
Session Information	SYMPOSIUM SESSION: BASIC - Pituitary Stem/Progenitor Cells (3:45 PM - 5:15 PM)
Title	Stem Cells, Differentiation & Cell Cycle Control in Pituitary
Author String	J Drouin Institut de Recherches Cliniques de Montréal, Montréal, Canada
Body	Stem cells constitute a pool of progenitors that may serve maintenance or reserve functions in adult tissues. Similar progenitors are the source of differentiated cells that contribute to organ development. We are using the developing pituitary gland in order to understand how pituitary stem/progenitors exit the cell cycle and initiate the differentiation process. Thus we showed that before any sign of cell differentiation, fetal pituitary progenitors exit the cell cycle under the action of the cell cycle inhibitor $p57^{Kip2}$. This mechanism of cell cycle exit appears to be unique to pituitary progenitors as re-entry into cycle of differentiated cells appears to be primarily prevented by the related inhibitor $p27^{Kip1}$. Using knockout models for these cell cycle inhibitors as well as for regulators of pituitary cell differentiation, we showed independent control of cell cycle and differentiation during pituitary organogenesis. Whereas expansion of undifferentiated progenitors appears to be the major mechanism for tissue growth during fetal pituitary development, normal maintenance of the adul anterior pituitary appears more dependent on self-replication of differentiated cells, at least for anterior pituitary corticotropes and in contrast to the related POMC-expressing melanotropes. Recent insight into the molecular mechanisms responsible for the differential identity of these two pituitary POMC lineages was gained from identification of a critical transcription factor that promotes the melanotrope gene expression program and represses the corticotrope program. The relationships between cell cycle control and differentiation will be further discussed in the context of pituitary tumorigenesis. Nothing to Disclose: JD

Pub #	S11-3
Session Information	SYMPOSIUM SESSION: BASIC - Pituitary Stem/Progenitor Cells (3:45 PM - 5:15 PM)
Title	RET/GFRa in Pituitary Stem/Progenitor Cells
Author String	CV Alvarez University of Santiago de Compostela, Santiago De Compostela, Spain
Body	The RET receptor belongs to a family of receptors having a double mechanism of activation. In the absence o ligand RET behaves as a dependence receptor inducing p53 and apoptosis. In the presence of ligand RET dimmerizes, activating its tyrosine kinase activity. In humans RET has four ligands and four co-receptors. Interestingly, the pituitary somatotrophs express RET together with the GFRa1 co-receptor and this complex is activated by a ligand called GDNF. We have discovered that the stem cells of the pituitary, the GPSs, express RET together with the GFRa2 co-receptor. This complex is activated by another ligand called NTN. We have used the GFRa2 co-receptor to study the properties of these adult pituitary stem cells in rodents and humans. Recently, we have further characterized the GPSs demonstrating that they express the four essential markers of stemness indicating the full potentiality of these cells. It is important to delineate the composition of the GPS niche, and how many types of cells are taking part in this physiological structure. In the other hand, new data demonstrate that RET regulates the ARF gene, the tumor suppressor implicated in p53 estability. How this RET-dependent system is regulated in differentiated versus stem cells will be an essential knowledge to understand the transition from a pituitary stem cell to a differentiated somatotroph and what could be wrong in pituitary derived tumors. Sources of Research Support: Xunta de Galicia-MICINN-FEDER 09CSA011208PR and BFU2010-16652. Nothing to Disclose: CVA

Pub #	S12-1
Session Information	SYMPOSIUM SESSION: BASIC - PI3 Kinase Regulation of Metabolism (3:45 PM - 5:15 PM)
Title	Activation of PI3Kα: From Cancer to Diabetes
Author String	S Gabelli Johns Hopkins University School of Medicine, Baltimore, MD
Body	Phosphoinoside 3-OH kinase α (PI3Kα), and in particular its catalytic subunit p110α, plays a crucial role in insulin signaling and is required for normal glucose and lipid homeostasis. Somatic mutations of PI3Kα result in a constitutively activated enzyme. Mutations are found throughout the gene that codes for the catalytic subunit, with the exception of the Ras binding domain. Mutations of the regulatory subunit, p85α, are mostly clustered in the two SH2 domains and the domain between them. Structural biology, biochemistry and mutational studies are beginning to unravel different mechanisms by which the mutations affect the activity of the heterodimer p110α/p85α. Hotspot mutations of the helical domain, E542K and E545K, relieve the inhibition of p85α on p110α by disrupting a charge-charge interaction between the helical domain of p110α and the nSH2 domain of p85α. This mechanism mimics the natural method of PI3K activation, in which phosphorylated IRS or RTK bind and disrupt this same interaction between p85α and p110α. Analysis of the structural data show that the helical domain loop that contains these two hotspot mutations is located precisely where phosphopeptide binds nSH2 domain. Another hotspot mutation, H1047R, causes a conformational change of two loops that affect membrane association, resulting in increased catalytic activity. These data suggest a previously undescribed mechanism for mutational activation of a kinase that involves perturbation of its interaction with the cellular membrane. Most mutations in both p110α and p85α are located at interfaces between domains or between conserved interfaces of p110α and p85. Disruptions of these interactions are likely to affect the regulation of kinase activity by p85α or the catalytic activity of the enzyme. Further studies of PI3Kα activation can lead to a better understanding of insulin/PI3K signaling. Nothing to Disclose: SG

Pub #	S12-2
Session Information	SYMPOSIUM SESSION: BASIC - PI3 Kinase Regulation of Metabolism (3:45 PM - 5:15 PM)
Title	Co-Activator p300 Is a Critical Mediator in the Development of Hyperglycemia in High-Fat Diet Induced Obesity & Diabetes
Author String	L He Johns Hopkins School of Medicine, Baltimore, MD
Body	A major cause of fasting hyperglycemia in obese and diabetic patients is unregulated hepatic glucose production (HGP). Glucagon increases hepatic gluconeogenesis by activating the cAMP-PKA signaling pathway, leading to the formation of the CREB-CBP transcriptional complex that activates the gluconeogenic gene program in the liver. Insulin suppresses HGP by phosphorylating CBP and disassemblying the CREB-CBP complex. We examined the role of p300, a protein closely related to CBP, in regulating gluconeogenesis and found that p300 bound constitutively to CREs due to lack of a phosphorylation site found on CBP. When a phosphorylation-competent version of p300 is expressed in the p300G442S knock-in mice, HGP becomes exquisitely sensitive to insulin and metformin suppression. P300G422S and hepatic-deleted p300 mice exhibi significant hypoglycemia, indicating a role for p300 in maintaining basal HGP. Strikingly, mice fed a high-fat diet (HFD) for only one week demonstrated induction of p300, increased p300 gluconeogenic gene promoter occupancy, and increased HGP. These changes were reversed in p300G442S and hepatic-depleted p300 mice. Unregulated p300 promoter occupancy protects against hypoglycemia, but a high-fat diet induces p300 protein levels, activating HGP and causes hyperglycemia. We also found that HFD feeding induced ER stress In cultured hepatocytes, experimental ER stress mimicked by tunicamycin and thapsigargin treatment led to the induction of p300; in contrast, pretreatment with the chemical chaperone tauroursodeoxycholic acid (TUDCA), known to reduce ER stress, blocked p300 induction by thapsigargin. In addition, TUDCA treatment blocked hepatic p300 induction by HFD feeding in animals. We then measured the lipopolysaccharide (LPS) levels in the liver of HFD fed mice and found HFD feeding resulted in a significant increase in LPS levels (>10 fold). In cultured hepatocytes, LPS treatment increased ER stress and p300 protein levels. Therefore, p300 is an early mediator of unregulated hepatic gluconeogenesis and contributes to the development of hyperglycemia in HFD induced obesity states. Nothing to Disclose: LH

Pub #	S12-3
Session Information	SYMPOSIUM SESSION: BASIC - PI3 Kinase Regulation of Metabolism (3:45 PM - 5:15 PM)
Title	The Regulation of Hepatic Metabolism by Insulin: Is It All about Akt?
Author String	MJ Birnbaum University Pennsylvania School of Medicine, Philadelphia, PA
Body	The regulation of hepatic metabolism after a meal involves a complex interplay of hormonal and nutritional signals. An immediate event is the suppression of hepatic glucose out, which occurs too rapidly to be attributed to changes in gene expression. Rather, it is due to an insulin-dependent reduction in glycogen mobilization, which is regulated by a signal transduced by Akt2 but, surprisingly, not Gsk3. Longer term responses involve decreases in gluconeogenic gene expression and increases in expression of genes encoding lipogenic proteins. Both processes depend on the presence of Akt, though expression of only gluconeogenic genes involves FoxO1. Induction of lipogenic genes is not mediated by inhibition of FoxO1 or FoxA2 but does depend on the activity of the mTORC1 complex. Nonetheless, activation of mTORC1 is not sufficient to induce lipogenesis, as other Akt-dependent pathways are required. Sources of Research Support: NIH Grant RO1-DK56886. Disclosures: MJB: Advisory Group Member, Pfizer, Inc.

Pub #	S13-1
Session Information	SYMPOSIUM SESSION: BASIC - The Steroid Trifecta of Breast Cancer (3:45 PM - 5:15 PM)
Title	On the Luminal Origin of Basal Breast Cancers: Hormone Therapies & Notch Signaling
Author String	KB Horwitz University of Colorado Health Science Center, Aurora, CO
Body	Breast cancers are classified by gene expression profiling and immuno-histochemical markers to provide guidelines for treatment. Tumors classified as [ldquo]Luminal[rdquo] are treated with endocrine therapies if a least 1% of cells are estrogen (ER) or progesterone (PR)-receptor positive. Tumors that overexpress the [ldquo]HER2[rdquo] oncogene are treated with targeted anti-HER2 antibodies. [ldquo]Basal-like[rdquo] tumors that lack ER, PR and HER2 are classified as triple negative (TN) if they express cytokeratin (CK) 5 and epidermal growth factor receptors (EGFR). They are treated with cytotoxic chemotherapies. Our analysis of primary human breast cancers shows, surprisingly, that among ER+PR+ Luminal tumors, many contain an ER-PR-CK5+ subpopulation of TN cells we call [ldquo]Luminobasal[rdquo] that would be resistant to endocrine therapies. We find that in Luminal tumor models, the Luminobasal cell fraction is rapidly upregulated by progestins *via* transcriptional and EGFR signaling pathways that reprogram Luminal cells. This has implications for the linkage between progestins in hormone replacement therapies and increased breast cancer risk. Additionally, we find that in models of Luminal disease, and following neoadjuvant treatment of patients, the Luminobasal cell subpopulation expands following estrogen withdrawal (EWD) or antiestrogen therapies. In 3D models, prolonged EWD leads to complete, irreversible, conversion of Luminal colonies into Basal-like colonies; confirmed by gene expression profiling. The expansion of Luminobasal cell subpopulations in response to endocrine therapies is accompanied by upregulation of Notch1 receptors. As a result, gamma-secretase inhibitors (GSI) that block Notch signaling prevent the EWD-dependent Luminobasa cell outgrowth, maintaining tumor colonies in an ER+ Luminal state. This suggests a strategy to slow development of hormone resistance and ER loss in response to endocrine therapies by using antiestrogen/GSI combinations. Our data demonstrate an unexpected plasticity of Luminal breast cancers, and provide support for a common origin of Luminal and Basal-like disease. Nothing to Disclose: KBH

Pub #	S13-2
Session Information	SYMPOSIUM SESSION: BASIC - The Steroid Trifecta of Breast Cancer (3:45 PM - 5:15 PM)
Title	Estrogen Receptor Signaling & Actions in Breast Cancer & Endocrine Resistance: Interplay of Receptors, Protein Kinases & microRNAs
Author String	BS Katzenellenbogen University of Illinois at Urbana-Champaign, Urbana, IL
Body	Estrogens regulate the gene transcriptional and proliferative programs of hormone-responsive cancers and the mechanisms by which estrogen hormones act are multi-faceted, involving two estrogen receptors (ERα and ERβ) and their dynamic interplay, and nuclear-initiated and extranuclear-initiated signaling pathways. Hormonal regulation of breast cancer involves cooperation and linkages between estrogen receptors (ERs) and protein kinases. The relative inputs from these two pathways are thought to determine whether the cancer is responsive vs. resistant to endocrine therapies, with the up-regulation of kinases being a hallmark of resistance, a major limitation to the treatment of ER-positive breast cancers. Estrogen and protein kinase signaling are interdependent and even convergent, and we have found that estrogen-stimulated cell proliferation and gene regulations require ERα action through nuclear and extranuclear-initiated pathways involving extracellular signal-regulated kinase (ERK2). The ER and kinase-mediated signaling pathways show genome-wide convergence at the level of chromatin and contribute to the aggressive behavior of breast cancer and to endocrine resistance through regulation of gene expression, miRNA production, and changes in cell properties. Understanding the crucial interplay between ER and protein kinases in controlling cell regulatory programs should assist in guiding the development of novel therapeutic strategies for suppressing breast cancer progression and preventing or overcoming endocrine therapy resistance in breast cancer. Sources of Research Support: Grants from NIH, BCRF, and DOD. Nothing to Disclose: BSK

Pub #	S13-3
Session Information	SYMPOSIUM SESSION: BASIC - The Steroid Trifecta of Breast Cancer (3:45 PM - 5:15 PM)
Title	Androgen Action in Breast Cancer: A Contemporary Perspective
Author String	WD Tilley University of Adelaide/Hanson Institute, Adelaide, Australia
Body	Androgens act via the androgen receptor (AR) to regulate hormone sensitive tissues and AR is abundantly expressed in a range of human tissues, including the breast. Clinical observations of endocrine disorders historically established the inhibitory influence of androgens on normal breast growth, and this action was effectively employed to oppose breast tumor growth prior to the establishment of tamoxifen, and later aromatase inhibitors, as first line therapies for estrogen sensitive breast cancer. Our research has focused on the interplay been AR and estrogen receptor-α (ERα) signaling in breast tissues, working under the general hypothesis that the oppositional balance between these two steroid receptor signaling pathways dictates normal and malignant breast tissue growth, with androgen acting as a direct hormonal brake for the stimulatory actions of estrogen. We and others have provided evidence that AR signaling can directly interfer with ERα signaling in breast cancer cells and that low levels of AR are associated with decreased survival in women with ERα positive breast cancer. Our more recent genome-wide data shows that there is a distinct set of estrogen-regulated genes that are influenced by androgen in the presence but not in the absence of estrogen and ChIP-seq experiments show that androgen can inhibit recruitment of ERα to certain binding sites. In addition, androgen regulates a distinct set of growth inhibitory gene networks that are not directly influenced by estrogen signaling. Collectively, these data suggest that AR signaling inhibits breast tumor growth by estrogen dependent pathways that involve competitive inhibition of ERα binding to DNA, and estrogen independent pathways that regulate growth inhibitory networks. A current research interest is the role of AR signaling in a subset of ERα negative breast cancer with a molecular apocrine phenotype, where AR signaling may exert estrogen-like effects that promote growth. Hence, AR inhibition is being trialed in women with advanced AR positive, ERα negative breast cancer. In summary, direct androgen action is an important growth regulatory mechanism in normal and malignant breast tissues, with the potential for dichotomous roles in breast cancer depending on the ERα status of the tumor. These findings have profound implications for the future hormonal treatment of breast cancer, understanding of treatment failure and the development of novel prevention strategies. Additional authors: Butler LM, Hickey TE. Nothing to Disclose: WDT

Pub #	S14-1
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Endocrine-Disrupting Chemicals & Human Health (3:45 PM - 5:15 PM)
Title	Endocrine-Disrupting Chemicals as Obesogens: Reprogramming of Mesenchymal Stem Cells through PPAR-γ
Author String	B Blumberg University of California, Irvine, CA
Body	Nothing to Disclose: BB

Pub #	S14-2
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Endocrine-Disrupting Chemicals & Human Health (3:45 PM - 5:15 PM)
Title	Bisphenol A as a Risk Factor for Type 2 Diabetes
Author String	A Nadal Miguel Hernandez University, Elche, Spain
Body	Bisphenol-A (BPA) is one of the most widespread endocrine disrupting chemicals (EDCs) used as the base compound in the manufacture of polycarbonate plastics. In humans, epidemiological evidence has associated Bisphenol-A exposure in adults with higher risk of type-2 diabetes and heart disease (Lang et al, 2008). We studied the action of environmentally relevant doses of BPA on glucose metabolism in adults (Alonso-Magdalena et al, 2006; Alonso-Magdalena et al, 2008) as well as during pregnancy and the subsequent impact of BPA exposure on these females later in life. We also investigated the consequences of *in utero* exposure to BPA on metabolic parameters and pancreatic function in offspring (Alonso-Magdalena et al, 2010). Pregnant mice were treated with either vehicle or BPA (10 or 100 microg/kg/day) during days 9-16 of gestation. To directly assess the impact of BPA treatment, glucose metabolism experiments were performed on pregnant mice and their offspring. Exposure to BPA aggravated the insulin resistance normally associated with pregnancy and was associated with glucose tolerance, hyperinsulinemia, hypertriglyceridemia, and hyperleptinemia. Insulin-stimulated Akt phosphorylation was reduced in skeletal muscle and liver of BPA-treated pregnant mice. Additionally, BPA exposure during gestation had long-term consequences for mothers; four months post-partum, these female mice weighed significantly more than untreated control females and were hyperinsulinemic, hyperleptinemic, dyslipidemic and insulin resistant, risk factors for type-2 diabetes and cardiovascular diseases. The male offspring of these females presented glucose intolerance, insulin resistance and altered blood parameters at six months of age. The insulin signaling cascade was altered in liver and muscle. Moreover, the islets of Langerhans from male offspring exposed *in utero* to BPA presented altered Ca^{2+} signaling and glucose-stimulated insulin secretion. Lang et al. (2008) JAMA; 300(11):1303 Alonso-Magdalena et al. (2006) Environ Health Perspect; 114:106 Alonso-Magdalena et al. (2008) Plos One; 3(4):e2069 Alonso-Magdalena et al. (2010) Environ Health Perspect; 118:1243 Sources of Research Support: MICINN Grant BFU2008-01492 and Genrealitat Valenicana. Nothing to Disclose: AN

Pub #	S14-3
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Endocrine-Disrupting Chemicals & Human Health (3:45 PM - 5:15 PM)
Title	Endocrine Disruptors & Human Development & Reproductive Health: The Evidence & Its Limitations
Author String	RB Hauser Harvard School of Public Health, Boston, MA
Body	Although the production and widespread use of synthetic chemicals date back many decades, we have a limited understanding of the potential clinical and public health impact of these ubiquitous chemicals of modern life. Among the 80,000+ chemicals, a subset is hormonally active, defined as chemicals that alter endocrine signaling. These chemicals, classified as endocrine disruptors, may adversely effect development and reproductive function. Although there is evidence in experimental animals of adverse developmental and reproductive health effects from several classes of synthetic chemicals, the evidence in humans is inconclusive. This is primarily a result of the paucity of epidemiologic studies rather than evidence showing no association of synthetic chemicals with human health effects. Despite the limited evidence for human health effects, there is data showing widespread human exposure to multiple endocrine disruptors, including phthalates, bisphenol A, dioxins and polychlorinated biphenyls. Data from human studies on developmental and reproductive health effects of these classes of chemicals will be presented. Specific endpoints of interest include male reproductive tract development, childhood growth and puberty, and adult reproductive function. The presentation will also discuss limitations of traditional epidemiologic study designs and methods, including the assessment of exposure at the appropriate etiologic window and defining sensitive relevant health endpoints. Nothing to Disclose: RBH

Pub #	S15-1
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Fat on Fire (3:45 PM - 5:15 PM)
Title	Obesity, Inflammation & Macrophages
Author String	AW Ferrante Columbia University, New York, NY
Body	Nothing to Disclose: AWF

Pub #	S15-2
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Fat on Fire (3:45 PM - 5:15 PM)
Title	Anti-Inflammatories in Type 2 Diabetes: Clinical Trial
Author String	AB Goldfine Joslin Diabetes Center, Boston, MA
Body	Nothing to Disclose: ABG

Pub #	S15-3
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Fat on Fire (3:45 PM - 5:15 PM)
Title	Oral Delivery of siRNA to Macrophages Targeting Inflammation
Author String	MP Czech University of Massachusetts Medical School, Worcester, MA
Body	Infiltration of macrophages and other immune cells into adipose tissue in animal models of obesity increases expression and secretion of many factors, including some inflammatory cytokines, which could potentially regulate lipogenesis and sequestration of fat in adipocytes. Work by many laboratories comparing lean versus obese human subjects has also revealed increased macrophage infiltration into omental adipose tissue in obesity. Recently our laboratory reported that omental adipose tissue was more highly infiltrated with macrophages in obese, insulin resistant subjects, even when matched to equally obese human subjects that were insulin sensitive. The data are consistent with the hypothesis that in human obesity adipose function may be altered in response to factors expressed on the surfaces of infiltrated macrophages or secreted by these cells. Based upon the above considerations, we have initiated strategies to target macrophages with siRNA to suppress expression of genes that promote adipose dysfunction and metabolic disease. We have silenced TNF-α as well as other genes that promote inflammation using siRNA encapsulated within hollow shells of β, 1-3 D-glucan prepared from yeast cell walls (denoted GeRPs, for Glucan-encapsulated RNAi particles). Both oral and intraperitoneal administration routes have been tested in mice with GeRPs loaded with siRNA anchored between cationic (polyethyleneimine) and anionic (tRNA and siRNA) layers that also contain an amphipathic peptide. We have now developed simplified versions of these formulations with fewer components in order to enhance reproducibility of manufacture, increase release kinetics of the siRNA and increase potency. The newly developed GeRPs have achieved significant gene silencing in mice targeting Map4k4, a protein kinase that controls inflammation in marcrophages, as well as the cell surface protein F4/8(which can be analyzed by FACS. The simplified GeRP system contains glucan shells loaded in a step-wise procedure under various pH conditions with only an amphipathic peptide, Endo-porter, and siRNA. Current experiments are devoted to testing whether gene silencing in macrophages in vivo by these simplified GeRP formulations can alleviate insulin resistance in obese mouse models and in other animal models where pathogenesis is known to be promoted by inflammation. Nothing to Disclose: MPC

Pub #	S16-1
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Molecular Mechanisms Involved in Male Fertility (3:45 PM - 5:15 PM)
Title	Tales of Fertility in Men: Modeling Meiosis through Post-Translational Modifications
Author String	PL Morris Population Council, New York, NY
Body	Underlying etiologies of spermatogenic maturation arrest (MA) in men are largely unknown--a pathology that reflects premature termination of germ cell development at either early stages (early arrest) or during spermiogenesis (late arrest). Errors in meiosis result in early arrest during chromosome synapsis or divisions or late arrest including progression failure. Meiotic prophase processes appear vulnerable to errors leading to arrest, i.e. homologous chromosome pairing, protein scaffold formation between paired chromosomes (synapsis) and exchange of DNA (recombination). Mutational studies in diverse model organisms show that gene mutations related to these events can present as MA phenotypes. Specific gene deletions or faulty protein interactions can interfere with meiosis resulting in sub- or infertility. Studies in yeast and animals have uncovered temporally important posttranslational (PT) events involved in meiotic prophase completion. Our studies on testis of men with normal spermatogenesis show a linear structure of the Small Ubiquitin Modifier (SUMO) SUMO1 coincident with the synaptonemal complex (SC) and SUMOylation of SC proteins 1 and 2. SUMOylation is involved in essential cell processes associated with protein stability and interactions, transcriptional regulation, nuclear-cytoplasmic transport, heterochromatin modification and DNA repair. Our studies show that SUMO proteins 1, 2 and 3 are present in human testis. SUMOs covalently bind to a lysine residue on a target molecule after activation by specific factors and conjugating enzymes and studies show expression of these SUMOylation pathway components in rodent testis. In men with normal spermatogenesis, we find SUMO2/3 as early as leptotene-zygotene; SUMO1 is first clearly identified at pachytene. In human germ cells, SUMO2/3's kinetochore association indicates a role during spindle attachment, chromosome alignment or movement, consistent with a developmental role in essential scaffold protein complexes and synapsis. In contrast, germ cells of some men with MA show abnormal PT modifications by SUMO and ubiquitin. Using modeling as diverse as human and fly, our data suggest SUMO may participate in meiosis onset, duration and progression. Given that fidelity and chromatin structure rearrangements are essential for DNA repair, chromatin structure, gene expression and chromosome segregation, SUMO PT errors may lead to DNA damage, chromosomal breakage and translocation in the male germ line. Nothing to Disclose: PLM

Pub #	S16-2
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Molecular Mechanisms Involved in Male Fertility (3:45 PM - 5:15 PM)
Title	Peritubular Cells of the Human Testis: Emerging Roles in Health & Disease
Author String	A Mayerhofer University of Muenchen, Munich, Germany

Body

The wall of the seminiferous tubules in the adult human testis consists of several layers of slender, smooth-muscle-like, peritubular cells and extracellular matrix (ECM). This normally inconspicuous compartment is il explored, but studies in rodents indicated that peritubular cells produce paracrine factors and that their contractile abilities are necessary to transport immotile sperm. In infertile men with impaired spermatogenesis this compartment typically is remodeled, implying fundamentally altered functions of the peritubular wall. In addition, mast cells accumulate. To be able to study human peritubular cells in health and disease, we established a culture method. It is based on small biopsies of patients with obstructive azoospermia but norma spermatogenesis (human testicular peritubular cells, HTPCs) and non-obstructive azoospermia, impaired spermatogenesis and testicular fibrosis (HTPCFs). Studies performed provide insights into the repertoire of secretion products, plasticity and contractile properties of these human testicular cells and their regulation by mast cell products (1-4). For example, GDNF was identified to be constitutively released by HTPC/Fs. Peritubular cells, in concert with Sertoli cells, may thus influence neighboring spermatogonial stem cells, which express receptors for GDNF. Other factors identified include IL-6 and MCP-1, which are stimulated by a mast cells product, TNFalpha. Thus peritubular cells participate in the inflammation-like events often associated with male infertility. The enzymatic activity of the major mast cell product tryptase may cleave secreted factors (e.g. proNGF/NGF), thus creating a complex microenvironment. HTPCFs provide a window into the infertile human testis. Like their counterparts in vivo they produce higher levels of the proteoglycan decorin, which has structural functions in the ECM and, importantly, interferes with growth factor receptors and their signaling. This may be an unexplored aspect of male infertility. Finally, human peritubular cells are contractile cells and HTPC/Fs retain this ability. Insights into the regulation and the loss of contractility, which is associated with impaired spermatogenesis in men, are being obtained. Thus, peritubular cells (and mast cells) are emerging as important cells of the human testis in health and disease, namely male infertility.

(1) Albrecht M et al., J Clin Endo Metabol 2006;91:1956
(2) Schell C et al., Endocrinology 2008;149:1678
(3) Schell C et al., Endocrinology 2010;151:1257
(4) Spinnler K et al., Hum Reprod 2010;25:2181

Sources of Research Support: DFG MA 1080/16-3; DAAD.

Nothing to Disclose: AM

Pub #	S16-3
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Molecular Mechanisms Involved in Male Fertility (3:45 PM - 5:15 PM)
Title	DNA Repair Deficiencies in Azoospermic Men
Author String	DJ Lamb Baylor College of Medicine, Houston, TX
Body	This project focuses on the molecular basis of non-obstructive azoospermia, a severe spermatogenic defect that causes male infertility. A diagnosis of non-obstructive azoospermia can destroy a couple's life plan of becoming parents. While some patients remain hopelessly infertile, rare foci of spermatogenesis may be present in an otherwise atrophic seminiferous tubule or with incomplete meiotic arrest. For these infertile men rare sperm can be surgically extracted from the testis, used to achieve fertilization using intracytoplamsic sperm injection (ICSI) and a pregnancy. Bypassing nature's barriers to fertilization by defective gametes is important because today researchers believe that the majority of male infertility results from genetic defects; although with few exceptions, methods to diagnose these patients are lacking. Our studies focus on DNA repair defects in men with nonobstructive azoospermia and build on the intriguing data obtained to date showing the presence of simultaneous deficiencies of mismatch repair proteins, as well as in other proteins in the DNA repair pathways (including those required for homologous recombination) in men with non-obstructive azoospermia. Functional deficiencies in DNA repair protein presence, localization and expression are observed both in testicular and somatic cells. The men with non-obstructive azoospermia display an abnormal growth response to akylating agent DNA damage, similar to that seen in Ataxia telangiectasia and other DNA repair protein deficiencies. These studies define a common cause of non-obstructive azoospermia and should provide insights into the non-reproductive related health risks that may be associated with this type of male infertility. Sources of Research Support: Eunice Kennedy Shriver National Institute of Child Health and Human Development Grant P01HD36289. Nothing to Disclose: DJL

Pub #	S17-1
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Who Wakes the Bugler? Mechanisms of Pubertal Onset (3:45 PM - 5:15 PM)
Title	Genetic Variation & Timing of Puberty
Author String	KK Ong Medical Research Council Epidemiology Unit, Cambridge, UK
Body	Age at menarche varies widely between girls, is highly heritable, but also highly dependent on nutritional status. While rare deleterious mutations are increasingly reported in genes of the hypothalamic-gonadal-sex hormone pathway, those are too infrequent to explain the normal variation in pubertal timing. Recent genome wide association (GWA) studies that genotype hundreds of thousands of single nucleotide polymorphisms (SNPs) located across the entire genome have identified many common genetic variants that are robustly associated with menarche timing. In 2009 four separate GWA studies identified common variants in the gene *LIN28B* on chromosome 6 as having 'genome-wide significant' ($P<5[\text{times}]10^{-8}$) associations with menarche timing. Effect sizes on menarche timing were ~6 weeks per allele, consistent associations were also seen with other puberty traits in both girls and boys, and a mouse model has since confirmed its biological role. *LIN28B* is homologous to the ancestral heterochromic gene *lin-28* in *Caenorhabditis elegans* and these findings point to a fundamental microRNA processing system that controls the tempo of both cellular and somatic development. In 2010 the large-scale [ldquo]REPROGEN[rdquo] collaboration, comprising the four original menarche GWA studies and also several others, identified a total of 32 loci for menarche timing. Notably, several menarche loci are also loci for adult body mass index, and others are implicated in energy homeostasis (*BSX*, *CRTC1* and *MCHR2*) and hormone regulation (*INHBA*, *PCSK2* and *RXRG*). Pathway analyses identified coenzyme A and fatty acid biosynthesis as biological processes related to menarche timing. In summary, GWA studies for age at menarche demonstrate how the genome-wide approach can help identify new mechanisms in pubertal timing and in particular how nutritional status might signal on the hypothalamic-gonadal pathway. Nothing to Disclose: KKO

Pub #	S17-2
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Who Wakes the Bugler? Mechanisms of Pubertal Onset (3:45 PM - 5:15 PM)
Title	Metabolic Control of Puberty Onset: New Players, New Mechanisms
Author String	VM Navarro University of Washington, Seattle, WA
Body	Puberty is a tightly regulated process by which an individual attains the ability to reproduce. An intricate network of central and peripheral factors, including metabolic cues, has been shown to play a role in this process; however, the mechanism triggering puberty onset remains largely unknown. Recent evidence suggests that the neuropeptides kisspeptin (encoded by Kiss1) and neurokinin B (NKB; encoded by TAC3 in humans and Tac2 in rodents) are essential gatekeepers of puberty onset. Studies in humans and rodents have shown that loss-of-function mutations in either KISS1 or TAC3, or their receptors, KISS1R and TACR3, respectively, lead to the absence of sexual maturation and infertility. As a result, reproductive neuroendocrinologists have begun to study the neurons that express these genes, attempting to discover how they interact and how they are regulated. We and others have shown that kisspeptin, NKB and dynorphin A (Pdyn) are co-expressed in neurons of the arcuate nucleus, so called KNDy neurons. Importantly, these neurons also co-express the NKB receptor, Tacr3. Kisspeptin stimulates the release of gonadotropin-relasing hormone (GnRH) by acting through Kiss1R expressed in GnRH neurons. Furthermore, NKB has been shown to stimulate LH release, presumably by acting directly on KNDy neurons to induce kisspeptin-mediated GnRH secretion. Additionally, we have shown that Tac2 and Tacr3 mRNA levels are increased at the time of puberty onset in the rat, and are inhibited by caloric restriction. Based on these observations, along with compelling evidence suggesting that GnRH pulses are associated with kisspeptin pulses, we have proposed a model whereby KNDy neurons drive pulsatile GnRH secretion. According to this model, the stimulatory action of NKB, coordinated with the delayed inhibitory action of dynorphin, generates the pulsatile release of kisspeptin from KNDy neurons by a mechanism that is dependent on sex steroid levels. Understanding how this pulse generator is activated during puberty and remains functional in adulthood is the goal of recent research in reproductive neuroendocrinology. Nothing to Disclose: VMN

Pub #	S17-3
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Who Wakes the Bugler? Mechanisms of Pubertal Onset (3:45 PM - 5:15 PM)
Title	Epigenetic Mechanisms Involved in the Control of the Onset of Puberty
Author String	SR Ojeda Oregon National Primate Research Center, Beaverton, OR
Body	The onset of puberty requires many genes, which play different roles along a developmental cascade leading to the pubertal increase in GnRH release. It is unclear, however, how inherited, permanent changes in DNA sequence can dynamically coordinate the expression of gene sets controlling the pubertal process. Epigenetics is able to do this. Here we propose that an epigenetic mechanism of *transcriptional repression* plays a significant role in timing the initiation of mammalian puberty. Using Illumina and Affymetrix arrays, Nimblegen genome-wide DNA methylation analyses, and quantitative PCR, we identified genes of the Polycomb group (PcG) of transcriptional silencers as major contributors to this repressive mechanism. Expression of two key PcG genes, *Cbx7* and *Eed*, decreases in the hypothalamus preceding the onset of puberty, and this decrease is accompanied by increased methylation of their promoters. In vivo inhibition of *de novo* DNA methylation with 5'-azacytidine (5-Aza), restored PcG expression, and resulted in pubertal failure (as assessed by the lack of ovulation). The treatment did not affect the ovarian estradiol response to gonadotropins, but it prevented the LH response to ovariectomy, suggesting that puberty is delayed due to a central, instead of an ovarian defect. Both the LH response to GnRH and the GnRH response to kisspeptin were normal, indicating that the defect is upstream of the GnRH network. ChIP assays showed that PcG proteins interact with the *Kiss1* promoter, and that puberty is accompanied by *Kiss1* promoter demethyation, association of activating histones (H3K9,14ac and H3K4me3), and loss of H3K27me3, a repressive histone form catalyzed by PcG proteins. Preventing the peripubertal decrease in hypothalamic Cbx7/Eed by microinjecting Cbx7 or Eed-producing lentiviruses into the arcuate nucleus of prepubertal rats resulted in association of these proteins to the *Kiss1* promoter, a striking delay in puberty, and disruption of estrous cyclicity. Some animals failed to become pregnant when exposed to a fertile male. These results suggest that an epigenetic mechanism of *transcriptional repression* plays a significant role in timing the initiation of mammalian puberty, and that the PcG group of transcriptional silencers is a major contributor to this repressive mechanism. PcG proteins appear to target downstream genes involved in the stimulatory control of GnRH secretion at puberty, such as the *Kiss1* gene. Authors and affiliations: Alejandro Lomniczi1, Alberto Loche1, Gerd Pfeifer2 and Sergio R Ojeda1 1Div. Neuroscience, Oregon National Primate Research Center, Beaverton, OR 97006; and 2City of Hope, Duarte, CA Sources of Research Support: NIH grants HD25123, U54-18185, RR000163. Nothing to Disclose: SRO

Pub #	S18-1
Session Information	SYMPOSIUM SESSION: CLINICAL - Aldosterone Excess: Technology, Strategies & Outcomes (3:45 PM - 5:15 PM)
Title	Adrenal Vein Sampling: State of the Art
Author String	T Nishikawa Yokohama Rosai Hospital, Yokohama City, Japan
Body	Objective: Primary aldosteronism (PA) due to unilateral hyperaldosteronemia, such as aldosterone-producing adenoma (APA) is a surgically curable secondary hypertension, and treated by unilateral total adrenalectomy. Adrenal vein sampling (AVS) is performed in patients who have been diagnosed with PA based on confirmation tests and wish to be treated surgically if there are indications. However, AVS can only differentiate the unilateral from the bilateral adrenal lesions. Thus, we had recently developed a new method of supper-selective ACTH-stimulated AVS (SS-ACTH-AVS) for detecting tiny adrenal masses. Moreover, w tried to remove partially the local lesions causing hyperaldosteronism detected by SS-ACTH-AVS. Methods: We performed SS-ACTH-AVS in 65 patients with PA by using a 2.2 Fr. strip-tip type microcatheter (Koshin Medical Inc. Japan). Adrenal effluents were sampled at central vein and tributary veins of each adrenal gland after ACTH stimulation. Hypersecretion of aldosterone was diagnosed when concentration of aldosterone was more than 1400ng/dl in effluents of any central vein(s) and tributary vein(s). Results: In patients with idiopathic hyperaldosteronism (IHA), plasma aldosterone concentration (PAC) in effluents sampled at the central and all tributaries veins in each adrenal were more than 1400ng/dl. On the other hand, PAC in effluents sampled at the central veins and one of all tributary veins in each adrenal were more than 1400ng/dl in patients with Blt-APAs. SS-ACTH-AVS revealed that there were 36 patients with unilateral APA, 4 with Blt-APAs, and 25 with IHA. The prevalence of Blt-APAs is 6% among 65 cases with PA, and 14% among 29 cases of bilateral hyperaldosteronism including IHA and Blt-APAs, diagnosed by SS-ACTH-AVS, of which procedure is almost safe. Conclusion; The present data clearly demonstrated that SS-ACTH-AVS could precisely detect the portion of localized or diffuse adrenal lesions inducing hyperaldosteronism. The present study also demonstrated that SS-ACTH-AVS clearly differentiates Blt-APAs from IHA. It is suggested that the prevalence of unilateral APA and Blt- APAs is much greater than that of IHA, and surgical indication for PA patients seems to be much higher than that previously expected. SS-ACTH-AVS is quite new method for detecting and localizing tiny adrenal lesions, and also giving us an important information how to treat the PA patients, such as partial adrenalectomy. Nishikawa T et al.,Jpn Clinic Med 2010;1:21 Sources of Research Support: Grant-in-Aid for Scientific Research of Adrenal Disorders from the Ministry of Public Health and Labor, Japan. Nothing to Disclose: TN

Pub #	S18-2
Session Information	SYMPOSIUM SESSION: CLINICAL - Aldosterone Excess: Technology, Strategies & Outcomes (3:45 PM - 5:15 PM)
Title	Workup of Primary Aldosteronism: Punt or Go Long?
Author String	WF Young Mayo Clinic, Rochester, MN
Body	The title for this presentation was provided by the Annual Meeting Steering Committee. [ldquo]Punt or go long[rdquo] is an American football phrase--when it is fourth down and the team has many yards to gain to get a first down, they can either kick the ball ([ldquo]punt[rdquo]) to the other team 40 or more yards down the field or they can gamble to gain the yardage ([ldquo]go long[rdquo]). If the team does not gain the needed yardage on fourth down it is considered a major failure, as the opposing team gets the ball at that site and not 40 yards down the field. The analogy for primary aldosteronism (PA) is in the patient with confirmed PA, whether to pursue subtype evaluation ([ldquo]go long[rdquo]) leading to possible surgery or whether to [ldquo]punt[rdquo] and forego subtype evaluation and proceed with medical management. Some of the factor that should impact this disease management decision include: patient preferences, patient age, surgical candidacy, availability of adrenal venous sampling (AVS), and probability of unilateral adrenal disease. It is important to note that medical management is not truly a [ldquo]punt.[rdquo] Medical management with a mineralocorticoid antagonist may be the best treatment approach in patients who decline surgery, are elderly, are not good surgical candidates, or are unlikely to have unilateral adrenal disease. In general, patients with aldosterone-producing adenomas (APA) have more severe hypertension, more frequent hypokalemia, higher plasma (>694 pmol/L; >25 ng/dL) and urinary (>83 nmol/d; >30 mcg/24-hour) levels of aldosterone, and are younger (<50 years) than those with bilateral idiopathic hyperaldosteronism. With that said, when these weak predictors of APA are absent in the patient with confirmed PA, subtype evaluation with computed tomography and possible AVS should be pursued when the patient desires a surgical resolution to PA. Nothing to Disclose: WFY

Pub #	S18-3
Session Information	SYMPOSIUM SESSION: CLINICAL - Aldosterone Excess: Technology, Strategies & Outcomes (3:45 PM - 5:15 PM)
Title	Benefits of Mineralocorticoid Antagonist Therapy
Author String	F Mantero University of Padova, Padova, Italy
Body	Recommended treatment in unilateral PA (APA or UHA) is laparoscopic adrenalectomy, while medical treatment with mineralocorticoid receptor antagonists (MRA) is indicated in patients with bilateral adrenal disease (idiopathic adrenal hyperplasia, bilateral APA, GRA) or those unable or unwilling to undergo surgery. MRA appear to be effective at controlling blood pressure and to provide blood pressure-independent target organ protection. The most used drugs are Spironolactone, K Canreonate or Canrenone, Eplerenone, Amiloride. The efficacy of spironolactone in the long-term treatment of PA was demonstrated more than 25 years ago. Eplerenone appears to be as effective as spironolactone in the management of PA, with the potential for fewer side effects, but is currently more expensive than spironolactone, and not available in many Countries. Several studies have shown the benefits of MRA treatment in PA patients. In a study from our and Rossi groups, in which we compared the efficacy of surgical versus MRA treatment in 151 PA patients, we demonstrated that in both there were a significant reduction of blood pressure and normalization of potassium levels, although more drugs were required to achieve a similar fall of BP in the patients treated with MRA.A significant reduction of LVMI, greater after 1year in adrenalectomized patients, subsequently similar in the two groups, was observed. Prevalence of hypertrophy decreased in both treated groups. MR antagonists have been beneficial in proteinuric patients with diabetic nephropathy or chronic kidney disease. Microalbuminuria is more frequent in PA than in essential Hypertension (EH). Analysis of renal outcomes in patients with PA after adrenalectomy or spironolactone therapy did not reveal significant difference and changes of glomerular filtration in patients with PA and EH were comparable. A recent study analyzed the effect of specific treatment of PA on common carotid IMT which is increased in PA patients. They demonstrated that MRA did not lead to significant decrease of common carotid IMT compared to surgical treatment. New non-steroidal mineralocorticoid receptor antagonists are being developed and will be available soon. In fact a number of dihydropyridine calcium channel blockers also have mineralocorticoid antagonist activity at high doses. Another treatment of PA are aldosterone-synthase inhibitors that are currently undergoing phase II clinical trials. Nothing to Disclose: FM

Pub #	S19-1
Session Information	SYMPOSIUM SESSION: CLINICAL - Genetics of Osteoporosis (3:45 PM - 5:15 PM)
Title	Genetic Explorations of Animal Models for Osteoporosis
Author String	CJ Rosen Maine Medical Center Research Institute, Scarborough, ME
Body	Genetic determinants influence peak bone mass and bone loss in mammals. Animal models of osteoporosis are numerous, but few can provide the kind of information required to map genes that influence bone acquisition and maintenance. Rodents are the most frequently used model for genetic exploration although spontaneous fractures are rare. On the other hand, phenotyping for bone mass has been refined dramatically such that both cortical and trabecular compartments can be quantitated, and mineralization assessed. Clearly, the mouse remains the most feasible model for identifying quantitative trait loci that influence bone mass inpart because of the large number of inbred strains with tremendous heterogeneity in their bone density. Although mouse genetics preceded large genome wide association studies, progress has been slow and few genes have been identified that contribute to peak bone mass. Moreover, there appear to be a vast array of genes that interact (i.e. epistasis)contributing to the complex trait of bone mass. Notwithstanding, there are opportunities to study not just the genes of interest but more importantly genetic by environmental or genetic by pharmacologic intervention. Here progress has been substantial, in part because of controlled environmental conditions and the ease of life long phenotyping. Several examples of successful mining of genes that are strongly influenced by environment will be presented and then discussed in light of human GWAS studies. Session supported by: Lilly USA, LLC Nothing to Disclose: CJR

Pub #	S19-2
Session Information	SYMPOSIUM SESSION: CLINICAL - Genetics of Osteoporosis (3:45 PM - 5:15 PM)
Title	Candidate Genes in Human Osteoporosis
Author String	SL Ferrari Geneva University Hospital (HUG), Geneva, Switzerland
Body	Additive genetic effects explain up to 80% of the variance in bone mineral density (BMD) and 50% of the susceptibility to fractures. The heritability of other constituents of bone strength /fragility, such as bone size, geometry and microstructure of cortical and trabecular bone, also ranges from 50 to 80%. Numerous genome-wide association studies (GWAS) have identified dozens of genes associated with BMD and/or fracture at genome-wide significance levels ($p<10-8$). Among them, the estrogen receptor alpha (ESR1), the RANKL/RANK/OPG pathway, as well as the LDL receptor-related protein 5 (LRP5) and other constituents o the Wnt-LRPx-beta-catenin signaling pathway, including sclerostin (SOST), have been consistently identified as candidate genes of human osteoporosis. These findings are in line with the key role of these signaling pathways in the regulation of bone resorption, respectively bone formation, and the severe skeletal phenotype (congenital osteoporosis, osteosclerosis and osteopetrosis) caused by mutations in these genes. GWAS have also identified numerous genes associated with BMD but which function in the regulation of bone mass and turnover currently remains unclear. However GWAS have failed to confirm the contribution of more than hundred candidate genes previously associated with BMD and/or fractures in smaller but perhaps more homogeneous cohorts, including the vitamin D receptor (VDR) and collagen 1 alpha 1 chain (Col1A1) genes. This observation, taken together with the rather small contribution of ascertained candidate genes to osteoporosis (i.e. less than 5% of BMD variance explained), raises questions about the pertinence of common SNPs used in GWAS vs rare and functional variants (eSNPs), and points towards gene-environment and gene gene interactions. For instance, the association of functional IL-6 promoter alleles with BMD has not been confirmed by GWAS so far, but this approach ignored the previously demonstrated interaction with sex and estrogen. Functional genomics (or system genetics) combining GWAS and gene expression data in humans, mice and cell cultures has started to identify novel gene polymorphisms which are more directly causal to osteoporosis. Hopefully the combination of GWAS into an even larger cohort (up to 100'000 subjects), the inclusion of environmental factors, and system genetics approaches will allow to uncover the many candidate genes that could explain the missing heritability of osteoporosis. Session supported by: Lilly USA, LLC Nothing to Disclose: SLF

Pub #	S19-3
Session Information	SYMPOSIUM SESSION: CLINICAL - Genetics of Osteoporosis (3:45 PM - 5:15 PM)
Title	Genetics of Osteoporosis: Applications for Clinical Management
Author String	NB Watts University of Cincinnati, Cincinnati, OH
Body	Osteoporosis is a polygenic disease. Rare genetic defects (RANK/RANKL/OPG, Wnt/DKK/LRP5-6/sclerostin) have provided new insights into the regulation of bone remodeling and pathophysiology involved in skeletal acquisition, bone loss and skeletal fragility and resulted in new treatments in the pharmacy and on the horizon. About 80% of the human genome is involved with structural variations, with differences in about 6 million SNPs (single nucleotide polymorphisms) between any two individuals. Obvious areas of difference are sex and race. A number of candidate genes for bone health and skeletal fragility have been explored, including genes for the vitamin D receptor, estrogen receptor and type 1 collagen. It is likely that specific genetic associations will be helpful to evaluate susceptibility to the disease, its severity and its progression. With proper markers, prevention strategies, based on personal genomics, might be offered early; for example,to susceptible adolescents and young adults, with hopes of reducing disease burden. Genomics could permit effective targeting of existing medications to patients whose genes point to best balance of benefits and risks. Knowledge of gene associations might decrease the cost of developing new treatment options by reducing the number of subjects required in clinical trials. It has only been eight years since sequencing of the human genome, and there are still many barriers to be overcome to develop practical applications. Session supported by: Lilly USA, LLC Nothing to Disclose: NBW

Pub #	S20-1
Session Information	SYMPOSIUM SESSION: CLINICAL - The Difficulties of Weight Loss Maintenance (3:45 PM - 5:15 PM)
Title	Why Is It so Hard To Keep Weight Off?
Author String	M Rosenbaum Columbia University College of Physicians & Surgeons, New York, NY
Body	The issue of whether there is a metabolic opposition to sustained weight reduction is critical to understanding the biological basis for the very high recidivism to obesity seen following otherwise successful weight loss and has multiple practical implications in the treatment of obesity. Classical studies of energy homeostasis in animals following hypothalamic lesions, weight perturbation, selective breeding, and during hibernation - plus more recent demonstrations of interactions between peripheral signals reflecting somatic energy stores with cellular-molecular substrates in the CNS regulating energy balance - all support the contention that body weight is regulated. Our studies of the bioenergetic responses of humans to sustained maintenance of experimentally altered body weight indicate that energy stores (fat) are asymmetrically regulated such that there is greater opposition to a sustained reduction than to sustained increase. This makes evolutionary sense in terms of the critical roles of fat in reproductive efficiency and survival in hostile environments. In rigorous in-patient studies of obese and non-obese humans we have found that attempts to weight loss are accompanied by changes in energy homeostatic systems that [ldquo]conspire[rdquo] to favor the regain of los weight. Individuals maintaining a 10% or greater reduced weight demonstrate an over 20% reduction in energy expenditure (due primarily to increased chemomechanical efficiency of skeletal muscle), as well as changes in neuroendocrine function (decreased circulating concentrations of bioactive thyroid hormones), autonomic function (decreased sympathetic nervous system tone and increased parasympathetic nervous system tone), that are further supported by changes in energy intake that also favor weight regain (decreased satiety). These broad changes in energy homeostatic systems following weight loss are mediated by hypothalamic and higher CNS responses, and conspire together to favor the regain of lost weight. Changes in circulating leptin concentrations, in response to reduced body fat, play a major role in these responses. The ability to manipulate this biology in service of helping to sustain weight loss could provide an important therapeutic resource. Keesey RE, Powley TL. Appetite 2009;51(3):442 Leibel RL. Int J Obes 2008;32 Suppl 7:S98 Rosenbaum M, et al. Am J Clin Nutr 2008;88:906 Rosenbaum M, et al, Brain Res, 2010; 1350:95 Rosenbaum M, Leibel, RL. Int. J. Obesity, 2010, 34: S47 Nothing to Disclose: MR

Pub #	S20-2
Session Information	SYMPOSIUM SESSION: CLINICAL - The Difficulties of Weight Loss Maintenance (3:45 PM - 5:15 PM)
Title	Fighting Weight Regain: The Role of Exercise
Author String	HJ Teede Monash University, Clayton, Australia
Body	During times of significant change such as the industrial revolution and now the information technology revolution, significant economic and societal changes occur. Whilst these changes often bring greater affluence and improved health overall, adverse health impacts are common whilst societies adjust to change. One of the key impacts of current social trends is the dramatic reduction in physical activity across the lifespan. Escalating weight globally has seen a rise in the proportion of the population overweight or obese. Obesity is now a major contributor to chronic disease and initiatives to halt progression have generally had very limited success. Despite clear benefits of even modest weight loss, short term interventions are commonly unsustainable and weight regain occurs in the vast majority of individuals who do achieve weight loss through lifestyle modification. Inadequate physical activity is a primary contributor to excess weight and the resultant burden of chronic disease. Increasingly, attaining and maintaining adequate physical activity is a challenge across the lifespan. Physical activity is recommended to prevent weight gain, to assist with weight loss and to prevent weight regain after loss. Surprisingly, there is a lack of high quality research on the type, intensities and duration of physical activity, but an even greater gap in the literature on how to successfully drive sustained behavior change and maximize adoption of greater physical activity at an individual, community and population level. This presentation will focus on: 1) Current physical activity recommendations, including for prevention of weight regain; 2) The role of behavior changes versus education and advice alone; 3) Identifying key enablers and barriers to behavior change; 4) Approaches to increasing physical activity at an individual, community and population level A greater focus on optimizing physical activity across the lifespan is vital and will require a public health approach including regulation, incentives and education to address current challenges. Nothing to Disclose: HJT

Pub #	S20-3
Session Information	SYMPOSIUM SESSION: CLINICAL - The Difficulties of Weight Loss Maintenance (3:45 PM - 5:15 PM)
Title	Strategies To Prevent Weight Regain: Lessons Learned from Successful Losers
Author String	HR Wyatt University of Colorado, Denver, CO
Body	Disclosures: HRW: Owner, Active planet; Principal Investigator, Amylin Pharmaceuticals; Advisory Group Member, Wellspring, Allergan, Arena; Editor, Up To Date.

Pub #	L2-1
Session Information	PLENARY: TRANSLATIONAL - Endocrine-Disrupting Contaminants & the Developing Reproductive System: The Wildlife to Human Connection (5:30 PM - 6:30 PM)
Title	Endocrine Disrupting Contaminants & the Developing Reproductive System: The Wildlife to Human Connection
Author String	LJ Guillette Medical University of South Carolina, Charleston, SC
Body	Nothing to Disclose: LJG

Pub #	L2-2
Session Information	PLENARY: TRANSLATIONAL - Sex Chromosome Evolution & Medicine (5:30 PM - 6:30 PM)
Title	Sex Chromosome Evolution & Medicine
Author String	DC Page Massachusetts Institute of Technology/Howard Hughes Medical Institute, Cambridge, MA
Body	In recent years, genomic analysis has revealed that the human X and Y chromosomes evolved from a pair of chromosomes - autosomes - that were identical in males and females of our reptilian ancestors. I will describe how these evolutionary and genomic perspectives provide insights into the roles of the X and Y chromosomes in health and disease, including sex differentiation, male infertility, and Turner syndrome. I will also discuss prominent predictions of the human Y chromosome's imminent demise. Nothing to Disclose: DCP

Pub #	M7
Session Information	MEET-THE-PROFESSOR: CLINICAL - Evaluation of the Transition Patient for Growth Hormone Replacement (5:30 PM - 6:15 PM)
Title	Evaluation of the Transition Patient for Growth Hormone Replacement
Author String	G Johannsson Sahlgrenska University Hospital, Gothenburg, Sweden
Body	Disclosures: GJ: Speaker, Eli Lilly & Company, MerckSerono; Clinical Researcher, Novo Nordisk, Pfizer, Inc.; Advisory Group Member, Otsuka; Chief Scientific Officer, DuoCort Pharma.

Pub #	M9
Session Information	MEET-THE-PROFESSOR: CLINICAL - Metabolic Management of Polycystic Ovary Syndrome (5:30 PM - 6:15 PM)
Title	Metabolic Management of Polycystic Ovary Syndrome
Author String	DA Ehrmann University of Chicago, Chicago, IL
Body	Nothing to Disclose: DAE

Pub #	CMF1-1
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Inpatient Management of Blood Glucose Levels (5:30 PM - 6:15 PM)
Title	Inpatient Management of Blood Glucose Levels
Author String	GE Umpierrez Emory University School of Medicine, Atlanta, GA
Body	Session supported by: Lilly USA, LLC Disclosures: GEU: Researcher, Sanofi-Aventis, Takeda.

Pub #	CMF1-2
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Inpatient Management of Blood Glucose Levels (5:30 PM - 6:15 PM)
Title	Inpatient Management of Blood Glucose Levels
Author String	JL Leahy University of Vermont College of Medicine, Colchester, VT
Body	Session supported by: Lilly USA, LLC Disclosures: JLL: Advisory Group Member, Bristol-Myers Squibb, Merck & Co., Novo Nordisk, Sanofi-Aventis; Research Funding, Takeda.

Pub #	CMF2-1
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Management of Apparent Secondary Hypogonadism due to Obesity, Opiates & Anabolic Steroids (5:30 PM - 6:15 PM)
Title	Management of Apparent Secondary Hypogonadism Due to Obesity, Opiates & Anabolic Steroids
Author String	FC Wu Manchester Royal Infirmary, Manchester, UK
Body	Disclosures: FCW: Consultant, Bayer Schering Pharma, Eli Lilly & Company.

Pub #	CMF2-2
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Management of Apparent Secondary Hypogonadism due to Obesity, Opiates & Anabolic Steroids (5:30 PM - 6:15 PM)
Title	Management of Apparent Secondary Hypogonadism Due to Obesity, Opiates & Anabolic Steroids
Author String	RA Bebb University of British Columbia, Vancouver, Canada
Body	Nothing to Disclose: RAB

Pub #	M6
Session Information	MEET-THE-PROFESSOR: CLINICAL - Disorders of Sexual Development in Adults (5:30 PM - 6:15 PM)
Title	Disorders of Sexual Development in Adults
Author String	GS Conway University College of London Hospital, London, UK
Body	Session supported by: Novo Nordisk Inc. & Pfizer, Inc. Nothing to Disclose: GSC

Pub #	M8
Session Information	MEET-THE-PROFESSOR: CLINICAL - Investigating Mineralocorticoid Hypertension (5:30 PM - 6:15 PM)
Title	Investigating Mineralocorticoid Hypertension
Author String	CE Kater Federal University of Sao Paulo, Sao Paulo, Brazil
Body	Nothing to Disclose: CEK

Pub #	M10
Session Information	MEET-THE-PROFESSOR: CLINICAL - New Options in the Investigation & Management of (Neuro-) Endocrine Tumors of the Digestive Tract & Pancreas (5:30 PM - 6:15 PM)
Title	New Options in the Investigation & Management of (Neuro) Endocrine Tumors of the Digestive Tract & Pancreas
Author String	WW de Herder Erasmus Medical Center, Rotterdam, Netherlands
Body	The majority of neuroendocrine tumors (NETs) of the gastrointestinal (GI) tract are non-functioning and their presentation is non-specific. Functioning NETs of the GI tract may present with the typical 'carcinoid syndrome', which is clinically manifested as cutaneous flushing and diarrhea. Most pancreatic NETs are non-functioning and are diagnosed either incidentally or by local mass effects. Insulinomas are the commonest functioning pancreatic NETs, mostly benign (95%), small sized tumors, characterized by insulin hypersecretion, neuroglycopenia and catecholamine responsive symptoms. Hypergastrinemia in gastrinomas causes acid hypersecretion with peptic ulceration of the upper GI tract and diarrhea. Glucagonomas are large tumors that present with weight loss, diabetes, cheilosis, diarrhea and necrolytic migratory erythema. VIPomas are rare, usually large metastatic tumors and present with severe secretory diarrhea. Surgery is essential in many phases of the management of NETs. Somatostatin analogs remain the mainstay of symptomatic therapy for GEP NETs and are usually well tolerated, safe drugs, with mild side effects. Their effects on tumor growth have been recently demonstrated in NETs of the gastrointestinal tract. Interferon alpha has high symptomatic and biological response rates, but has substantial adverse effects. Etoposide plus cisplatin is effective for poorly differentiated GEP NETs, whereas streptozotocin + 5-FU + doxorubicin has some benefit in poorly differentiated pancreatic NETs. Traditional cytotoxic agents are of limited efficacy in the treatment of well differentiated GEP-NETs. However, their high expression of several pro-angiogenic molecules including VEGF render them potentially susceptible to antiangiogenic strategies. Other therapeutic targets include epithelial grown factor receptor (EGFR), insulin-like growth factor receptor (IGFR), and dowr signaling pathway components PI3K/AKT/MTOR which are overexpressed, with loss of the related tumor suppressor protein PTEN. In this spectrum, Everolimus and Sunitinib have shown promising results in phase III trials in GEP-NETs. GEP-NETs over-express somatostatin receptors and are, therefore, attractive targets for radiolabelled somatostatins. Peptide radioreceptor therapy has utilized high dosages of [^{90}Y-DOTA^{0},Tyr^{3} octreotide, [^{90}Y-DOTA]lanreotide, [^{90}Y-DOTA^{0},Tyr^{3}]octreotate and [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Very promising results have also been achieved using these targeted therapies. Disclosures: WWdH: Advisory Group Member, Novartis Pharmaceuticals, Ipsen.

Pub #	M11
Session Information	MEET-THE-PROFESSOR: CLINICAL - Phosphate Disorders in Children & Adolescents (5:30 PM - 6:15 PM)
Title	Phosphate Disorders in Children & Adolescents
Author String	TO Carpenter Yale University School of Medicine, New Haven, CT
Body	Disclosures: TOC: Consultant, Kyowa Hakko Kirin Pharma.

Pub #	M12
Session Information	MEET-THE-PROFESSOR: CLINICAL - Surgical Management of Thyroid Cancer (5:30 PM - 6:15 PM)
Title	Surgical Management of Thyroid Cancer
Author String	E Kebebew National Cancer Institute/National Institutes of Health, Bethesda, MD
Body	Nothing to Disclose: EK

Pub #	M13
Session Information	MEET-THE-PROFESSOR: CLINICAL - When & How To Use Vitamin D (5:30 PM - 6:15 PM)
Title	When & How To Use Vitamin D
Author String	DD Bikle Veterans Affairs Medical Center 111N-University of California, San Francisco, CA
Body	Nothing to Disclose: DDB

Pub #	
Session Information	CONFERENCE EVENT: Adrenal MOC Live Learning Session (6:30 PM - 9:30 PM)
Title	Adrenal MOC Live Learning Session
Author String	
Body	Registration and fee required

Pub #	
Session Information	CONFERENCE EVENT: Women in Endocrinology Dinner (6:30 PM - 10:00 PM)
Title	Women in Endocrinology Dinner
Author String	
Body	Fee required

Pub #	
Session Information	CMES SYMPOSIA: Your Questions Answered: A "Hot Seat" Discussion on GLP-1 Receptor Agonists (6:30 PM - 9:00 PM)
Title	Your Questions Answered: A "Hot Seat" Discussion on GLP-1 Receptor Agonists
Author String	
Body	Novo Nordisk, Inc.

Pub #	
Session Information	CMES SYMPOSIA: Advances in Anti-Resporptive Therapies To Treat Bone Disease (6:30 PM - 9:30 PM)
Title	Advances in Anti-Resporptive Therapies To Treat Bone Disease
Author String	
Body	Amgen

Pub #	
Session Information	CMES SYMPOSIA: New Development in Therapy for Adult Growth Hormone Deficiency (6:00 AM - 8:00 AM)
Title	New Development in Therapy for Adult Growth Hormone Deficiency
Author String	
Body	Novo Nordisk, Inc.

Pub #	
Session Information	CMES SYMPOSIA: What the Practicing Endocrinologist Needs To Know about Uncommon Metabolic Bone Diseases: Pathogenesis and New Treatments (6:00 AM - 8:00 AM)
Title	What the Practicing Endocrinologist Needs To Know about Uncommon Metabolic Bone Diseases: Pathogenesis and New Treatments
Author String	
Body	Enobia

Pub #	NS1-1
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Diabetes Mellitus in Special Populations (7:30 AM - 8:30 AM)
Title	Diabetes Mellitus in Special Populations: Presentations of Doctoral Dissertations Funded by ENS
Author String	DM Weiler Boise State University, Nampa, ID
Body	This presentation highlights two doctoral dissertations which were supported by the Endocrine nurses society. The first study explored the socio-cultural influences and social context associated with living with type 2 diabetes among rural, migrant Latino adults. A qualitative descriptive study using grounded theory techniques was conducted to provide a comprehensive summary of events in the everyday terms of those events. In-depth semi-structured interviews were completed with ten participants (6 female and 4 male) ranging in age from 46-65 years and duration of diabetes diagnosis ranging from 1.5- 40 years. An over-arching meta-theme Self Management in a Social Environment emerged. Every aspect of the process of self-management, as described in the four major themes, (1) Family Cohesion, (2) Social Stigma of Disease, (3) Social Expectations/ Perception of [ldquo]Illness,[rdquo] and (4) Disease Knowledge and Understanding, was influenced by the social context. This study revealed several socio-cultural influences that impact diabetes self-management practices for the migrant Latino adult. The familist traditions, central to the Mexican culture had both positive and negative consequences on diabetes management. Application of study findings will be addressed during the presentation. The second study emphasizes the transformative potential of praxis as described by Newman in the theory, [ldquo]Health as Expanding Consciousness[rdquo] (HEC). This study addresses the following research questions: What are the patterns of self-care decision making of women of African descent with Type 2 diabetes and limited financial resources? and [ldquo]How can nursing practice support new understanding of self-care decision making with study -participants?[rdquo] A purposive sample of 16 women of African descent with Type 2 diabetes was recruited for the study. Follow-up interviews were conducted with 13/16 participants. Participants were provided audio copies of the recorded interview after each was completed. An overview of action methods will be presented. The presentation will include individual patterns identified, patterns across the narratives and a representational graphic of the labyrinth of care. Session supported by: Endocrine Nurses Society Research Grants Sources of Research Support: The Endocrine Nurses Society; Sigma Theta Tau/Western Institute of Nursing Research Grant; Boise State University, School of Nursing Faculty Research Grant. Disclosure Incomplete: DMW

Pub #	NS1-2
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Diabetes Mellitus in Special Populations (7:30 AM - 8:30 AM)
Title	Diabetes Mellitus in Special Populations: Presentation of Two Doctoral Dissertations Funded by the Endocrine Nurses Society
Author String	ME Eckert-Norton State University of New York Downstate, Brooklyn, NY
Body	This presentation highlights two doctoral dissertations which were supported by the Endocrine Nurses Society. The first study explores the socio-cultural influences and social context associated with living with type 2 diabetes among rural, migrant Latino adults. A qualitative descriptive study using grounded theory techniques was conducted to provide a comprehensive summary of events in the everyday terms of those events. In-depth semi-structured interviews were completed with ten participants (6 female and 4 male) ranging in age from 46-65 years and duration of diabetes diagnosis ranging from 1.5- 40 years. An over-arching meta-theme *Self Management in a Social Environment* emerged. Every aspect of the process of self-management, as described in the four major themes, (1) Family Cohesion, (2) Social Stigma of Disease, (3) Social Expectations/ Perception of [ldquo]Illness,[rdquo] and (4) Disease Knowledge and Understanding, was influenced by the social context. This study revealed several socio-cultural influences that impact diabetes self-management practices for the migrant Latino adult. The familist traditions, central to the Mexican culture had both positive and negative consequences on diabetes management. Application of study findings will be addressed during the presentation. The second study emphasizes the transformative potential of praxis as described by Newman in the theory, [ldquo]Health as Expanding Consciousness[rdquo] (HEC). This study addresses the following research questions: What are the patterns of self-care decision making of women of African descent with Type 2 diabetes and limited financial resources? and [ldquo]How can nursing practice support new understanding of self-care decision making with study -participants?[rdquo] A purposive sample of 16 women of African descent with Type 2 diabetes was recruited for the study. Follow-up interviews were conducted with 13/16 participants. Participants were provided audio copies of the recorded interview after each was completed. An overview of action methods will be presented. The presentation will include individual patterns identified, patterns across the narratives and a representational graphic of the labyrinth of care. Session supported by: Endocrine Nurses Society Research Grants Sources of Research Support: Endocrine Nurses Society. Disclosure Incomplete: MEE-N

Pub #	L3-1
Session Information	PLENARY: TRANSLATIONAL - Gerald D Aurbach Award Lecture: Obesity, Type 2 Diabetes & Cancer: The Insulin & Insulin-Like Growth Factor Connection (8:00 AM - 9:15 AM)
Title	Gerald D Aurbach Award Lecture: Obesity, Type 2 Diabetes & Cancer: The Insulin & Insulin-Like Growth Factor Connection
Author String	D LeRoith Mount Sinai School of Medicine, New York, NY
Body	Recent studies have demonstrated a role for both the insulin receptor (IR) and the IGF-1 receptor (IGF-1R) in cancer development, growth and cancer-related mortality. Epidemiological studies showed a relationship between total IGF-1 circulating levels and the relative risk of developing most of the common epithelial cancers including prostate, colon, and breast cancer. Tissue and cell culture studies have shown an increased expression of the IGF-1R in cancer cells. The increased gene expression is the result of both enhanced promoter activity and increased translation. The formed is seen in many cancer cells that express mutated tumor suppressor gene products such as p53, WT1 and BRACA1/2. Enhanced translation is see with deletion of PTEN, another common tumor suppressor that is mutated in cancers. Blocking the activation of the IGF-1R inhibits cancer growth both in vitro and in vivo. This has led to the development of numerous humanized IGF 1R blocking antibodies and a number of tyrosine kinase inhibitors (TKI, that have entered various phases of preclinical and clinical testing in various epithelial cancers and sarcomas.. On the hand, interest has also focused on the role of insulin in promoting cancer in obesity and Type 2 diabetes (T2D). Again, interest has arisen from epidemiological studies that find an association between C-peptide and serum insulin levels and cancer risk and cancer-related mortality in obesity and/or T2D. In the case of breast cancer, studies have convincingly shown that prognosis is worse when the breast cancer samples express higher levels of IR that is activated. In these case the expression of IR-A, a mitogenic sub-type of the IR is also expressed at high levels. To demonstrate the direct causality between endogenous hyperinsulinemia and cancer growth and metastases, we have utilized such a mouse model. Our results show that reducing the hyperinsulinemia or blocking the IR/IGF-1R tyrosine kinases blocks the extra growth of the tumors considered secondary to the hyperinsulinemia. Thus the insulin and IGF-1 systems are involved in cancer growth and development and afford a novel new adjunct to convention chemotherapy. Session supported by: Lilly USA, LLC & Novo Nordisk Inc. Nothing to Disclose: DL

Pub #	L3-2
Session Information	PLENARY: TRANSLATIONAL - Clinical Investigator Award Lecture: From Base Change to Better Care in Neonatal Diabetes (8:00 AM - 9:15 AM)
Title	Clinical Investigator Award Lecture: From Base Change to Better Care in Neonatal Diabetes
Author String	A Hattersley Peninsula Medical School, Exeter, UK
Body	Nothing to Disclose: AH

Pub #	CMF3-1
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Risk Assessment for Osteoporotic Fracture (8:30 AM - 9:15 AM)
Title	Risk Assessment for Osteoporotic Fracture
Author String	TJ Weber Duke University Medical Center, Durham, NC
Body	Disclosures: TJW: Speaker, Eli Lilly & Company, Amgen, Genentech, Inc., Novartis Pharmaceuticals.

Pub #	CMF3-2
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Risk Assessment for Osteoporotic Fracture (8:30 AM - 9:15 AM)
Title	Risk Assessment for Osteoporotic Fracture
Author String	R Eastell University of Sheffield, Sheffield, UK
Body	Nothing to Disclose: RE

Pub #	CMF4-1
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Thyroid Nodules & Cancer in Children (8:30 AM - 9:15 AM)
Title	Thyroid Nodules & Cancer in Children
Author String	GL Francis Medical College of Virginia, Children's Hospital of Richmond, Richmond, VA
Body	Nothing to Disclose: GLF

Pub #	CMF4-2
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Thyroid Nodules & Cancer in Children (8:30 AM - 9:15 AM)
Title	Thyroid Nodules & Cancer in Children
Author String	SG Waguespack University of Texas MD Anderson Cancer Center, Houston, TX
Body	Nothing to Disclose: SGW

Pub #	M14
Session Information	MEET-THE-PROFESSOR: CLINICAL - Amenorrhea (8:30 AM - 9:15 AM)
Title	Amenorrhea
Author String	CK Welt Massachusetts General Hospital, Boston, MA
Body	Nothing to Disclose: CKW

Pub #	M16
Session Information	MEET-THE-PROFESSOR: CLINICAL - Management of Hypogonadism through Puberty (8:30 AM - 9:15 AM)
Title	Management of Hypogonadism through Puberty
Author String	N Pitteloud University of Lausanne, Lausanne, Switzerland
Body	Nothing to Disclose: NP

Pub #	M18
Session Information	MEET-THE-PROFESSOR: CLINICAL - Pheochromocytoma & Paraganglioma Syndromes: What's New? (8:30 AM - 9:15 AM)
Title	Pheochromocytoma & Paraganglioma Syndromes: What's New?
Author String	C Jimenez University of Texas MD Anderson Cancer Center, Houston, TX
Body	Nothing to Disclose: CJ

Pub #	M19
Session Information	MEET-THE-PROFESSOR: CLINICAL - Premature Adrenarche (8:30 AM - 9:15 AM)
Title	Premature Adrenarche
Author String	SE Oberfield Columbia University Medical Center, New York, NY
Body	Nothing to Disclose: SEO

Pub #	M20
Session Information	MEET-THE-PROFESSOR: CLINICAL - The Value of Medical Therapy before Pituitary Surgery (8:30 AM - 9:15 AM)
Title	The Value of Medical Therapy before Pituitary Surgery
Author String	L Katznelson Stanford University, Stanford, CA
Body	There has been growing interest regarding the value of preoperative medical therapy to improve surgical outcomes in patients with pituitary disorders. This is particularly the case with acromegaly, where preoperative administration of medical therapy, especially with somatostatin analogs (SSAs), has been considered both to improve surgical efficacy and limit perioperative anesthetic complications. The literature regarding use of SSAs to improve transsphenoidal surgical cure rates for acromegaly consists mostly of open label trials that have suggested improved biochemical outcomes. In a recent randomized trial involving administration of SSAs for 6 months versus no medical therapy prior to surgery, preoperative administration of SSAs improved surgical cure rates in patients with macroadenomas compared to those who did not receive preoperative SSAs (1). Since the majority of patients with acromegaly have macroadenomas, these data are compelling to support a case that SSAs should be administered prior to surgery to improve surgical efficacy in such patients. Further investigations are important to validate these findings before a blanket guideline is issued. Additionally, because patients with acromegaly often have sleep apnea syndrome and heightened cardiovascular risk, there is risk of anesthetic complications during surgery, including traumatic intubation, postoperative airway management difficulties, and cardiovascular complications. Although there are scant data regarding the utility of preoperative medical management to improve such outcomes, medical therapy is sometimes administered prior to surgery for this purpose. In the setting of Cushing's disease, preoperative medical therapy has been considered to reduce hypercortisolemia and thereby reverse medical comorbidities, such as diabetes, hypertension, and poor wound healing, which may lead to peri and postoperative complications. However, there are limited data to support such use. Whether medical therapy can improve surgical outcome in Cushing's disease is unknown. Session supported by: Pfizer, Inc. & Ipsen, US (1) Carlsen SM et al., JCEM 2008;93:2984 Disclosures: LK: Researcher, Novartis Pharmaceuticals; Speaker, Ipsen.

Pub #	M21
Session Information	MEET-THE-PROFESSOR: CLINICAL - What To Do for Sweating & Flushing (8:30 AM - 9:15 AM)
Title	What To Do for Sweating & Flushing
Author String	P-MG Bouloux Royal Free Hospital, London, UK
Body	Nothing to Disclose: P-MGB

Pub #	NS2-1
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Inpatient Diabetes Mellitus Collaborative Practice: Sugar Busters - Improving Inpatient Glycemic Control with Collaborative Resident & Nurse Education (8:30 AM - 9:30 AM)
Title	Sugar Busters: Improving Inpatient Glycemic Control with Collaborative Resident & Nurse Education
Author String	J Najarian Lehigh Valley Health Network, Allentown, PA
Body	This presentation will describe how a large academic multi-site community hospital's glycemic control initiative was enhanced through the development of an innovative partnership. The "Sugar Busters" created a collaborative appropriate to increase resident and nursing knowledge and skills related to the care of patients with hyperglycemia in the acute care setting. Through joint education and multi-disciplinary rounding on complex diabetes cases, the presenters have seen measurable improvements in inpatient glycemic control, enhanced staff knowledge, improved relationships between team members, and increased emphasis on patient preparation for discharge. This session will provide practical information on strategies used to improve inpatient glycemic control. Presenters will share their experience and lessons learned in an understandable way to help others improve the care of hospitalized patients with diabetes and/or hyperglycemia in their facility. Nothing to Disclose: JN

Pub #	NS2-2
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Inpatient Diabetes Mellitus Collaborative Practice: Sugar Busters - Improving Inpatient Glycemic Control with Collaborative Resident & Nurse Education (8:30 AM - 9:30 AM)
Title	Inpatient Diabetes Mellitus Collaborative Practice: Sugar Busters - Improving Inpatient Glycemic Control with Collaborative Resident & Nurse Education
Author String	GA Perilli Helwig Health & Diabetes Center/Lehigh Valley Health Network, Allentown, PA
Body	Nothing to Disclose: GAP

Pub #	M15
Session Information	MEET-THE-PROFESSOR: CLINICAL - Interpretation of Challenging Thyroid Function Tests (8:30 AM - 9:15 AM)
Title	Interpretation of Challenging Thyroid Function Tests
Author String	VJ Bernet Mayo Clinic Jacksonville, Ponte Vedra Beach, FL
Body	Nothing to Disclose: VJB

Pub #	M17
Session Information	MEET-THE-PROFESSOR: CLINICAL - Managing Cardiovascular Risk in Type 2 Diabetes (8:30 AM - 9:15 AM)
Title	Managing Cardiovascular Risk in Type 2 Diabetes
Author String	S Dagogo-Jack University of Tennessee, Memphis, TN
Body	Session supported by: Kowa Pharmaceuticals America, Inc. and Lilly USA, LLC and Merck & Co., Inc. Disclosures: SD-J: Speaker, Eli Lilly & Company, GlaxoSmithKline; Consultant, Merck & Co., Roche Pharmaceuticals; Principal Investigator, Astra Zeneca, Bristol-Myers Squibb.

Pub #	S26-1
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Insulin-Like Growth Factor System & Growth: Basic Mechanisms (9:30 AM - 11:00 AM)
Title	IGF-I Role in Skeletal Acquisition: Lessons from Mouse Models
Author String	S Yakar Mount Sinai School of Medicine, New York, NY
Body	The best protection against future osteoporosis and bone fragility is to [ldquo]grow the right skeleton[rdquo]. The GH/IGF-1 axis plays a major role in determining bone size and mass acquired during growth in humans and animals. Nonetheless, over the last decade, much of the research on the GH/IGF-1 axis in bone has focused on its potential role in post-menopausal and age-related osteoporoses. The overwhelming consensus of these studies is that decreases in IGF-1 and GH with menopause and aging coincide and do not appear to be the major cause driving osteoporosis. Studies from our as well as other laboratories clearly show that modulation of the GH/IGF-1 axis during growth has major effects on skeletal integrity that are carried on into adulthood and aging. As GH/IGF-1 axis has two modes of actions i.e. the endocrine (serum) and autocrine/paracrine (tissue), studies in the last decade addressed how selective changes in each of the modes affect skeletal integrity. We found that alterations in serum IGF-1 levels have minimal effects on bone length, but appear to control radial/transversal bone growth. Likewise, mouse models with reduced serum IGF-1 levels, such as the liver IGF-1 deficient (LID) or the acid labile subunit KO mice (ALSKO), develop slender bones, owing to inhibition of periosteal osteogenesis, which can be reversed by GH treatment. In contrast, mice overexpressing hepatic IGF-1 transgene (HIT), with elevations in serum IGF-1 show more robust bones, independent of tissue IGF-1 (KO-HIT model). Interestingly, however, knocking out the GHR impairs both longitudinal and transverse bone growth, such that periosteal apposition cannot be rescued by elevations in serum IGF-1. This may suggest that GH-dependent tissue factors control transversal bone growth. Since transversal bone growth is one of the most critical determinants of bone strength throughout life, future studie should uncover how intrinsic tissue (bone/muscle) GH/IGF-1 signaling pathways control transversal/periosteal bone growth. Session supported by: Pfizer, Inc. & Ipsen, US Sources of Research Support: NIH NIAMS (AR054919 & AR055141). Nothing to Disclose: SY

Pub #	S26-2
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Insulin-Like Growth Factor System & Growth: Basic Mechanisms (9:30 AM - 11:00 AM)
Title	Mechanisms Regulating Prepubertal Bone Growth
Author String	S Mohan Loma Linda Veterans Affairs Healthcare System, Loma Linda, CA
Body	Low peak bone mass is an important risk factor for osteoporosis. Therefore, studies to understand the mechanisms regulating bone accretion during postnatal growth periods are of considerable importance in the prevention and treatment of osteoporosis. With regard to potential signaling molecules which contribute to skeletal growth, the findings from a variety of transgenic mouse models and clinical studies of mutations in genes that regulate IGF-I action have illustrated a key role for IGF -I in the regulation of peak bone mass. In terms of mechanisms for IGF-I regulation, there is now irrefutable evidence for the involvement of GH axis in the regulation of IGF-I action during pubertal growth period. Based on the findings that the magnitude of deficit in bone mineral density (BMD) and bone size is much greater in IGF-I knockout mice compared to GH deficient mice at the end of prepubertal growth period, we predicted that IGF-I regulation of bone accretion during prepubertal growth period is mediated by a GH-independent mechanism. In our efforts to identify the key molecules that regulate IGF-I expression during the prepubertal growth period, we focused on thyroid hormone (TH) axis because the rapid increase in serum IGF-I levels during prepubertal growth period were preceded by increased serum T3 and correlated highly with serum T3. The cause and effect relationship between changes in serum T3 and IGF-I levels was demonstrated by using two mutant mouse models, $Tshr^{hyt/hyt}$ and $Duox2^{-/-}$, that exhibit TH deficiency. Furthermore, treatment of $Tshr^{hyt/hyt}$ mice during prepubertal growth period (day 5-14) with daily administration of replacement doses of T3/T4 rescued deficits in serum IGF-I, skeletal IGF-I and femur BMD. Accordingly, T3 treatment increased IGF-I expression at both mRNA and protein levels in a dose and time-dependent manner in osteoblasts. Studies on the molecular pathway for TH regulation of IGF-I expression in osteoblasts revealed that ligand bound TH receptor α1 binds to TH response element in the first intron of IGF-I gene and thereby regulates the promoter activity of IGF-I gene. Based on these and other findings, we conclude that IGF-I expression and bone accretion during prepubertal growth is predominantly mediated by TH axis in mice. Session supported by: Pfizer, Inc. & Ipsen, US Nothing to Disclose: SM

Pub #	S26-3
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Insulin-Like Growth Factor System & Growth: Basic Mechanisms (9:30 AM - 11:00 AM)
Title	Insulin-Like Growth Factor Deficiency & Growth Failure: Lessons from Mutational Analysis
Author String	RG Rosenfeld Stat 5 LLC, Los Altos, CA
Body	IGF Deficiency (IGFD) can result from either growth hormone (GH) deficiency or GH insensitivity. As experience with clinical conditions associated with IGFD has increased, it has become apparent that IGFD in the presence of normal GH secretion (primary IGFD) constitutes a spectrum of clinical conditions, with a range of phenotypes, biochemical characteristics and molecular defects. Over the last five years, we have performed biochemical and/or molecular analyses on ~500 patients with short stature, of whom ~200 have ha heights < -3 SD. Of these, ~50 have had identifiable molecular defects resulting in either primary IGFD or IGF resistance. The molecular basis for an IGFD continuum is predicated on the following observations: 1) defects of a variety of genes may result in primary IGFD; 2) different mutations of the same gene may result in a range of phenotypes: 3) mutations can result in immunodetectable, but bioinactive proteins; 4) dominant negative mutations in classic autosomal recessive disorders may lead to a mild phenotype; 5) heterozygosity for some autosomal recessive disorders may result in an attenuated phenotype; 6) even with identical mutations of the same gene, a range of phenotypes may be observed, presumably reflecting the effects of interacting genes and polymorphisms. In an effort to catalog these defects, a website has been established for genetic defects of the GH-IGF axis: http://growthgeneticsconsortium.org Session supported by: Pfizer, Inc. & Ipsen, US Nothing to Disclose: RGR

Pub #	S27-1
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Nutritional Regulation of Growth Hormone Secretion (9:30 AM - 11:00 AM)
Title	Effect of Body Mass & Body Composition on Stimulated Growth Hormone Secretion in Children
Author String	TL Stanley Massachusetts General Hospital, Boston, MA
Body	The diagnosis of GH deficiency in children relies heavily on the use of stimulation testing. Peak stimulated GH is poorly reproducible, however, and is influenced by multiple factors, including body mass and body composition. Numerous studies have determined that both spontaneous and stimulated GH secretion are lowe in obese individuals, particularly in relationship to increased visceral adiposity. In children, previous investigations demonstrate that spontaneous GH secretion is inversely associated with body mass index (BMI), even in children of normal weight. We have shown that, in children presenting with short stature, BMI standard deviation score (SDS) is a strong negative determinant of peak stimulated GH even within the normal weight range, such that BMI SDS significantly affects the likelihood of being diagnosed with GHD in children with short stature. Thus BMI SDS, in addition to pubertal status and recent nutritive intake, is an important consideration when interpreting the results of GH provocative testing. An important related issue is whether the decreased GH secretion seen with increased adiposity contributes to cardiometabolic abnormalities in children. Studies in adults demonstrate that reduced stimulated peak GH is associated with increased carotid intimal thickness, dyslipidemia, hypoadiponectinemia, and increased systemic circulating inflammatory markers. Similar findings have recently been reported in adolescents, in whom decreased growth hormone is associated with less favorable lipid parameters and increased circulating inflammatory markers. Further studies are needed, however, to determine if endogenous GH secretion is an independent determinant of cardiometabolic risk in children and to investigate the effects of GH treatment on cardiometabolic parameters. Session supported by: Novo Nordisk Inc. & Pfizer, Inc. Nothing to Disclose: TLS

Pub #	S27-2
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Nutritional Regulation of Growth Hormone Secretion (9:30 AM - 11:00 AM)
Title	Ghrelin: Physiological Significance & Therapeutic Potential
Author String	K Kangawa National Cardiovascular Center Research Institute, Osaka, Japan
Body	A complex network of cell-cell communication system by peptide hormones works for maintaining the mammalian homeostatic balance. To further clarify the intricate mechanisms of the regulation, it is important to discover unidentified bioactive peptides. By using our own methods, we discovered novel bioactive peptides such as neuromedins, three natriuretic peptides (ANP, BNP, CNP), and adrenomedullin. Moreover in 1999, we discovered ghrelin from stomach as an endogenous ligand for the growth hormone (GH) secretagogue receptor (GHS-R), an orphan G-protein coupled receptor. Ghrelin is a 28-amino acid peptide with a marvelous structure modified by a fatty acid, n-octanoic acid, which is essential for its activity. Ghrelin potently induces GH release both in rats and humans. Ghrelin is primarily produced in distinct endocrine cells X/A-like cells, in the stomach, and its secretion is regulated under conditions of energy balance. Ghrelin-producing neurones are also present in the hypothalamic arcuate nucleus, a region that regulates GH release and food intake. In addition to the stimulation of GH secretion and orexigenic effects, ghrelin has various physiological functions such as regulation of cardiovascular system and anti-inflammatory effects, and also sympatho-inhibitory actions have been identified. Thus, ghrelin has multifaceted roles by GH-dependent and -independent mechanisms for maintaining the homeostatic balance in such as cardiovascular and metabolic systems. Furthermore, translational research and clinical trial of ghrelin have already begun for many diseases such as cachexia, chronic obstructive pulmonary disease, anorexia nervosa and gastric cancer. In this symposium, I will make an overview on ghrelin; from its discovery to translational research. Session supported by: Novo Nordisk Inc. & Pfizer, Inc. Disclosure Incomplete: KK

Pub #	S27-3
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Nutritional Regulation of Growth Hormone Secretion (9:30 AM - 11:00 AM)
Title	New Evidence Linking Orexigenic/Anorexigenic Signals & Growth Hormone
Author String	C Dieguez University of Santiago Faculty of Medicine, S Francisco s/n, Santiago de Compostela, Spain
Body	Growth hormone secretion is markedly influence by energy status. Thus, blunted GH secretion is present in states of increased BMI. In contrast, food deprivation in humans elcited a marked increase in basal and stimulated circulating GH levels. Data gleaned over the last few years have shown that some of the most relevant signals involved in energy homoestasis such as leptin and ghrelin also play a major role in GH secretion. Since these signals were shown to exert their effects on food intake by influencing the expression o neuropeptides such as NPY, AgRP and POMC ; earlier work focused on assessing their role on GH secretion as well as the direct effects of leptin/ghrelin at pituitary level. More recently the mechanisms by which these and other similar signals regulate food intake have been uncovered. Of particular interest in this context is the data implicating hypothalamic AMPK and lipid metabolism in ghrelin orexigenic effect and ghrelin-induced GH secretion. Taken together these findings indicate the existence of specific cellular sensors at hypothalamic level that help to set up an integrated homeostatic response in the face of altered nutritional status in terms of food intake, energy homeostasis and pituitary hormone secretion . Session supported by: Novo Nordisk Inc. & Pfizer, Inc. Nothing to Disclose: CD

Pub #	S21-1
Session Information	SYMPOSIUM SESSION: BASIC - Chromatin Modulation by Nuclear Receptors (9:30 AM - 11:00 AM)
Title	Steroid Receptors Operate in a Chromatin Environment
Author String	TK Archer National Institute Environmental Health Science/National Institutes of Health, Research Triangle Park, NC
Body	Nothing to Disclose: TKA

Pub #	S21-2
Session Information	SYMPOSIUM SESSION: BASIC - Chromatin Modulation by Nuclear Receptors (9:30 AM - 11:00 AM)
Title	Regulation of Long Range Chromatin Interactions by Estrogen Receptor
Author String	E Cheung Genome Institute of Singapore, Singapore, Singapore
Body	Estrogen receptor α (ERα) is key player in the progression of breast cancer. Recently, we mapped the cistrome and interactome of ERα in breast cancer cells, revealing the importance of spatial organization in nuclear receptor-mediated transcription. However, the underlying mechanism in this process is unclear. Here, we show that AP-2 motifs are highly enriched in ERα binding sites (ERBS) identified from the ChIA-PET of ERα. More importantly, we demonstrate that AP-2γ (also known as TFAP2C), a member of the AP-2 family which has been implicated in breast cancer oncogenesis, binds to ERBS in a ligand-independent manner. Furthermore, pertubation of AP-2γ expression disrupts ERα DNA binding, long-range chromatin interactions, and gene transcription. Using ChIP-seq, we show that AP-2γ and ERα binding events occur in close proximity on a genome-wide scale. The majority of these shared genomic regions are also occupied by the pioneer factor, FoxA1. From our molecular studies, we provide evidence that AP-2γ is required for efficient FoxA1 binding and vice versa. Finally, we show that most ERBS associated with long-range chromatin interactions are co-localized with both AP-2γ and FoxA1. Together, our results suggest AP-2γ is an essential factor in ERα-mediated transcription, primarily working together with FoxA1 to facilitate ERα binding and long-range chromatin interactions. Nothing to Disclose: EC

Pub #	S21-3
Session Information	SYMPOSIUM SESSION: BASIC - Chromatin Modulation by Nuclear Receptors (9:30 AM - 11:00 AM)
Title	Epigenetic Regulation in Breast Cancer - Role in Endocrine Treatment Resistance
Author String	S Oesterreich University of Pittsburgh Cancer Institute, Pittsburgh, PA
Body	Postmenopausal breast cancer patients benefit from aromatase inhibitors (AIs) that reduce the levels of estrogens. Mechanisms underlying the hormonal resistance are complex, and not fully understood. To date, epigenetic contributions to hormonal therapy resistance have not been well characterized, with only a limited understanding of antiestrogen mediated epigenetic modification of chromatin and altered gene expression. We hypothesized that estrogen deprivation results in altered methylation, and therefore altered expression of critical target genes in breast cancer cells, which ultimately would contribute to acquired AI resistance in breast cancer patients. We used previously established MCF-7 cell clones, termed C4-12 and LTED (Long Term Estrogen Deprivation) that were isolated after being cultured in estrogen-free media for 9 months and 18-24 months, respectively. A genome-wide methylation screen was done using Methyl CpG binding Domain (MBD) pull down assay followed by hybridization into Affymetrix Promoter 1.0R Array. Altered DNA methylations were validated by bisulfite sequencing assays, and gene expression was studied by qRT PCR. We found that long term estrogen deprivation results in widespread genomic hyper- and hypomethylation events, with hypermethylation being more prominent. A gene which was heavily methylated in both cell line systems was HOXC10. Silencing of HOXC10 in MCF-7 cells resulted in increased cell proliferation and decreased apoptosis in estrogen deprived medium. In contrast to control MCF-7 cells, which did not grow as xenograft in the absence of estrogen, cells with low HOXC10 survived in vivo in the absence of estrogen. These data suggest a potential role of HOXC10 in developing resistance to endocrine therapy in breast cancer patients, a hypothesis which we are currently testing in clinical samples. Nothing to Disclose: SO

Pub #	S22-1
Session Information	SYMPOSIUM SESSION: BASIC - microRNAs: No Small Role in Skeletal Regulation (9:30 AM - 11:00 AM)
Title	Estrogen & Selective Estrogen Receptor Modulator Regulation of microRNAs
Author String	CM Klinge University of Louisville School of Medicine, Louisville, KY
Body	Estrogens have diverse physiological effects including stimulation of breast carcinogenesis. Estrogens regulate genes directly through binding to estrogen receptors alpha and beta (ERa and ERb) and indirectly by activating plasma membrane-associated ER which, in turns, activates intracellular signaling cascades leading to altered gene expression. MicroRNAs (miRNAs) are short (~22 nucleotides), non-coding RNAs that base-pair with the 3' untranslated region of target mRNAs within the RISC complex. This interaction blocks translation of the mRNA and results in mRNA degradation. miRNAs are key regulators of gene expression and aberrant patterns of miRNA expression are implicated in human diseases including breast cancer. Recent studies have identified miRNAs regulated by estrogens in human breast cancer cells and other cells and tissues (1). We reported that estradiol reduced miR-21 expression in MCF-7 breast cancer cells (2). Although changes in miRNA expression correlate with breast cancer diagnostic markers, comparatively little is known about miRNA regulation by selective estrogen receptor modulators (SERMs), *e.g.*, tamoxifen (TAM), or their role in endocrine-resistance. A microarray approach identified miRNAs differentially expressed in MCF-7 *versus* MCF-7/LY2 endocrine-resistant human breast cancer cells. The expression of specific miRNAs was validated by quantitative, real-time PCR. Bioinformatic analyses identified 36 predicted TAM-regulated gene targets of the miRNAs differentially expressed in MCF-7 versus LY2 cells. Twelve of the putative targets were regulated in anticipated direction in the TAM-treated cells, supporting biological significance of the differentially expressed miRNAs. Among the miRNAs differentially expressed are: miRs -93, 200abc (MCF-7> LY2) and miRs- 10a, 22, 29, 125b, 221, and 222 (MCF-7< LY2). ERa was lower in LY2 than MCF-7, commensurate with higher miR-221/222 in LY2. Reflecting an inverse association with miR-200, the expression of ZEB1 was higher in LY2 than MCF-7 cells and concomitantly, E-cadherin expression was absent in LY2 cells, indicating that LY2 have undergone epithelial-to-mesenchymal transition. Our studies identifying miRNAs with opposite expression between the two cell lines indicate the involvement of these miRNAs in endocrine resistance. 1. Klinge CM 2009 Current Genomics 10:169-183 2. Wickramasinghe et al. Klinge CM 2009 Nucleic Acids Res 37:2584-2595 Nothing to Disclose: CMK

Pub #	S22-2
Session Information	SYMPOSIUM SESSION: BASIC - microRNAs: No Small Role in Skeletal Regulation (9:30 AM - 11:00 AM)
Title	Role of miR-29 in Bone
Author String	AM Delany University of Connecticut Health Center, Farmington, CT
Body	MicroRNAs (miRNAs) are key post-transcriptional regulators of gene expression, and the miR-29 family is one of the best characterized miRNA families with regard to osteoblast function. miR-29a/b1 and miR-29c/b2 are transcribed from distinct loci, and these miRNAs are important positive regulators of osteoblast differentiation. The expression of miR-29 family members is low during the early, matrix deposition phases o osteoblastogenesis. A low level of miR-29 expression is important at this time since miR-29 targets bone matrix RNAs including COL1A1, COL3A1, and osteonectin/SPARC. The co-regulation of osteonectin and fibrillar collagens is not unexpected, since osteonectin is critical for collagen fibril organization. Later, as the matrix matures and osteoblasts express more differentiated markers, miR-29 levels increase. Over expression of miR-29 family members promotes osteoblastic differentiation, whereas knockdown of miR-29 decreases differentiation markers. Notably, other validated targets for miR-29, important in osteoblast function, include several inhibitors of osteoblastic differentiation. Canonical Wnt signaling is a critical positive regulator of osteoblast differentiation, and it rapidly induces the expression of miR-29a and -29c. Since activation of canonical Wnt signaling tends to increase during osteoblastic differentiation, it is possible that Wnt signaling plays a role in up regulating the expression of miR-29 during this process. Moreover, miR-29a modulates canonical Wnt signaling in a positive feedback loop, promoting human osteoblast differentiation. In human osteoblasts, transcription of miR-29a is induced by canonical Wnt signaling, and two TCF/LEF binding sites in the miR-29a promoter region are necessary for this induction. miR-29a also targets three inhibitors of Wnt signaling: Dkk1 (dikkopf-1), Kremen2 (kringle domain-containing transmembrane protein), and sFRP2 (secreted frizzled related protein 2). The induction of miR-29a transcription, in response to canonical Wnt signaling, results in decreased Dkk1, Kremen2, and sFRP2 levels, which potentiates Wnt signaling. This loop provides an additional mechanism by which miR-29 can promote osteoblast differentiation, and a mechanism for fine tuning the expression of specific components in the Wnt signaling pathway. Sources of Research Support: NIH Grant AR44877. Nothing to Disclose: AMD

Pub #	S22-3
Session Information	SYMPOSIUM SESSION: BASIC - microRNAs: No Small Role in Skeletal Regulation (9:30 AM - 11:00 AM)
Title	microRNA Regulation of Osteoblast Commitment & Bone Mass
Author String	JB Lian University of Massachusetts Medical School, Worcester, MA
Body	MicroRNAs (miR) attenuation of protein translation has emerged as an important regulator of developmental osteogenic signaling pathways, osteoblast growth and differentiation, and bone homeostasis in the adult skeleton. Several miRs have been identified with key roles in regulating bone formation. In response to the BMP osteogenic signal, a program of microRNAs are downregulated to allow for commitment of MSCs to the osteoblast lineage, while upregulated miRs suppress alternate lineages (1). Furthermore, during osteoblast differentiation, approximately 40 miRs are continuously upregulated from the proliferation stage of pre-osteoblasts reaching peak levels during mineralization of the extracellular matrix (2). Many of these miRs target genes to limit bone mass and prevent fibrosis. Other miRs provide intricate control over regulatory genes for feed-forward and feed-reverse mechanisms that regulate the timing for expression of osteoblast related genes during differentiation. The biological significance of these programs was established *in vivo* by conditional deletion in osteoblast lineage cells of the Dicer enzyme, which processes pre-miRs to the functional mature miRs (3). By crossing collagen-Cre and osteocalcin-Cre mice with $Dicer^{F1/F1}$ mice, our findings show that Dicer generated miRs are essential for two periods of bone formation, to promote osteoblast differentiation before birth and to control bone mass accrual in the adult. Excision of Dicer in mature osteoblasts increased bone mass continuously from 2 to 9 month age. While microRNAs have been characterized as markers in relation to tumor growth and metastasis of several cancers (breast, prostate, leukemia), more recent studies from other groups have identified miRs associated with osteoporosis and osteoarthritis. These discoveries provide novel opportunities for treating skeletal disorders with miRs and antagomirs. (1) Li Z et al., Proc Natl Acad Sci USA 2008; 105(37):13906-11. (2) Li Z et al., J Biol Chem 2009; 284(23):15676-15684. (3) Gaur T et al., Dev Biol 2010; 340(1):10-21. Sources of Research Support: NIH grant R37DE012528 awarded to JBL; NIH grant P01CA082834 awarded to GSS; NIH grant R01AR039588 awarded to GSS and JBL. Nothing to Disclose: JBL

Pub #	S23-1
Session Information	SYMPOSIUM SESSION: BASIC - New Insights in Thyroid Biology from Mouse Models (9:30 AM - 11:00 AM)
Title	Novel Cyclic AMP Pathways in Thyroid Mitogenesis
Author String	DL Altschuler University of Pittsburgh, Pittsburgh, PA
Body	cAMP is a ubiquitous second messenger mediating a plethora of cellular functions. Particularly in the thyroid gland, it mediates the proliferative action of TSH. For many years PKA represented the only known effector of cAMP action; however, recently a role for Epac1 (Exchange Protein Activated by cAMP) was established. Both cAMP effectors, Epac1 and PKA, are required synergistically for TSH-mediated cell proliferation and consistent with this synergistic response, most elements of the pathway (i.e. adenylate cyclase, cAMP, Epac, PKA, and Rap1) co-localize in the same compartment. We will discuss the role of the ERM member radixin as a scaffolding unit for both PKA and Epac1, thus delimiting a new functional compartment for cAMP action. Epac1-radixin-PKA complex localizes in clusters present at a sub-membrane compartment and maneuvers that disrupt this compartmentalization abrogate TSH-mediated proliferation. Deletion studies indicate that Epac's ERM binding domain (EBD) resides in its N-terminal 1-52 domain; (1-52)-dsRed localized in clusters indicating that Epac EBD is sufficient for proper targeting. Utilizing peptide arrays Epac's EBD was further mapped down to a minimal 15aa peptide sufficient for binding and its use as specific inhibitors with effector pathway selectivity will be discussed. A deletion DEBD-Epac protein is no longer associated with radixin in clusters but enriched in the nucleus. This result suggests that Epac-radixin interaction might be subjected to regulation. Consistent with this, TSH stimulation induces a PKA-dependent translocation of Epac to the nucleus. A new PKA-mediated Epac phosphorylation site was identified and its role in regulating Epac-radixin interaction will be discussed. Rap1 is a substrate for both Epac1 and PKA; Rap1 expression rescues the inhibitory effect of cluster disruption, however, only in its constitutively active (GTP-bound) and phosphorylated state (i.e. G12V-S179D). This result is consistent with our previous observation that Rap activation and phosphorylation were required to efficiently transduce cAMP proliferative response. We propose that radixin scaffolds both cAMP effectors in a functional cAMP-sensing compartment for efficient transduction, using Rap1 as a downstream signal integrator. Nothing to Disclose: DLA

Pub #	S23-2
Session Information	SYMPOSIUM SESSION: BASIC - New Insights in Thyroid Biology from Mouse Models (9:30 AM - 11:00 AM)
Title	Oncogenic Synergies & Metabolic Alterations in Advanced Thyroid Cancer: Lessons from Mouse Models
Author String	A Di Cristofano Albert Einstein College of Medicine, Bronx, NY
Body	Aberrant activation of the PI3K/AKT pathway, activating mutations of Ras family members, and p53 loss of function are common features of advanced, aggressive thyroid tumors originating from the follicular epithelium. We have generated a series of mouse strains recapitulating single and compound alterations in these clinically relevant pathways, and have used them to model in vivo increasingly aggressive thyroid neoplastic lesions. These mouse models are allowing us to deconstruct the intricate network of molecular interactions that synergize to induce rapidly growing, poorly differentiated and anaplastic tumors, as well as to characterize novel mechanisms responsible for the metabolic alterations that are necessary to support cancer growth. At the same time, these models are an invaluable tool to test in a clinically relevant setting novel therapeutic approaches harnessing the specific genetic and metabolic alterations characterizing advanced thyroid tumors. Nothing to Disclose: ADC

Pub #	S23-3
Session Information	SYMPOSIUM SESSION: BASIC - New Insights in Thyroid Biology from Mouse Models (9:30 AM - 11:00 AM)
Title	New Insights from Mouse Models of Graves Hyperthyroidism
Author String	SM McLachlan Cedars-Sinai Medical Center, Los Angeles, CA
Body	Hyperthyroidism caused by thyroid stimulating antibodies, as in human Graves' disease, is induced in mice by repeated injections of plasmid or adenovirus vectors expressing the human TSHR. These Graves' models provide insights into the nature of the immunogen, the genetic basis for antibody-mediated hyperthyroidism and the breakdown in self-tolerance to the TSHR:- *First*, in vivo TSHR expression, not injected purified TSHR- protein plus adjuvant, is required to induce thyroid stimulating antibodies. In contrast, lymphocytic infiltration and thyroid damage develop in some mouse strains after conventional immunization with adjuvant + purified thyroglobulin (Tg) or thyroid peroxidase (TPO). *Second*, as suggested by the properties of Graves' autoantibodies, the optimal immunogen is not the full-length TSHR but the A-subunit cleaved and shed from the receptor expressed on the thyrocyte surface. *Third*, development of Graves' disease is strain dependent; some strains are susceptible while others are resistant to induced hyperthyroidism. Exploiting the availability of genetically typed recombinant inbred mice, strain variability for development of TSHR antibodies versus hyperthyroidism was linked to different chromosomes/loci. Moreover, these studies implicate a role for immunoglobulin heavy chain variable-region genes in the development of thyroid stimulating antibodies. *Fourth*, regulatory T cells (Treg) influence the balance between thyroid stimulation and thyroid damage: in mice with the human A-subunit targeted transgenically to the thyroid, Treg deletion before A-subunit immunization induced Hashimoto's disease with hypothyroidism, elevated TSH, thyroid lymphocytic infiltration and autoantibodies to Tg and TPO. *Fifth,* breaking self tolerance does not involve Treg but implicates a role for central tolerance mediated by intrathymic TSHR expression. Thus, responses to mouse TSHR A-subunit adenovirus could be induced in TSHR knock-out (not wild-type) mice. Moreover, consistent with decreased intrathymic TSHR expression, mice defective for the Autoimmune regulator had decreased tolerance (versus wild-type mice) as reflected by their enhanced responses to immunization. *Finally*, we hypothesize that, unlike all non-primate mammals, the presence of a fifth glycosylation site in the human TSHR A-subunit contributes to enhanced A-subunit immunogenicity and provides (in part) an explanation for the lack of spontaneous Graves' disease in mammals other than humans. Sources of Research Support: NIH grants DK54684 and DK082390. Nothing to Disclose: SMM

Pub #	S24-1
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Adipose Tissue: Adipocyte Fate (9:30 AM - 11:00 AM)
Title	Molecular Mechanisms of Adipose Fate
Author String	BM Spiegelman Dana Farber Cancer Institute/Harvard Medical School, Boston, MA
Body	Nothing to Disclose: BMS

Pub #	S24-2
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Adipose Tissue: Adipocyte Fate (9:30 AM - 11:00 AM)
Title	Resistin & Inflammation
Author String	RS Ahima University of Pennsylvania School of Medicine, Philadelphia, PA
Body	Resistin is secreted by adipocytes in mice and induces insulin resistance. We have found that murine resistin promotes hepatic steatosis, inflammation and insulin resistance by signaling through hypothalamic neurons expressing neuropeptide Y (NPY)-Y1 receptors. Although resistin has been associated with insulin resistance, atherosclerosis and inflammatory diseases in humans, many studies investigating the link between resistin and metabolic disease in humans have produced conflicting results. Unlike rodents, human resistin is produced by monocytes and macrophages, and induced by inflammatory signals. To investigate the role of human resistin on glucose homeostasis in inflammatory states, we generated mice lacking murine resistin but transgenic for a bacterial artificial chromosome containing human resistin (BAC-Retn). The metabolic and molecular phenotypes of BAC-Retn mice were assessed after acute or chronic endotoximea (i.e. lipopolysaccharide treatment). BAC-Retn mice displayed circulating resistin levels within the normal human range, and similar to humans, lipopolysaccharide markedly increased serum resistin levels. Acute endotoxemia caused hypoglycemia in mice lacking murine resistin, and this was attenuated in BAC-Retn mice. In addition, BAC-Retn mice developed severe hepatic insulin resistance under chronic endotoxemia, accompanied by increased inflammatory responses in liver and skeletal muscle. These results demonstrate an important role of human resistin in the development of insulin resistance in inflammation. Sources of Research Support: NIH Grants PO1DK049210, and P30DK19525. Nothing to Disclose: RSA

Pub #	S24-3
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Adipose Tissue: Adipocyte Fate (9:30 AM - 11:00 AM)
Title	Adiponectin & Adipose Tissue
Author String	T Kadowaki University of Tokyo, Tokyo, Japan
Body	Disclosure Incomplete: TK

Pub #	S25-1
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Extra-Renal Actions of Aldosterone (9:30 AM - 11:00 AM)
Title	Aldosterone as a Regulator of Inflammation
Author String	L Pojoga Brigham & Women's Hospital, Boston, MA
Body	Nothing to Disclose: LP

Pub #	S25-2
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Extra-Renal Actions of Aldosterone (9:30 AM - 11:00 AM)
Title	Aldosterone Regulation of Cardiac Rhythms
Author String	F Jaisser Institut National de la Santé et de la Recherche Médicale U872, Paris, France
Body	Nothing to Disclose: FJ

Pub #	S25-3
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Extra-Renal Actions of Aldosterone (9:30 AM - 11:00 AM)
Title	Aldosterone Regulation of Vascular Function
Author String	IZ Jaffe Tufts Medical Center, Boston, MA
Body	In clinical trials, aldosterone antagonists prevent cardiovascular ischemia and mortality with only modest changes in blood pressure. The mineralocortocoid receptor (MR) is a ligand-activated transcription factor that we have previously shown is expressed in human vascular cells, where it regulates gene transcription and promotes inflammation and vascular calcification. We now show in mouse models of vascular injury, that the MR ligand aldosterone promotes vessel wall thickening, smooth muscle cell (SMC) proliferation, and extracellular matrix deposition. We hypothesize that aldosterone-activated vascular MR regulates genes that promote vascular injury and atherosclerosis and that identification of MR-regulated vascular gene pathways will provide novel targets to prevent and treat cardiovascular diseases. We have now explored the aldosterone stimulated vascular transcriptome in mouse aortae and identified 72 vascular MR-regulated genes that are enriched in pathways involved in vascular function and disease. In endothelium-denuded vessels, aldosterone-stimulated gene expression is enhanced for a subset of genes that are dependent on vascular oxidative stress. Our recent studies explore one of these genes, placental growth factor (PGF), a pro-proliferative, secreted growth factor member of the vascular endothelial growth factor (VEGF) family that has been implicated in atherogenesis in animals and in cardiovascular ischemia in humans. Activation of MR in the mouse aorta and carotid arteries by aldosterone at physiologic concentrations induces PGF gene transcription, protein expression, and secretion. In the mouse carotid injury model, aldosterone-stimulated vascular remodeling observed in WT mice is inhibited in PGF knockout mice supporting that regulation of PGF by vascular MR is a new and important mechanism of aldosterone-mediated vascular injury. In human vessels from patients with atherosclerosis, aldosterone further enhances expression of PGF and its transmembrane receptor, Flt1, and MR antagonists inhibit PGF expression and secretion. The identification of vascular MR target genes with enhanced expression in the setting of vascular damage supports a new mechanism for the pathologic effects of aldosterone. These studies illuminate the mechanisms underlying the substantial vascular protective effects of aldosterone antagonists in cardiovascular patients and suggest novel drug targets for ischemic cardiovascular diseases. Sources of Research Support: National Institutes of Health, HL074892 (IZJ) and HL056069 (MEM), and American Heart Association, GIA0855920D (IZJ). Nothing to Disclose: IZJ

Pub #	S28-1
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Stop Right There! Emerging Targets To Block Sex Steroid Synthesis (9:30 AM - 11:00 AM)
Title	P450 Aromatase Structure: Molecular Basis for Next-Generation Aromatase Inhibitors
Author String	D Ghosh State University of New York Upstate Medical University, Syracuse, NY
Body	Aromatase, the only enzyme known to catalyze the biosynthesis of estrogens in vertebrates, has been a prime therapeutic and prevention target for estrogen-dependent breast cancer. Having elucidated the details of the molecular architecture of the intact human placental enzyme [1], our investigation has moved towards design, synthesis and evaluation of the next generation of aromatase inhibitors (AI) that are highly specific for the target. The structure-guided de novo design and synthesis approach has yielded a number of steroidal compounds that rival the affinities of the FDA-approved AIs. The virtual screening method has produced leads to novel non-steroidal compounds. A 96 well-based colorimetric assay has been developed for high throughput enzymatic evaluation of newly synthesized compounds. To understand how dynamical motion and higher order organization could influence its catalytic function and ligand-binding ability at the active site, we have conducted normal mode analysis on the aromatase monomer and oligomers. The aromatase core is found to be quite rigid. Nevertheless, some evidence of ligand-induced fit is observed at the catalytic cleft. Mutational studies with a truncated recombinant enzyme have provided new functional insights for structure-based observations. [1] Ghosh D et al., Nature 2009; 457:219 Sources of Research Support: Grant GM086893 from the National Institute of Health. Nothing to Disclose: DG

Pub #	S28-2
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Stop Right There! Emerging Targets To Block Sex Steroid Synthesis (9:30 AM - 11:00 AM)
Title	17beta-Hydroxysteroid Dehydrogenases as Biomarkers of Disease & Targets for Inhibitor Development
Author String	J Adamski Helmholtz Zentrum Muenchen, Neuherberg, Germany
Body	Mechanism of estrogen and androgen action involves binding to a nuclear steroid receptor to elicit canonical genomic pathway or binding to other sensory molecule mediating non-genomic effects. The binding efficiency itself is determined by the position 17 of the steroid scaffold. 17β-Hydroxysteroid dehydrogenases (17β-HSDs) are oxidoreductases which act like as pre-receptor molecular switches by a NAD(P)H or NAD(P)+ dependent reductions/oxidations in 17-position of the steroids, respectively. Up to now 14 different subtype have been identified in mammals. The crucial role of estrogens and androgens in the development of hormone-dependent diseases has been demonstrated. The 17β-HSDs are promising therapeutic targets for diseases like breast/prostate cancer, endometriosis and osteoporosis. Moreover, several 17β-HSDs, e.g. type 1 4, 7 and 12, were shown to serve as biomarkers of disease prognosis. The selective inhibition of the 17β-HSDs might provide an effective treatment and a good alternative to the existing endocrine therapies. Several approaches including development of steroidal and non-steroidal inhibitors will be reviewed including ligand- and structure-based pharmacophore models, in silico screening of compound databases, experimental validation by enzymatic efficacy tests of selected virtual hits and metabolomic test for off-target inhibitor action. Nothing to Disclose: JA

Pub #	S28-3
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Stop Right There! Emerging Targets To Block Sex Steroid Synthesis (9:30 AM - 11:00 AM)
Title	Steroid Sulfatase Inhibitors for the Treatment of Hormone-Dependent Cancers: Where Are We Now?
Author String	A Purohit Hammersmith Hospital, Imperial College London, London, UK
Body	Steroid sulfatase (STS) has a crucial role in regulating the hydrolysis of steroid sulfates such as estrone sulfate (E1S) and dehydroepiandrosterone sulfate (DHEAS) to form estrone and dehydroepiandrosterone, respectively, which can then be converted to the potently estrogenic steroids, estradiol (E2) and androstenediol (Adiol) by 17β-hydroxysteroid dehydrogenase type 1. STS is thus a new target for the treatment of steroid hormone-dependent diseases such as breast, prostate and endometrial cancer. Despite current endocrine therapies, improvement is still necessary to achieve better disease control and outcome. BN83495 (Irosustat) is a non-steroidal, non-estrogenic, highly potent, irreversible STS inhibitor that inhibits the growth of E1S-stimulated NMU-induced mammary tumors in ovariectomised rats. A first ever, first-in-class, Phase I trial of Irosustat in 14 postmenopausal women with advanced breast cancer revealed that STS activity was effectively blocked (>98%) and this resulted in significant reduction in serum Adiol concentrations but only moderate reductions in serum levels of E1 and E2. Four patients, all of whom had previously progressed on aromatase inhibitors, showed evidence of stable disease for up to 7 months. A second dose-escalation study in postmenopausal women with ER+ve advanced metastatic breast cancer was conducted. Three to six patients were recruited into each of 5 sequential dose cohorts (1, 5, 20, 40 and 80mg). Irosustat was well tolerated and the main toxicity seen was dry skin (Grade [le]2). OBD was determined to be 40mg. Stable disease of >6 months was noted in 3 patients. Further phase I/II trials of this promising agent are currently in progress in breast, prostate and endometrial cancer patients. Sources of Research Support: Cancer Research UK and the Ipsen Group. Disclosures: AP: Principal Investigator, Consultant, Ipsen.

Pub #	S29-1
Session Information	SYMPOSIUM SESSION: CLINICAL - Advances in the Management of Acromegaly (9:30 AM - 11:00 AM)
Title	Reproductive Function & Pregnancy in Acromegaly
Author String	P Chanson Hospital Bicetre, Le Kremlin-Bicetre, France
Body	The menstrual cycle is often abnormal in women with acromegaly. The analysis of the causes of ovarian dysfunction in a large series of women aged from 17 to less than 45 years (1), showed that only one third of them were eugonadal, as they had regular menstrual cycles and/or conceived spontaneously, while two-thirds had anovulatory cycles, related to hyperprolactinemia and/or hypogonadism due to a mass effect; alternatively, the gonadal dysfunction was likely related to the GH/IGF-1 excess which exerts a direct effect on gonadotropic axis in some patients. Finally, few women had polycystic ovary syndrome which disappeared after control of acromegaly, suggesting that GH/IGF-I excess may also have a direct effect on the ovary. Few data are available on pregnancy in acromegaly. We reviewed the outcomes of 59 pregnancies in a large French multicenter study (2). Pregnancy was rarely complicated by gestational diabetes and hypertension. Both may be more frequent than in the general population when GH/IGF-1 hypersecretion was not controlled before pregnancy. Visual field defects or headache and/or increase in size of the adenoma on MRI occurred very rarely. Birth weight of the babies was generally normal. No significant increase in malformation rate was observed in the children of patients treated with dopamine agonists (DA) and/or somatostatin analogues (SA) at the onset of pregnancy. Birth of small-for-gestational-age infant may be the consequence of treatment with SA during pregnancy. Hemodynamic changes in the materno-fetal barrier tissues, as recently reported under high dose of subcutaneous SA during pregnancy (3) lead to recommend SA discontinuation when pregnancy is considered or confirmed, as withdrawal is usually safe. The significant decrease in mean IGF-I level during the first trimester observed in some pregnant women in parallel with improvement in acromegalic symptoms is likely related to the GH-antagonistic properties of estrogens; by contrast, in general, GH concentration did not change significantly. In conclusion, causes of infertility are multiple in acromegaly; pregnancy in women with active or uncontrolled acromegaly may be associated with an increased risk of gestational diabetes and gravid hypertension; pregnancy is occasionally associated with symptomatic enlargement of GH-secreting pituitary macroadenomas; GH-suppressive treatment can be safely withdrawn after conception in most acromegalic women. Session supported by: Pfizer, Inc. & Ipsen, US (1) Grynberg M et al., J Clin Endocrinol Metab 2010;95:4518 (2) Caron P et al., J Clin Endocrinol Metab 2010;95:4680 (3) Maffei P et al., Clin Endocrinol (Oxf) 2010;72:668 Sources of Research Support: Club Fran[ccedil]ais de l'Hypophyse. Disclosures: PC: Advisory Group Member, Eli Lilly & Company; Clinical Researcher, Ipsen, Novartis Pharmaceuticals, Novo Nordisk, Pfizer, Inc., Merck & Co.

Pub #	S29-2
Session Information	SYMPOSIUM SESSION: CLINICAL - Advances in the Management of Acromegaly (9:30 AM - 11:00 AM)
Title	How Good Are Non-Selective Somatostatin Analogues in Acromegaly?
Author String	S Petersenn ENDOC Center for Endocrine Tumors, Hamburg, Germany

Body

Transsphenoidal surgery is the first-line option for patients with acromegaly. However, many patients with macroadenomas will require adjuvant medical therapy. Currently licensed somatostatin analogues achieve disease control in 48-67%. They have also been suggested as primary treatment, with combined control of GH and IGF-1 in approximately 25% of patients. Their action is transduced by preferential binding to the somatostatin receptor subtype 2 (sst2), with lower affinity to sst5.

Due to the expression of additional subtypes in GH-secreting pituitary adenomas, a non-selective somatostatin analogue with high affinity for sst1,2,3 and sst5 was developed to improve the control of GH excess and tumor proliferation (1). Compared with octreotide, pasireotide has a binding affinity 40-, 30- and 5-fold higher for sst5, sst1 and sst3, respectively, and 2-fold lower for sst2.

Results from an early proof-of-concept study showed that single doses of subcutaneous pasireotide 100 and 250 [mu]g suppressed GH and IGF-I levels in a dose-dependent manner in 12 patients with acromegaly (2). Although GH was suppressed to a similar extent with pasireotide and octreotide in 8 patients, a significantly greater GH suppression was seen with pasireotide in 3 patients. A more recent Phase II, randomized, multicenter, open-label, three-way, crossover study included 60 patients with acromegaly (3). After treatment with octreotide 100 [mu]g sc tid for 28 days, each patient received pasireotide 200, 400 and 600 [mu]g sc bid in random order for 28 days. A biochemical response was defined as a reduction in GH to [le]2.5 [mu]g/liter and normalization of IGF-I. After 4 weeks of octreotide, 9% of patients achieved a biochemical response. After 4 weeks of pasireotide 200-600 [mu]g sc bid, 19% of patients achieved a biochemical response, which increased to 27% after 3 months of pasireotide; 39% of patients had a >20% reduction in pituitary tumor volume. The side effects of pasireotide resembled those of currently available somatostatin analogues. The mean HBA1C increased from 6.01% at pasireotide baseline to 6.45% at study end.

It is currently unknown whether the observed response rate in this study was influenced by a bias to include especially difficult to treat patients. The potential of pasireotide may only become clear in an ongoing randomized, Phase III study comparing octreotide and pasireotide.

Session supported by: Pfizer, Inc. & Ipsen, US

(1) Bruns C et al., EJE 2002; 146:707
(2) Van der Hoek J et al., JCEM 2004; 89:638
(3) Petersenn S et al., JCEM 2010; 95:2781

Disclosures: SP: Advisory Group Member, Speaker, Novartis Pharmaceuticals; Advisory Group Member, Speaker, Ipsen.

Pub #	S29-3
Session Information	SYMPOSIUM SESSION: CLINICAL - Advances in the Management of Acromegaly (9:30 AM - 11:00 AM)
Title	Long-Term Safety of Growth Hormone Receptor Antagonist
Author String	CJ Strasburger Charite Campus Mitte, Berlin, Germany
Body	The currently only available GH receptor antagonist for the treatment of patients of acromegaly is pegvisomant. In pivotal studies it had been demonstrated that disease activity can be adequately controlled in up to 90 % of all patients with acromegaly previously uncontrolled by other approved treatment modalities. Immediately after marketing authorization of pegvisomant, the German Pegvisomant Observational Study (GPOS) started documenting the use of this new medical treatment modality and until today succeeded in documenting 84 % of all patients receiving pegvisomant. By this drug's mechanism of action it had been feared initially that peripheral GH receptor blockade in analogy to Nelson's syndrome after bilateral adrenalectomy might promote expansion of the somatotrope pituitary adenoma. For this reason in GPOS and subsequently in the global ACROSTUDY surveillance program, central MRI reading by experts was offered in the context of the surveillance program. Within GPOS, in 5 % of patients adenoma enlargement had been suspected by local staff, but was confirmed after central reading of serial MRIs as a de-novo tumour enlargement after the onset of pegvisomant treatment only in less than 1 % of the currently 450 patients documented in GPOS. This tumour enlargement rate is well comparable with the rate observed under other medical treatment regimens. In 6 % of patients followed in GPOS, liver function test (LFT) elevation above 3 x upper limit of normal was observed with typically ALT being the leading enzyme elevated. In more than half of the patients, in whom LFT-elevations occurred, this resolved spontaneously without an alteration in pegvisomant dose. In all patients in whom the treatment was discontinued, LFT normalised thereafter. Initially, several cases of lipohypertrophy at the injection site were reported, prompted the recommendation to treating physicions to instruct their patients on rotating the injection site, which led to a remarkable reduction in frequency of such side effects being observed and hardly being reported in the recent years. In summary, the long term treatment with the peripheral GH receptor blocking drug pegvisomant is not associated with concerning safety issues, regular monitoring of pituitary adenoma size and liver function tests however, continues to be advisable. Session supported by: Pfizer, Inc. & Ipsen, US Disclosures: CJS: Speaker, Novartis Pharmaceuticals, Novo Nordisk; Scientific Board Member, Lilly USA, LLC; Advisory Group Member, Speaker, Pfizer, Inc.; Clinical Researcher, Ipsen.

Pub #	S30-1
Session Information	SYMPOSIUM SESSION: CLINICAL - Biological Mechanisms for Global Disparities in Type 2 Diabetes & Its Complications: Controversies & Advances (9:30 AM - 11:00 AM)
Title	Flatbush Diabetes: Unique Pathophysiologic Features of Type 2 Diabetes in Patients of African or Southeastern Asian Descent
Author String	MA Banerji State University of New York Health Science Center, Brooklyn, NY
Body	Disclosure Incomplete: MAB

Pub #	S30-2
Session Information	SYMPOSIUM SESSION: CLINICAL - Biological Mechanisms for Global Disparities in Type 2 Diabetes & Its Complications: Controversies & Advances (9:30 AM - 11:00 AM)
Title	Disparities in Type 2 Diabetes among Indigenous Populations around the World
Author String	J Toumilehto University of Helsinki, Helsinki, Finland
Body	Nothing to Disclose: JT

Pub #	S30-3
Session Information	SYMPOSIUM SESSION: CLINICAL - Biological Mechanisms for Global Disparities in Type 2 Diabetes & Its Complications: Controversies & Advances (9:30 AM - 11:00 AM)
Title	Genetic & Ethnic Considerations in Type 2 Diabetes: Complications around the World: How Much Does Biology Matter?
Author String	HE Lebovitz State University of New York Health Science Center at Brooklyn, Staten Island, NY
Body	Minority diabetic populations have different outcomes than Caucasian diabetic populations. African American and Hispanic type 2 diabetic populations have more visual problems and end stage renal disease. Mortality from cardiovascular disease is higher in minority diabetic populations. A confounding factor in assessing ethnic differences is the heterogeneity of ethnic minorities. Individuals of African descent have their origins from different African tribes. Hispanic populations are composed of individuals from Cuban, Mexican, Puerto Rican and other Caribbean and South American backgrounds. Asians are from Oriental or South Asian Indian origins. Consequently the results of studies depend on the origins of the ethnic population recruited. African Americans with the same BMI and percent body fat as Caucasians have smaller visceral adipose tissue pools, lower hepatic triglyceride content, and a less atherogenic lipid profile (lower LDL-cholesterol, lower plasma triglyceride, and higher HDL-cholesterol). They are more insulin resistant and have greater basal hyperinsulinemia than Caucasians. However they are not able to increase insulin secretion in response to increases in glucose as well as Caucasians and have greater insulin insufficiency than Caucasians. African Americans have lower basal hepatic glucose production than Caucasians. Past studies have shown a lower prevalence of coronary heart disease in African Americans as compared to Caucasians. Hispanic Americans as well as Caucasians with type 2 diabetes have increased visceral adipose tissue pools, increased hepatic steatosis and dyslipidemia compared to their non-diabetic controls. Asian Indians have greater body fat and visceral fat than Caucasians of comparable BMI. Asian Indians are more insulin resistant than comparable other ethnic populations. They have higher plasma free fatty acids, lower adiponectin levels and more hepatic steatosis. The prevalence of coronary artery disease is higher in Asian Indian diabetic populations than in Caucasian diabetic populations. Oriental Asians have a more severe insulin deficiency than other Asian or Caucasian populations. Metabolic differences in ethnic populations need to be considered in developing treatment strategies. It is important to determine the extent to which ethnic and racial disparities in the prevalence, treatment and clinical outcomes are related to social factors versus differences in genetic background and pathophysiology. Nothing to Disclose: HEL

Pub #	S31-1
Session Information	SYMPOSIUM SESSION: CLINICAL - Controversies in the Clinical Guidelines for Diagnosis & Management of Male Hypogonadism (9:30 AM - 11:00 AM)
Title	Diagnosis of Male Hypogonadism
Author String	FJ Hayes Saint Vincent's University Hospital, Dublin, Ireland
Body	Nothing to Disclose: FJH

Pub #	S31-2
Session Information	SYMPOSIUM SESSION: CLINICAL - Controversies in the Clinical Guidelines for Diagnosis & Management of Male Hypogonadism (9:30 AM - 11:00 AM)
Title	Management of Male Hypogonadism: Who, When, What & How?
Author String	AM Matsumoto Veterans Affairs Puget Sound Health Care System, Seattle, WA
Body	Guidelines[1,2] for the management of male hypogonadism are based on a limited body of evidence, resulting in controversial recommendations regarding who, when, what and how to treat men with non-specific symptoms and signs of androgen deficiency and consistently low testosterone (T) levels. In the absence of long-term outcome studies, practitioners cannot be overly dogmatic about treating or not treating specific men and how to monitor individuals during therapy. Guidelines provide a framework that should be used with sound clinician judgment and informed patient decisions to direct management. Treatment and monitoring decisions should be based on the balance of potential clinical benefits versus risks. Men with poor sexual development and severe manifestations of androgen deficiency or castrate serum T levels will benefit the most, while those with few manifestations or slightly low T levels are less likely to benefit. Treatment is more likely to be beneficial in men with loss of libido, spontaneous erections or body hair; symptomatic gynecomastia; low serum free T levels and an organic versus functional etiology, and less likely in men with multiple co-morbidities. The risk of T treatment is unacceptably high in men with active prostate and breast cancer, while the risks in men with baseline high hematocrits, severe lower urinary tract symptoms, untreated obstructive sleep apnea and uncontrolled heart failure are less clear. The choice of T formulations is based on patient preference, pharmacokinetics, burden and cost. During T treatment, monitoring of both the efficacy and potential adverse effects should be performed with a goal of mid-normal T levels. Lack of clinical response to normalization of T should prompt re-evaluation of continued therapy. Despite a lack of evidence, there is trepidation that T therapy will stimulate prostate cancer growth, and assessment of baseline risk and monitoring for prostate cancer using digital rectal examination and PSA are recommended. In this regard, it is important to distinguish *screening* for prostate cancer in the general population from *monitoring* for prostate cancer growth in hypogonadal men during T therapy, as the latter may alter the natural history of prostate cancer progression. Because prostate cancer is common and may be aggressive in some men, it is prudent to assess baseline risk and monitor for clinical prostate cancer shortly after starting T therapy, but longer-term monitoring is more controversial. 1. Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, Montori VM; Task Force, Endocrine Society. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2010;95:2536-59. 2. Wang C, Nieschlag E, Swerdloff R, Behre HM, Hellstrom WJ, Gooren LJ, Kaufman JM, Legros JJ, Lunenfeld B, Morales A, Morley JE, Schulman C, Thompson IM, Weidner W, Wu FC. Investigation, treatment and monitoring of late-onset hypogonadism in males. Int J Androl. 2009;32:1-10. Disclosures: AMM: Principal Investigator, GlaxoSmithKline; Investigator, Solvay Pharmaceuticals, Inc.; Editor, Up To Date.

Pub #	S31-3
Session Information	SYMPOSIUM SESSION: CLINICAL - Controversies in the Clinical Guidelines for Diagnosis & Management of Male Hypogonadism (9:30 AM - 11:00 AM)
Title	Adverse Effects of Testosterone Therapy
Author String	SS Basaria Boston University, Chestnut Hill, MA
Body	Recently there has been a growing interest in the use of testosterone replacement in men. This interest extends beyond those men who have classic symptoms of androgen deficiency. Large numbers of testosterone prescriptions are being given to men with age-related decline in serum testosterone levels, even though benefits of long-term testosterone replacement in this subset of men remain unknown. The risks of testosterone replacement in men, particularly in older men, also remain unclear. The Expert Panel formed by the Institute of Medicine has recommended a series of efficacy trials of testosterone replacement in older men and has suggested that plans for safety trials be deferred until efficacy is established. In the meantime, there is a need to educate practicing clinicians regarding the potential risks of testosterone replacement. Polycythemia remains a common adverse effect of testosterone replacement that is encountered in both clinical and research settings, and is seen more frequently in older men and in men who are on intramuscular preparations. Meta-analyses of clinical trials evaluating the effect of testosterone replacement on lipids have shown a slight decrease in HDL cholesterol in men randomized to testosterone without any significant effect on other lipoproteins. The effect of testosterone replacement on obstructive sleep apnea remains inconclusive and only well-designed trials will broaden our understanding of this association. Prostate health has been a major concern for both the patients and the clinicians. A previous meta-analysis of randomized controlled trials had shown that testosterone replacement was associated with a significant increase in overall prostate events; however, a recent meta-analysis that included larger number of randomized trials did not confirm this finding. The issue of prostate safety can only be resolved conclusively by large long-term safety trials. Although cardiovascular safety of testosterone therapy has always been a concern among the clinicians, clinical trials in healthy older men have not shown increased cardiovascular adverse effects in men randomized to testosterone. A recent trial of testosterone replacement in older men with mobility limitation was prematurely stopped due to increased cardiovascular adverse events in men randomized to testosterone. This talk will mainly focus on prostate and cardiovascular adverse effects of testosterone replacement in men (particularly in older men). 1. Bhasin S et al., JCEM 2010; 95:2536. 2. Haddad RM et al., Mayo Clin Proc 2007; 82:29. 3. Fernandez-Balsells MM et al., JCEM 2010; 95:2560. 4. Basaria S et al., NEJM 2010; 363:109 Nothing to Disclose: SSB

Pub #	NS3-1
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Cushing (9:45 AM - 10:45 AM)
Title	Diagnosis & Management of Cushing Syndrome
Author String	AL Utz Vanderbilt University, Nashville, TN
Body	Cushing's syndrome is a rare disorder, but due to its significant morbidity and mortality it is important to identify individuals with this disease. Many of the clinical findings of Cushing's syndrome are common in the general population, making it difficult to determine when to screen for the disorder. The specificity of the clinical signs and symptoms of Cushing's syndrome will be discussed. The biochemical tests used to establish that an individual has elevated cortisol levels will be explained, with a comment on dealing with individuals who potentially have reactive elevations in cortisol, known pseudo-Cushing's syndrome. Cushing's syndrome is most commonly due to a pituitary adenoma but may be due to tumors in the adrenal gland(s) or elsewhere, known as ectopic tumors. Methods for localizing the responsible tumor will be described. Surgery is the first line therapy for most cases of Cushing's syndrome. However in certain individuals, or those with unsuccessful surgical resection, other therapies are utilized, including radiation and medications. Transient, and in some cases permanent, adrenal insufficiency is present following successful surgical intervention for Cushing's syndrome and appropriate management is critical. Due to the possibility of Cushing's syndrome recurrence, long-term follow up of these patients is necessary. Nothing to Disclose: ALU

Pub #	NS4-1
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Complexities of Congenital Adrenal Hyperplasia: Implications for Adolescents & Adults (10:45 AM - 11:45 AM)
Title	Complexities of Congenital Adrenal Hyperplasia: Implications for Adolescents & Adults
Author String	SF Witchel Children's Hospital of Pittsburg/University of Pittsburgh, Pittsburgh, PA
Body	CAH is a common autosomal recessive disorder. The clinical spectrum ranges from classical salt-losing CAH associated with significant mineralocorticoid deficiency to simple virilizing to non-classical CAH (NCAH). Male & female infants with the salt-losing form may present with dehydration, hyponatremia, & hyperkalemi during the first weeks of life. Boys & girls with simple virilizing CAH may present with early development o pubic hair, phallic enlargement, & tall stature. Infant girls with salt-losing & simple virilizing forms often have genital ambiguity (prominent phallus, labio-scrotal fusion, & non-palpable gonads). Boys & girls with the severe forms of CAH are often detected through state NBS programs. Children with NCAH often present with premature pubic hair development. Pediatric endocrinologists have traditionally provided care for infants, children, & adolescents with CAH. As young adults transition to college & the workforce, the availability of informed medical care often changes. Increasingly, data indicate the benefits of defined transition planning for such young adults (1). In preparation for this process, health care providers & parents should understand the evolution of cognitive & emotional maturation in adolescents & young adults. Review of genital anatomy, vaginal dilation, potential for additional surgery, sexual intercourse following feminizing genitoplasty, pregnancy, & childbirth is important (2). Discussions regarding fertility & genetic counseling ar warranted. Rather than viewing the young adult as non-compliant, it is important to consider the young adult's schedule & work together to develop an appropriate therapeutic regimen for hormone replacement. Young adults need to comprehend the biology & genetics of CAH to be able to advocate for their own health care since they may encounter health care professionals who are less knowledgeable about CAH. A structured program in which young adults have ownership of their medical records has been demonstrated to be more effective (1). The precise age at transition depends on the young adult's specific needs & their environment. The overall goal is to transition the young adult from being the consumer to being the chief executive officer (CEO) of his/her health. Collaboration, cooperation, & teamwork among patients, families, & the network of pediatric & adult health care providers increase the chances for successful transition into the adult world. 1. Cadario F, et al., Clin Endocrinol(Oxf) 2009;71:346 2. Auchus RJ., et al. Int J Pediatr Endocrinol 2010;(in press) Disclosure Incomplete: SFW

Pub #	
Session Information	CONFERENCE EVENT: ENDO's Got Talent (11:00 AM - 12:30 PM)
Title	ENDO's Got Talent
Author String	
Body	

Pub #	OR10-1
Session Information	ORAL SESSION: BASIC - Gene Regulation of Growth Control & Homeostasis (11:15 AM-12:45 PM)
Title	Evidence That Transcription Factor E2f3 Contributes to the Down-Regulation of Multiple Growth-Promoting Genes during Juvenile Growth Deceleration
Author String	JCK Lui, J Baron NICHD, Bethesda, MD

Body

In mammals, body growth is rapid in early life but decelerates with age and ceases as the adult body size is reached. We recently showed evidence that body growth deceleration results in part from the downregulation of a large set of growth-promoting genes with age in multiple organs. These genes include growth factors (e.g *Igf2, Mdk*), transcription factors (e.g. *Ezh2, Plagl1, Mycn*), and cell cycle regulators (*Cdk4, Cdc6, Skp2*). We hypothesized that this downregulation of hundreds of genes with age is orchestrated by transcription factors that commonly bind to the promoters of a large set of genes. To explore this possibility, we analyzed the 235 genes that are uniformly downregulated with age in heart, kidney, and lung using DiRE (Distant Regulatory Elements of co-regulated genes). This bioinformatic method most strongly implicated the *E2f* family of transcription factors. Expression microarray in mouse showed that of the eight *E2f* family members, *E2f3* was significantly downregulated from 1 to 4 wks of age in kidney, lung, and heart (all $P < 0.001$ and >1.5-fold). These findings were then confirmed by quantitative real-time PCR (all $P < 0.05$) and western blot analysis in kidney, lung, and liver at 1, 4, and 8 wks. Using chromatin immunoprecipitation, we next found that, in mouse kidney, *E2f3* binding was downregulated at the promoter regions of *Cdk4, Cdc6, Ezh2, Mycn, Peg3,* and *Bub1* from 1 to 4 wks of age (all $P < 0.05$). This decreased binding of *E2f3* with age may cause the downregulation of these growth-promoting genes and thus help drive juvenile growth deceleration.
Taken together, our findings suggest that juvenile growth deceleration results from the downregulation of a large set of genes important for cell cycle progression and cell proliferation. This downregulation may be orchestrated in part by the decreased expression and reduced promoter binding of *E2f3* in a subset of genes. Although *E2f3* is a well-established activator of cell proliferation *in vitro*, this is the first study exploring its role in juvenile growth deceleration.

Sources of Research Support: Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH.

Nothing to Disclose: JCKL, JB

Pub #	OR10-2
Session Information	ORAL SESSION: BASIC - Gene Regulation of Growth Control & Homeostasis (11:15 AM-12:45 PM)
Title	Direct Linkage of Noncoding Transcription to the Activity of the *hGH-N* Long-Range Enhancer
Author String	EJ Yoo, SA Liebhaber, NE Cooke University of Pennsylvania School of Medicine, Philadelphia, PA
Body	The expression of the *human Growth Hormone (hGH-N)* gene is dependent on the actions of a set of remote regulatory elements that comprise the *hGH* locus control region (LCR). The components of the *hGH* LCR are marked by a set of DNase I hypersensitive sites (HS) distributed from -14.5 kb to -32 kb 5' to the *hGH-N* gene. This LCR is necessary and sufficient to establish an autonomous chromatin domain and supports robust and tissue-specific *hGH-N* expression in the somatotropes of the anterior pituitary. The pituitary-specific HSI, located at -14.5 kb from the *hGH-N* promoter, constitutes the major enhancer element of *hGH-N* expression. The mechanism(s) that underlie the ability of HSI to act as a remote enhancer of *hGH-N* expression remain to be fully determined. We previously identified a discrete domain of noncoding transcription immediately 3' to HSI that is pituitary-specific and linked to HSI actions. Whereas recent mammalian genome-wide studies hav identified multiple regions of noncoding transcription that map to enhancers, critical questions regarding how these noncoding transcription units are activated and mediate their putative enhancer function(s) remain unanswered. In the current study we have used a set of transgenic mouse models to explore the relationship between HSI enhancer activity and its noncoding transcription. The data revealed that this domain of noncoding transcription can be initiated in an autonomous manner by HSI, independent of the presence of the target *hGH-N* promoter. By constructing a 'synthetic' noncoding domain within the *hGH/BAC* transgene we were able to further demonstrate that HSI enhancer activity was quantitatively linked to the levels of noncoding transcription and that the enhancer-linked activity of this domain is a direct function of its transcriptional activity, independent of its encoded RNA. These results lead us to propose a novel pathway of pituitary-specific long-range enhancement of *hGH-N* expression by HSI. This pathway is initiated via the establishment of a domain of robust noncoding PolII-mediated transcription adjacent to the HSI. Subsequent higher-order chromatin 'looping' then places the *hGH-N* promoter within this PolII-enriched enhancer environment. This juxtaposition of the *hGH-N* promoter with the domain of PolII enrichment supports sustained and robust *hGH-N* gene expression in the terminally-differentiated somatotrope. Sources of Research Support: NIH Grants R01 HD/DK25147 and R01 HD/DK046737 (to NEC and SAL). Nothing to Disclose: EJY, SAL, NEC

Pub #	OR10-3
Session Information	ORAL SESSION: BASIC - Gene Regulation of Growth Control & Homeostasis (11:15 AM-12:45 PM)
Title	High-Throughput Molecular Characterization of Epigenetic and Genetic Defects in Patients with Pseudohypoparathyroidism (PHP) 1b
Author String	JA Danzig, S Jan de Beur, MA Levine The Children's Hospital of Philadelphia, Philadelphia, PA; Johns Hopkins University, Baltimore, MD
Body	PHP1b is characterized by parathyroid hormone (PTH) resistance, which in most patients is due to an epigenetic defect within the maternal *GNAS* allele that abrogates expression of the α-subunit of the stimulatory G protein (Gsα) in renal proximal tubule cells. In addition to Gsα, the *GNAS* locus encodes additional imprinted transcripts, including the maternally expressed NESP55 and the paternally expressed XLαs, antisense and 1A transcripts that are associated with differentially methylated regions (DMRs). Abnormal methylation of one or more maternal *GNAS* DMRs is often associated with small deletions in the NESP55 and XLαs antisense exons or the *STX16* gene in familial PHP1b, but genetic defects have not been identified in sporadic PHP1b. We used high throughput techniques to characterize the epigenetic and genetic defects in 40 PHP1b patients (9 families with 14 affected members and 26 sporadic cases) and 3 unaffected PHP1b carriers. Controls included 13 normals and 3 patients with PHP1a. We used pyrosequencing to analyz ^{me}CpG in NESP55, XLαs and 1A DMRs and performed high resolution array comparative genomic hybridization (aCGH) with custom-made microarrays (135,000 oligonucleotide probes with 10 bp tiling coverage of *STX16* through *GNAS* on 20q13.3) to identify genetic mutations. 33 of 40 (83%) of PHP1b patients had loss of methylation at exon 1A DMR. Most patients showed one of two patterns of DMR methylation: gain of methylation at NESP55 and loss of methylation at XLαs and 1A (HLL) or normal methylation for NESP55 and XLαs and loss of methylation at 1A (NNL). 8 of 9 families (89%) were NNL. Sporadic cases were HLL (38%), NNL (15%), HNL (12%) or NNN (15%). All carriers, normals and PHP1a were NNN. 4 of 19 PHP1b patients who underwent aCGH had deletions, including a novel 9-kbp *NESP55* deletion, which were confirmed by quantitative PCR. We conclude that pyrosequencing and targeted aCGH affords high throughput and quantitative analysis of epigenetic and genetic defects in PHP1b. Patients with familial PHP1b usually have NNL methylation and an *STX16* deletion or HLL methylation with deletions in or near NESP55. By contrast, patients with sporadic PHP1b have variable methylation patterns and rare deletions. We propose that sporadic PHP1b with HLL methylation pattern results from imprint erasure defects that occur in female primordial germ cells. In this case, a paternal methylation imprint may not be erased and is transmitted through the oocyte to the embryo. Nothing to Disclose: JAD, SJdB, MAL

Pub #	OR10-4
Session Information	ORAL SESSION: BASIC - Gene Regulation of Growth Control & Homeostasis (11:15 AM-12:45 PM)
Title	Ultradian Glucocorticoid Exposure Results in Distinct Cycles of Transcriptional Co-Activator Recruitment and Chromatin Acetylation at the *Period 1* Promoter
Author String	CL George, JR Pooley, SL Lightman, BL Conway-Campbell University of Bristol, Bristol, UK
Body	During basal (non-stressed) conditions mammals secrete glucocorticoids (GCs) in distinct pulses [1]. Peak plasma GC levels occur at approximately 60 min intervals in rats during the circadian GC rise, thus resulting in hourly 'ultradian' fluctuations in GC exposure & glucocorticoid receptor (GR) activation [2,3]. However the dynamics of GR-induced recruitment of transcriptional co-activators (e.g. histone acetyl transferases CBP & p300) & the effects on chromatin acetylation at the promoters of GC-regulated genes following pulsatile GC exposure remain unknown. Using chromatin immunoprecipitation of murine pituitary AtT-20 cells we tested whether pulsatile (hourly) GC exposure statically or cyclically recruited transcriptional co-activators p300 & CBP, or altered chromatin acetylation at the glucocorticoid response element (GRE) in the *Period 1* promoter. In accordance with *in vivo* studies [2,3], within 15 min each GC pulse resulted in a wave of GR binding at the GRE that returned to basal levels 60 min later, prior to the addition of the second GC pulse which subsequently resulted in a second cycle of GR binding. Notably we found that both p300 and CBP were recruited & dispelled rapidly from the *Period 1* promoter within the 60 min following each GC exposure. Furthermore the binding of GR, CBP & p300 at the GRE correlated with increased promoter acetylation, that sharply returned to basal levels 1 hr after the start of each GC pulse. Therefore we present evidence that pulsatile GC exposure results in temporally dynamic cyclical recruitment of transcriptional co-activators, producing rapidly reversing alterations in acetylation status at the promoter of a GC-target gene. Given that previous studies into GC-induced transcriptional regulation and chromatin modification have typically used models of prolonged GC exposure & GR activation, we demonstrate the first insight into the rapid temporal changes in GC-induced transcriptional co-factor recruitment & chromatin modification stimulated during a physiologically relevant ultradian exposure to GCs. [1] Lightman SL et al., Eur J of Pharmacol 2008; 583: 255-262. [2] Stavreva DA et al., Nat Cell Biol 2009; 11: 1093-1102. [3] Conway-Campbell BL et al., J Neuroendocrinol 2010; 22: 1093-1100 Sources of Research Support: Wellcome Trust Programme Grant (074112/Z/04/Z); Needham Cooper Trust UK Studentship. Nothing to Disclose: CLG, JRP, SLL, BLC-C

Pub #	OR10-5
Session Information	ORAL SESSION: BASIC - Gene Regulation of Growth Control & Homeostasis (11:15 AM-12:45 PM)
Title	Transcriptional Up-Regulation of 11β-Hydroxysteroid Dehydrogenase Type 1 by Pro-Inflammatory Cytokine Is Mediated by C/EBPβ Transcription Factor
Author String	CL Esteves, EA Rog-Zielinska, V Kelly, M Nixon, JR Seckl, KE Chapman University of Edinburgh, Edinburgh, UK
Body	Metabolic syndrome, an inflammatory condition comprising a constellation of cardiovascular risk factors (visceral obesity, type 2 diabetes, hypertension, dyslipidaemia) resembles Cushing's syndrome of chronic plasma glucocorticoid excess. Although plasma glucocorticoids are normal in metabolic syndrome, glucocorticoid action within adipose tissue is increased by selective overexpression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) which converts inert cortisone into active cortisol. The mechanisms underlying increased adipose 11β-HSD1 in obesity remain unclear, but expression is potently induced, including in pre-adipocytes, by the pro-inflammatory cytokines IL-1 and TNFα. Here, we show that these pro inflammatory cytokines increased 11β-HSD1 mRNA levels in human SGBS adipocytes (TNFα, 41.5±7.5-fold; $p<0.05$) and A549 lung epithelial cells (IL-1, 56.6±4.3-fold; TNFα, 11.7±1.8-fold; both $p<0.001$). In A549 cells, this effect was attenuated by inhibition of p38 MAPK signalling (IL-1, 3.3±0.03-fold; TNFα, 1.9±0.01-fold; both $p<0.05$), while MEK and PKA inhibition had the opposite effect. In contrast, induction of 11β-HSD1 by the synthetic glucocorticoid dexamethasone (20.8±1.9-fold; $p<0.001$), previously showed to be mediated by C/EBPβ, was not affected by inhibition of any of these signalling pathways. Concomitantly with transcriptional up-regulation of 11β-HSD1 by cytokines, there was an increase in mRNA levels encoding the transcription factors C/EBPβ (IL-1, 2.7±0.3-fold; TNFα, 2.1±0.3-fold; both $p<0.05$) and C/EBP[delta] (IL-1, 1.6±0.1-fold; TNFα, 1.7±0.1-fold; both $p<0.05$). siRNA-mediated knock-down demonstrated that only C/EBPβ is essential for the induction of 11β-HSD1 expression by cytokines. Chromatin immunoprecipitation showed increased binding of C/EBPβ to the 11β-HSD1 promoter upon cytokine treatment. These results indicate that C/EBPβ is central to the induction of 11β-HSD1 transcription in response to both pro- (cytokine) and anti-inflammatory (glucocorticoid) stimuli. Pro-inflammatory cytokines plausibly contribute to the up-regulation of 11β-HSD1 in adipose tissue in obesity. Sources of Research Support: Wellcome Trust Programme Grant awarded to JRS and KEC. Nothing to Disclose: CLE, EAR-Z, VK, MN, JRS, KEC

Pub #	OR10-6
Session Information	ORAL SESSION: BASIC - Gene Regulation of Growth Control & Homeostasis (11:15 AM-12:45 PM)
Title	Androgen-Induced Activation of Gonadotropin-Regulated Testicular RNA Helicase (GRTH/DDX25) Transcription: Essential Role of a Non-Classical Androgen Response Element Half-Site
Author String	J Villar, C-H Tsai-Morris, ML Dufau National Institutes of Health, Bethesda, MD
Body	GRTH, a testis-specific member of the DEAD-box family of RNA helicases essential for spermatogenesis is present in Leydig cells (LC) and germ cells. In the LC, GRTH exerts a negative role in StAR mRNA stability with consequent reduction in mitochondrial cholesterol and androgen production by gonadotropin (1). GRTH is transcriptionally up-regulated in LC by gonadotropin via cAMP/androgen through androgen receptor present in these cells. To study the mechanism of androgen regulation, we utilized GT1-7 cells stably expressing the AR. Androgen-induced GRTH expression was prevented by the AR antagonist. Two putative atypical ARE half-sites are present at -800 (ARE2) and -200 bp (ARE1) of the GRTH 5'-UTR. Point mutation of ARE2 prevented androgen-induced up-regulation of GRTH transcription. DNA precipitation-assay using nuclear GT1-7 extract showed androgen-induced AR binding to ARE2 prevented by a point mutation and adjacent sequences do not participate in AR binding or function. ChIP assay in GT1-7 showed recruitment of AR as well of coactivator SRC-1, Med-1, TFIIB and PolII to GRTH ARE2 (-980/-702 bp) and to (-80/+63 bp). Chip-3D revealed chromosomal looping between AR/ARE2 and the core transcriptional machinery at the promoter presumably through mediator complex. Knock-down of SRC-1 and/or Med 1 demonstrated their essential role in the transcriptional activation of GRTH by AR. The in vitro findings were corroborated by in vivo studies in LC of mice with stimulation androgen induced by hCG where significant increased GRTH expression was observed. Our findings provide valuable insights on the molecular mechanism of androgen regulated GRTH transcription. Thus, GRTH gene transcription induced by androgen in turn regulates androgen production. (1)Fukushima et al., Endocrine Society's Annual Meeting Abstract #P2-155, 2010 Nothing to Disclose: JV, C-HT-M, MLD

Pub #	OR11-1
Session Information	ORAL SESSION: BASIC/TRANSLATIONAL - Adipogenesis, Adiposity & Adipocyte Biology: Novel Findings in Human Adipose Tissue (11:15 AM-12:45 PM)
Title	Human Subcutaneous Preadipocytes Enhance HIV-1 Replication in Primary CD4+ Memory T Cells
Author String	J Couturier, A Balasubramanyam, DE Lewis University of Texas Health Science Center at Houston, Houston, TX; Baylor College of Medicine, Houston, TX
Body	The mechanisms underlying adipocyte abnormalities in patients with HIV-associated lipodystrophy are unclear and likely to be complex, with possible contributions from the effects of antiretroviral drugs and a proinflammatory state secondary to HIV infection or immune reconstitution following treatment. T cells and macrophages are the principal immune cells infected by HIV; their migration into adipose tissues is increased during HIV infection, leading to the possibility of pathogenic cross-talk between HIV-infected immune cells and adipocytes. We hypothesized that adipocytes in close proximity to HIV infected T cells or macrophages might affect viral replication, since they produce cytokines and chemokines (e.g., IL-6, TNFα, RANTES, MIP-1α, MIP-1β) known to influence HIV production. Hence, we conducted in vitro co-culture experiments (using transwells) between HIV-infected human CD4+ memory T cells (the main T cell subset infected by HIV which also routinely migrate into adipose tissues) and primary human subcutaneous preadipocytes. The T cells were infected with 3 pathogenic HIV-1 strains (primary X4-, R5-, or R5X4-tropic) just prior to co-culture with preadipocytes. After 3 and 6 days of co-culture, X4- and R5-tropic HIV replication was consistently increased by preadipocytes as determined by extracellular HIV p24 levels. Other important characteristics associated with increased HIV replication in T cells were also found to be affected by preadipocytes, such as T cell activation and proliferation, and cell cycle effects. Surface expression of the early T cell activation marker CD69 was increased nearly 2-fold by preadipocytes, as measured by flow cytometry. Preadipocytes also increased T cell proliferation, as measured by flow cytometry bead counting. Finally, preadipocytes enhanced G2 arrest, a classic feature of HIV-infected T cells, as measured by flow cytometry cell cycle analysis. These findings suggest that adipose tissues accelerate HIV replication in infected resident T cells, which may promote a localized, self-amplifying pathogenic cascade leading to HIV lipodystrophy. Furthermore, there was an effect of HIV-infected T cells upon preadiopcytes, manifested by a block in adipogenic differentiation. Ongoing work is aimed at characterizing how preadipocytes increase HIV production, and the nature of the inimical response to HIV that leads in turn to defects in preadipocyte differentiation and adipocyte dysfunction. Nothing to Disclose: JC, AB, DEL

Pub #	OR11-2
Session Information	ORAL SESSION: BASIC/TRANSLATIONAL - Adipogenesis, Adiposity & Adipocyte Biology: Novel Findings in Human Adipose Tissue (11:15 AM-12:45 PM)
Title	Determinants of the PPARγ Posttranslation Modification Code during Human Adipogenesis
Author String	SM Hartig, MA Mancini Baylor College of Medicine, Houston, TX
Body	Tight regulation of peroxisome proliferator-activated receptor gamma (PPARγ) is required to maintain adipocyte function and energy balance. PPARγ mRNA and protein levels are increased during adipogenesis due to the action of several transcription factors and coregulators, while PPARγ activity is regulated by post-translational modifications (PTMs). Different PPARγ ligands induce unique conformational changes that expose PTM motifs to direct gene networks for distinct biological effects. Although PTMs are central for nuclear receptor activity, PTMs regulating PPARγ function are largely unidentified. Using automated microscopy-based high content analysis (HCA), we have recently discovered a novel coactivator setpoint in human adipocytes that controls PPARγ-dependent lipogenesis and cell-cell phenotypic heterogeneity by attenuating a negative regulatory PTM on PPARγ at S114 (Hartig et al, 2011. J. Cell Biol. 192:55). Follow-up studies showed that phospho-PPARγ S114 is decreased in a ligand-dependent manner with rosiglitazone being the most repressive agonist. In parallel HCA-based screening of all human nuclear receptors (48) and 303 coregulators, we have identified a small ubiquitin modifier (SUMO) E2-conjugation enzyme (Ubc9) as a repressor of human adipogenesis. Ubc9 knockdown increased lipid content, lipid droplet size and number, suggesting that Ubc9-mediated SUMOylation of PPARγ attenuates lipogenesis. Previous studies have proposed that K109 SUMOylation, by Ubc9, represents an additional PPARγ PTM that couples with S114 phosphorylation to reinforce repression of PPARγ target genes. Thus, we hypothesize that N-terminal phosphorylation and upstream SUMOylation selectively modulates the action of therapeutic PPARγ ligands. mRNA profiling during adipocyte differentiation showed that Ubc9 expression during adipocyte differentiation is decreased and inversely proportional with classic adipocyte and lipogenic genes. Consistent with our previous findings, adipocyte differentiation with natural or synthetic PPARγ ligands downregulated Ubc9 protein levels, coincident with decreased PPARγ S114 phosphorylation. Quantitative imaging and FRET approaches are ongoing to identify differential and selective coregulator recruitment to the PPARγ phospho-SUMOyl motif that defines the PTM code of natural or synthetic PPARγ agonists. The translation of this code is fundamental to the development of current and future drugs that target PPARγ in type 2 diabetes. Nothing to Disclose: SMH, MAM

Pub #	OR11-3
Session Information	ORAL SESSION: BASIC/TRANSLATIONAL - Adipogenesis, Adiposity & Adipocyte Biology: Novel Findings in Human Adipose Tissue (11:15 AM-12:45 PM)
Title	Reduction in the Expression of β-Amyloid Precursor Protein (APP) Following Roux-en-Y Gastric Bypass Surgery (RYGB) and Weight Loss
Author String	P Dandona, H Ghanim, S Monte, CL Sia, J Schentag, S Dhindsa, J Caruana State University of New York at Buffalo, Buffalo, NY; State University of New York at Buffalo, Buffalo, NY; Sisters of Charity Hospital, Buffalo, NY
Body	Obesity and type 2 diabetes are associated with an increase in the incidence and prevalence of Alzheimer's disease (AD). We have recently demonstrated that peripheral blood mononuclear cells (MNC) express APP, the precursor of β-amyloid, which forms the pathognomonic plaques in the brain. We hypothesized that APP expression diminishes after the marked caloric restriction and weight loss associated with RYGB. Fifteen type 2 diabetic patients with morbid obesity underwent RYGB following which their caloric intake diminished and they lost 38.5 ±2.9 Kg in weight over 6 months. BMI fell from 54.4±3.1 to 40.5± 2.9 Kg/m2. There was a significant fall in plasma concentrations of glucose (from 148±8 to 101±4mg/dl), insulin (from 18.5±2.2 to 8.6±1.0[micro]U/ml) and HOMA-IR (from 7.1±1.1 to 2.1±0.3).). The expression of APP mRNA fell by 31±9% and that of protein fell by 22±12%. In addition, there was a reduction in other AD related genes including presinilin 2 (PN2) which fell by 27±10%, ADAM-9 which fell by 35±12% and GSK-3β which fell by 28±6% ($P<0.01$ for all). PN2 mediates the conversion of APP into β-amyloid while GSK-3β hyperphosphorylates tau protein to form the neurofibrillary tangles in the brains of patients with AD. These changes occurred in parallel with reductions in other pro-inflammatory mediators including CRP (from 10.7±1.6 to 5.8±1.0mg/L, $P<0.001$). Thus, the reversal of the pro-inflammatory state of obesity is associated with a concomitant reduction in the expression of APP and other AD related genes in MNC. We conclude that obesity and caloric intake modulate the expression of APP in MNC. If indeed, this effect also occurs in the brain, this may have implications for the pathogenesis and the treatment of AD. It is relevant that cognitive function has been shown to improve with weight loss following bariatric surgery. Sources of Research Support: NIH Grants R01-DK075877 and R01-DK069805 awarded to PD; American diabetes Grant awarded to PD; American Diabetes Grant 10-JF-13 awarded to SD. Disclosures: PD: Principal Investigator, GlaxoSmithKline; Clinical Researcher, Sanofi-Aventis; Speaker, Novartis Pharmaceuticals; Eli Lilly & Company; GlaxoSmithKline; Sanofi-Aventis. SD: Speaker, Abbott Laboratories. Nothing to Disclose: HG, SM, CLS, JS, JC

Pub #	OR11-4
Session Information	ORAL SESSION: BASIC/TRANSLATIONAL - Adipogenesis, Adiposity & Adipocyte Biology: Novel Findings in Human Adipose Tissue (11:15 AM-12:45 PM)
Title	Abdominal Subcutaneous White Adipose Tissue in Surgically Slimmed Ex-Obese Shows the Same Molecular Features as Morbidly Obese Adipose Tissue
Author String	R Cancello, A Zulian, D Gentilini, M Maffei, A Della Barba, A Liuzzi, AM Di Blasio Istituto Auxologico Italiano, Cusano Milanino, Italy; Istituto Auxologico Italiano, Milano, Italy; University of Pisa, Pisa, Italy; Casa di Cura Igea, Milano, Italy; Istituto Auxologico Italiano, Verbania, Italy
Body	Bariatric surgery represents a fast and powerful treatment for morbid obesity. However, after stabilization of weight loss, ex-obese patients face the problem of removing residual tissues such as flabby skin, abdominal skin overhang, pendulous breast. Moreover, it is not known whether their adipose tissue is restored to a non-obese condition. In order to clarify this issue, we compared gene expression profile of ex-obese abdominal subcutaneous white adipose tissue (SAT) with that of SAT from lean, overweight and obese patients of different classes of body mass index (BMI), according to the WHO classification. A total of 32 samples of human SAT were collected from patients undergoing different surgical interventions (n= 32, female, mean BMI±SD:32.4±9.5 kg/m^2, mean age±SD: 46.9±12.3 years). These samples were classified and grouped, according to the BMI as: normal-weight (NW, BMI[le]25), over-weight and never obese (OW, 25< BMI[le] 30), slimmed ex-obese (ExOB, 25< BMI[le]30), first degree obese (OB1, 3040) subjects. Gene expression levels were assessed using high-density microarray technique (Illumina bead arrays, Illumina Inc.) and validated by real time quantitative PCR (RT-qPCR). Histological SAT sections were observed by light microscopy and then processed for estimation of adipocyte hypertrophy, inflammatory infiltration and fibrosis. Using hierarchical clustering procedures, gene expression of SAT from ExOB patients was closely related to that of SAT from morbidly obese patients (OB3), showing metallothioneins (MT2A and MT3) as key over-expressed genes. Adipocyte hypertrophy and inflammatory infiltration improved after weight loss, despite a persistent fibrosis. In conclusion, these data suggest that, although bariatric surgery induces an extensive and rapid weight loss in obese patients, ExOB SAT gene expression is not fully restored to that of NW SAT. Based on these observations, it is tempting to speculate that in adipose tissue the molecular signatures induced by obesity are not strictly dependent on weight loss and may need longer time period to completely disappear. Nothing to Disclose: RC, AZ, DG, MM, ADB, AL, AMDB

Pub #	OR11-5
Session Information	ORAL SESSION: BASIC/TRANSLATIONAL - Adipogenesis, Adiposity & Adipocyte Biology: Novel Findings in Human Adipose Tissue (11:15 AM-12:45 PM)
Title	Novel Pathway of Adipogenesis through Cross-Talk between Adipose Tissue Macrophages (ATMs), Adipose Stem Cells (ASCs) and Adipocytes: Evidence of Cell Plasticity
Author String	G Chazenbalk, M Jamubay, E Keller, K Yoshimura, R Azziz, D Dumesic David Geffen School of Medicine at UCLA, Los Angeles, CA; David Geffen School of Medicine at UCLA, Los Angeles, CA; University of Tokyo, Tokyo, Japan; Medical College of Georgia, Augusta, GA
Body	**Introduction:** Understanding adipogenesis is crucial to unlock cellular and molecular biological mechanisms governing obesity, diabetes and ASC plasticity. We have recently demonstrated that co-culture between human adipocytes, ATMs and ASCs causes robust proliferation of new preadipocytes due in part to differentiation of ATMs to preadipocytes suggesting a high degree of plasticity between these cell types. The goal of the present study is to further characterize the interaction between adipocytes with ATMs/ASCs in adipogenesis. **Research Design and Methods:** Human adipocytes and the stromal vascular containing ATMs and ASCs were isolated from human adipose tissue and co-cultured for 24 hours. Preadipocytes generated after co-culture were characterized by DLK (preadipocytes), CD68 (ATMs) and CD34 (ASCs), and Nile Red immunostaining. A novel fluorescent nanobead lineage tracing was utilized before co-culture so that fluorescent nanobeads could be internalized by ATMs. qRT-PCR was used to quantified adipogenic markers such as CBP/a and PPARg. **Results:** Co-culture of adipocytes with ATMs and ASCs increased the formation of new preadipocytes, thereby increasing lipid accumulation and CBP/a and PPARg gene expression. Preadipocytes originating after co-culture were positive for DLK, CD68 and CD34. Moreover, fluorescent nanobeads were internalized by ATMs before co-culture and the new preadipocytes formed after co-culture also contained fluorescent nanobeads, suggesting that new preadipocytes were originated in part from ATMs. The presence of DLK (+)/CD68(+)/CD34(+) cells grouped in spheres indicate that paracrine interactions between these cell types plays an important role in the generation and proliferation of new preadipocytes. **Conclusions:** Cross-talk between adipocytes, ATMs and ASCs promotes preadipocyte formation. The regulation of this novel adipogenic pathway involves differentiation of ATMs to preadipocytes as well as the formation of DLK (+)CD68(+)/CD34(+) cell spheres. This phenomenon may reflect the in vivo plasticity of adipose tissue in which ATMs play an additional role during inflammatory and disease states. Understanding this novel pathway could influence adipogenesis, leading to new treatments for obesity, inflammation, type 2 diabetes mellitus and polycystic ovary syndrome. Nothing to Disclose: GC, MJ, EK, KY, RA, DD

Pub #	OR11-6
Session Information	ORAL SESSION: BASIC/TRANSLATIONAL - Adipogenesis, Adiposity & Adipocyte Biology: Novel Findings in Human Adipose Tissue (11:15 AM-12:45 PM)
Title	Anatomical Localization, Gene Expression Profiling, and Bioenergetic Potential of Brown Adipose Tissue in Humans
Author String	AM Cypess, AP White, C Vernochet, TJ Schulz, C Sass, C Sze, A Chacko, L Deschamps, N Truchan, AR Holman, Y-H Tseng, CR Kahn Joslin Diabetes Center, Boston, MA; Beth Israel Deaconess Medical Center, Boston, MA
Body	It has now been shown that adult humans have functional brown adipose tissue (BAT) that can expend energy via thermogenesis. This process is regulated by the tissue-specific mitochondrial inner-membrane protein uncoupling protein-1 (UCP1), which uncouples oxidative phosphorylation to generate heat. While BAT holds great potential for being utilized as an obesity treatment, studies in humans have been limited because the precise locations of brown adipocytes in a given individual are unpredictable, discontinuous, and variable ove time. To gain a more specific description of where BAT is located as well as its gene expression and bioenergetic profiles, we resected adipose tissue from defined regions within the neck from 10 (6F/4M) patients undergoing routine surgery and measured the expression of brown and white-specific genes using qRT-PCR. The stromal vascular fraction (SVF) was also isolated from some patients and differentiated in vitro, and oxygen consumption rate (OCR) was measured. We found that UCP1 expression was enriched in deeper cervical fat near the carotid sheath, longus colli muscle, and prevertebral region, but not in the more superficial subcutaneous and subplatysmal fat. In 7/10 patients, maximal UCP1 expression was >10-fold above that in the subcutaneous depot. In some individuals, UCP1 expression was detectable in only a single region, whereas in others, expression could be found in several sites. Over 90% of the SVF fibroblasts from the deeper depots differentiated into adipocytes that expressed PPARγ, adipsin, and UCP1. Compared with undifferentiated cells, these adipocytes had basal and maximally-stimulated OCRs that were 3.5- and 6.5-fold higher, respectively (both $p<0.05$). In summary, the primary sites where human BAT is found are in a continuous fascial plane near the carotid sheath, longus colli, and prevertebral areas. While there is substantia variation in UCP1 expression, fibroblasts from the depots readily differentiate into UCP1+ adipocytes with increased metabolic activity. These findings indicate that human BAT is in a defined anatomical location and can be induced to differentiate into metabolically active tissue, supporting its utility for treating obesity. Sources of Research Support: NIH, Eli Lilly Foundation. Nothing to Disclose: AMC, APW, CV, TJS, CS, CS, AC, LD, NT, ARH, Y-HT, CRK

Pub #	OR12-1
Session Information	ORAL SESSION: BASIC/TRANSLATIONAL - Prolactin, Growth Hormone & IGF Signaling: A Cytokine Concert (11:15 AM-12:45 PM)
Title	Mice Lacking Prolactin Receptor Resist High-Fat Diet-Induced Obesity by Browning of Adipose Tissue
Author String	J Auffret, S Viengchareun, A Muscat, B Feve, M Lombes, N Binart Inserm U693, Kremlin Bicetre, France; Inserm U938 CHU Saint Antoine, Paris, France

Body

In humans and rodents, the pituitary lactogenic hormone prolactin (PRL) exerts a large variety of physiological actions most notably in reproduction, mammary development and lactation (1). These effects are mediated via binding of lactogens to the prolactin receptor (PRLR), a class I cytokine receptor, linked to activation of the JAK/STAT, MAPK and PI3K signalling pathways. It has been reported that PRL plays important roles in carbohydrate metabolism through effects on pancreatic beta cell mass and insulin production but could also impact energy homeostasis through modulation of lipid metabolism (2). We have previously shown that functional PRLR are highly expressed in both white (WAT) and brown adipose tissue (BAT) essential for adipogenesis and adaptive thermogenesis. Adult PRLR-/- mice show impaired adipose tissue development due to decreased adipocyte number (3). However, the molecular mechanisms of PRL action on adipocyte plasticity remain unclear. To study the metabolic impact of PRL signalling, we subjected male mice to High Fat Diet (HFD) during 16 wks and showed that PRLR-/- mice exhibited a strong resistance of weight gain correlated with a significant decrease of fat mass index and reduced adipose depots as compared to WT animals. Despite increased food intake under chow diet, PRLR-/- mice remained leaner than controls and were partially protected against HFD-induced obesity. Histomorphometric analyses of different adipose tissues revealed a 20% reduction of adipocyte surface and the appearance of massive brown-like adipocyte foci defined as browning. Of interest, expression of adipogenic markers (ZFP423, PPARγ2, C/EBPα) was unaffected, whereas expression of specific BAT markers (PRDM16, UCP1, PGC1α) was 2 to 3-fold increased in PRLR-/- fat depots under HFD as compared to those of WT animals. We hypothesized tha change in overall energy homeostasis is at least in part responsible for this phenotype. This strongly reinforces the fact that browning of WAT depot could be a major event leading to protection of HFD-induced obesity in PRLR-/- mice. Ongoing analyzes are aimed at identifying the multiple cascades and key target genes modulated by PRLR signalling in adipocytes. Our findings highlight a specific role for PRL action in adipocyte physiology and pathophysiology. Stimulating white to brown fat conversion through PRL blockade could hopefully be translated into adjunct therapy to raise energy expenditure and promote weight loss.

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2. Ben Jonathan et al. Endocr. Rev. 29, 1-41 (2008)
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Sources of Research Support: ANR- 09-BLAN-0246-01.

Nothing to Disclose: JA, SV, AM, BF, ML, NB

Pub #	OR12-2
Session Information	ORAL SESSION: BASIC/TRANSLATIONAL - Prolactin, Growth Hormone & IGF Signaling: A Cytokine Concert (11:15 AM-12:45 PM)
Title	Generation and Initial Characterization of Growth Hormone (GH) Knockout Mice: Phenotypic Rescue in Gene Dosage-Dependent Manner by Introduction of Human GH Transgenes under Intact Pituitary Control
Author String	N Yang, AF Yanac, EJ Yoo, NE Cooke, SA Liebhaber, H Rui Thomas Jefferson University, Philadelphia, PA; University of Pennsylvania, Philadelphia, PA

Body

GH is a polypeptide hormone important for postnatal somatic growth. Furthermore, the involvement of GH in mammary gland and breast cancer development is manifold. GH activates GH receptors (GHRs) and stimulates secretion of insulin-like growth factor (IGF)-I, an important growth factor. The endocrine and local GH/IGF-1 axis is believed to be critical for terminal end bud development and ductal elongation in the mammary gland development. In addition, human GH (hGH) has dual actions on breast tissues both as a potent lactogen via PRLRs on epithelial cells and as a somatogen via GHRs on epithelial and stromal cells of both human and murine origin. In contrast, murine GH (mGH) is not a lactogen and also lacks agonist activity toward human GHRs. This hormonal [ldquo]deficiency[rdquo] of mice with respect to human GHR influences the biology of xenotransplanted human GHR-expressing tumors, and may alter responses to preclinical therapies tested in mice. Having recognized this limitation of current mouse models for human xenotransplants, we set out to generate a humanized mouse model that expresses physiological levels of hGH in the absence of mGH. We first generated GH knockout mice and subsequently crossed these mice with two separate lines of hGH/P1 transgenic mice, with either 4 (clone 811D) or 19 (clone 813I) copies of the transgene [1]. The hGH/P1 transgene is an 87 kb genomic fragment of human GH locus containing the cluster locus control region, and maintains pituitary-specific expression of hGH in transgenic mice [1]. The GH knockout mice displayed a severely dwarf phenotype similar to GHR knockout mice [2]. The GH null mice also had impaired mammary gland development. Crossing GH knockout mice with two lines of hGH/P1 transgenic mice yielded mice humanized for GH with either intermediate or high levels of hGH. Introduction of hGH/P1 transgenes restored the defects in somatic growth and mammary gland development in GH null mice in a dose-dependent manner. Offspring of line GHKO-hGH/P1_811D, carrying 4 copies of the hGH transgene, were of similar size as wildtype mice, while offspring of line GHKO-GH/P1_813I, carrying 19 copies of the hGH transgene, were approximately 20% larger than wildtype mice. Further characterization of GH null mice and GH-humanized mice is under way. After crossing into appropriate immunodeficient background, the new mouse lines are expected to be useful for improved endocrine modeling and drug response testing of GHR expressing human tumors.

1. Su, Y., S.A. Liebhaber, and N.E. Cooke, The Human Growth Hormone Gene Cluster Locus Control Region Supports Position-independent Pituitary- and Placenta-specific Expression in the Transgenic Mouse. Journal of Biological Chemistry 275; 7902-7909, 20002.
2. Zhou, Y., et al., A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse). Proceedings of the National Academy of Sciences of the United States of America, 94, 13215-13220,1997.

Sources of Research Support: Susan G. Komen for the Cure Postdoctoral Fellowship KG080129 awarded to NY.

Nothing to Disclose: NY, AFY, EJY, NEC, SAL, HR

Pub #	OR12-3
Session Information	ORAL SESSION: BASIC/TRANSLATIONAL - Prolactin, Growth Hormone & IGF Signaling: A Cytokine Concert (11:15 AM-12:45 PM)
Title	Genome-Wide Analysis of STAT5 and Bcl6 Binding in Adult Male and Female Mouse Liver Elucidates Growth Hormone (GH)-Dependent, Sex-Specific Hepatic Gene Expression
Author String	Y Zhang, EV Laz, DJ Waxman Boston University, Boston, MA
Body	Sex-specific plasma GH profiles regulate sex differences in liver gene expression by transcriptional mechanisms. We used ChIP-Seq to characterize the DNA binding profiles of STAT5, a GH-responsive transcription factor previously implicated in the sex-dependent actions of GH (1), and Bcl6, a male-predominant transcription repressor regulated by GH whose binding site overlaps that of STAT5 (2). ChIP-Seq of STAT5 and Bcl6 binding was carried out using liver chromatin from individual male mice killed at a peak of GH/peak of STAT5 binding activity, or between plasma GH peaks, when liver STAT5 activity is low as revealed by STAT5 EMSA analysis. STAT5 and Bcl6 ChIP-Seq was also carried out in individual female mouse livers. Liver STAT5 activity status showed a positive correlation with the number of STAT5-binding peaks identified, while Bcl6 binding generally showed a negative relationship to liver STAT5 status. Sites showing significant differences in STAT5 binding between male and female liver chromatin were identified using CisGenome. Gene Set Enrichment Analysis of the sex-specificity of genes at or near these STAT5 binding sites indicated male-predominant STAT5 binding (at 7640 sites) was enriched near male-biased genes, while female-predominant STAT5 binding (at 7823 sites) was enriched near female-biased genes ($p = 0$). Fewer Bcl6 binding sites were identified in female (730 sites) compared to male mouse liver (3002 sites). A subset (22%) of the Bcl6 binding sites in male liver overlapped with STAT5 sites. De novo motif discovery indicated that STAT5 and Bcl6 have distinct binding motifs, with STAT5 binding to the consensus sequence TTC-NNN-GAA, while Bcl6 showed binding preference for TC-NAG-GAA. In male liver, Bcl6 showed a tendency to bind sequences at or near genes more highly expressed in female liver. That female bias in expression can now be explained by the more extensive Bcl6 binding in males, leading to gene repression. These findings provide a genome-wide view of the sex-specific binding of two factors implicated in GH-regulated sex differences in liver gene expression. Together, they support the hypothesis that sex differences in STAT5 binding contribute to the sex-dependent expression of its target genes, and that the transcriptional repressor Bcl6 modulates STAT5 transcriptional responses by preferentially binding to and down regulating a subset of STAT5 target genes. (1) Clodfelter KH, Holloway MG, Hodor P, Park SH, Ray WJ, Waxman DJ. Sex-dependent liver gene expression is extensive and largely dependent upon signal transducer and activator of transcription 5b (STAT5b): STAT5b-dependent activation of male genes and repression of female genes revealed by microarray analysis. Mol Endocrinol (2006) 20:1333-1351. (2) Meyer RD, Laz EV, Su T, Waxman DJ. Male-specific hepatic Bcl6: growth hormone-induced block of transcription elongation in females and binding to target genes inversely coordinated with STAT5. Mol Endocrinol (2009) 23:1914-1926. Sources of Research Support: In part by NIH grant DK33765 (to DJW). Nothing to Disclose: YZ, EVL, DJW

Pub #	OR12-4
Session Information	ORAL SESSION: BASIC/TRANSLATIONAL - Prolactin, Growth Hormone & IGF Signaling: A Cytokine Concert (11:15 AM-12:45 PM)
Title	Serum IGF-I Is Insufficient to Restore Body Size of the GHR Null Mouse
Author String	Y Wu, H Sun, S Yakar Mount Sinai School of Medicine, New York, NY
Body	In our previous studies we showed that hepatic IGF-1 transgene (HIT) driven by the transthyretin (TTR) promoter was able to restore postnatal growth of the IGF-1 null mice (KO-HIT mouse model), such that their body size was indistinguishable from controls. Interestingly however, despite 2 fold elevations in serum IGF-levels in the KO-HIT mice, GH levels remained normal. Thus, we could not rule out direct effects of GH on body size, which are tissue IGF-1- independent. We therefore developed a new mouse model where the hepatic IGF-1 transgene (HIT) driven by the transthyretin (TTR) promoter was expressed in a mouse model o GHRKO (GHRKO-HIT). Consistent with our previous results, expression of the hepatic IGF-1 transgene (HIT) under the TTR promoter led to 2fold increase in serum IGF-1 levels (648+/-49ng/ml vs 292+/-13ng/ml, respectively). However, in GHRKO-HIT mice serum IGF-1 levels were not elevated and were comparable to those of control mice (320+/-1ng/ml vs 292+/-13ng/ml, respectively), likely due to reduced IGFBP-3 and the acid labile subunit in the absence of GH action. Despite normal levels of serum IGF-1, post-natal growth of the GHRKO-HIT mice was impaired as evident by reduced body weight (by 40% throughout 16 weeks postnatally) and body length (by 20% throughout 16 weeks postnatally) in both males and females of two different genetic backgrounds (i.e. C57Bl/6 and FVB/N). These striking results show that in the absence of tissue GHR activity, serum IGF-1 is insufficient to restore normal growth and development. These data imply that GHR in tissues is the master regulator of body size. Sources of Research Support: NIH Grants AR054919 & AR055141 to SY. Nothing to Disclose: YW, HS, SY

Pub #	OR12-5
Session Information	ORAL SESSION: BASIC/TRANSLATIONAL - Prolactin, Growth Hormone & IGF Signaling: A Cytokine Concert (11:15 AM-12:45 PM)
Title	Autocrine Effects of Mesenchymal Stem Cells Expressing IGF-I Rescue the Fracture-Healing Defects of Irs1 Knockout Mice
Author String	F Granero-Molto, TJ Myers, JA Weis, L Longobardi, T Li, Y Yan, N Case, J Rubin, A Spagnoli University of North Carolina, Chapel Hill, NC; Vanderbilt University, Nashville, TN; University of North Carolina at Chapel Hill, Chapel Hill, NC
Body	Failures of fracture repair (nonunions) occur in 10% of the fractures. Clinical and animal studies have indicated that the use of mesemchymal stem cells (MSC) in tissue regeneration is safe and effective. The use of MSC expressing IGF-I enhanced such effects increasing callus bone formation. Objectives: To determine, 1) the intracellular pathway mediating osteoblastic differentiation of MSC by IGF-I and 2) whether IGF-I expressed by MSC, through autocrine effects, improved the fracture healing promoting new bone formation ir a model of fracture non-union with a defective IGF signaling. Methods: 1) MSC were induced to differentiate into osteoblasts in serum free conditions. The effect of IGF-I on the differentiation process was assayed by quantification of the RNA expression levels of osteogenic markers. The contribution of the intracellular signaling pathways initiated by IGF-I was characterized using shRNA mediated gene silencing. 2) MSC isolated from WT mice were engineered to express human IGF-I (MSCIGF). A stabilized tibia fracture was produced in adult Irs1 knockout females (Irs1KO). Mice were transplanted with 106 MSC or MSCIGF by IV injection; untransplanted mice were used as controls. Fractured tibias were analyzed 14 days postfracture by [mu]CT analysis. Calluses were subjected to histological studies to determine the distribution of bone and cartilage. Results: We determined that IGF-I induced osteoblastic differentiation of MSC in vitro. IGF-I induced significant expression of the osteoblast specific genes Osterix and Osteocalcin and a significant accumulation of calcium deposits. We characterized the intracellular pathway mediating the differentiation process and found that is dependent of an intact IRS1-PI3K pathway. Using Irs1KO as nonunion model of fracture healing through altered IGF-I signaling, we demonstrated, by [mu]CT analysis, that, through autocrine properties, MSCIGF improved the fracture healing process inducing the formation of a callus that bridged the gap. Histological analysis confirmed these observations. In addition, MSCIGF transplant induced a significant increase of the callus new bone and soft tissue content when compared with control group (bone: 1.747±0.518 mm3, n=2 vs 1.063±0.037 mm3, n=3, $p<0.05$; soft tissue: 1.207±0.396 mm3; n=2 vs 0.689±0.078 mm3, n=3; $p<0.05$). Conclusions: We provided evidence of the effects and mechanism of MSCIGF in fracture repair and showed their therapeutic potential to treat fracture non-unions. Sources of Research Support: NIH-NIDDK Grant R01 DK70929 awarded to AS. Nothing to Disclose: FG-M, TJM, JAW, LL, TL, YY, NC, JR, AS

Pub #	OR12-6
Session Information	ORAL SESSION: BASIC/TRANSLATIONAL - Prolactin, Growth Hormone & IGF Signaling: A Cytokine Concert (11:15 AM-12:45 PM)
Title	Determination of the Mechanism by Which IGFBP-2 Regulates PTEN/AKT Signal Transduction in Vascular Smooth Muscle Cells (VSMC)
Author String	X Shen, G Xi, LA Maile, CJ Rosen, DR Clemmons The University of North Carolina at Chapel Hill, Chapel Hill, NC; Maine Medical Center Research Institute, Scarborough, ME
Body	Similar to other members of the IGFBP family IGFBP-2 (BP2) can enhance or inhibit the actions of IGFs. In BP2-/- mice there is an inverse relationship between BP2 and PTEN levels in aorta and bone leading to reduced cell growth and survival therefore, these studies were undertaken to determine the mechanism of BP2 mediated PTEN suppression and its consequences for IGF-I signaling. PTEN phosphorylation typically occur on Ser/Thr residues but previous studies have shown that PTEN tyrosine phosphorylation can down-regulate PTEN activity. Initially we showed that BP2 stimulated PTEN tyrosine phosphorylation leading to increased IGF-I stimulated AKT activation. To investigate the mechanism mediating this response we focused on the receptor protein tyrosine phosphatase β (RPTPβ). RPTPβ is inactivated via ligand-induced oligomerization that occurs when it binds to the heparin-binding growth factors (midkine and PTN). Since BP2 contains a heparin-binding motif (HBD) we hypothesized that BP2 binds to RPTPβ and inactivates its catalytic activity resulting in enhanced PTEN tyrosine phosphorylation and suppression.To test this hypothesis, we used an in vitro binding assay and biotinylated BP2. BP2 bound to cell surface which was disrupted by a peptide containing the BP2 (HBD) sequence. Furthermore co-incubation with IGF-I enhanced BP2 binding. Next we determined whether BP2 bound directly to RPTPβ. Using affinity cross-linking we showed that BP2 bound specifically to RPTPβ and IGF-I enhanced binding. This was associated with attenuated RPTPβ catalytic activity, increased PTEN tyrosine phosphorylation and instability. Utilizing BP2 knockdown we showed that in the absence of BP2, IGF-I did not alter RPTPβ activation and it had no ability to regulate PTEN tyrosine phosphorylation. This resulted in decreased IGF-I stimulated AKT activation compared to control cells. These changes could be reversed by adding back BP2. The results show that the extracellular domain of RPTPβ binds to BP2 leading to enhanced RPTPβ oligomerization, which attenuates its phosphatase activity leading to PTEN instability. These changes lead to enhancement of IGF-I stimulated AKT phosphorylation and biologic actions such as increased cell proliferation and decreased apoptosis that are linked to this response. This unique demonstration of how an IGFBP receptor functions to alter IGF-I signaling has important implications for understanding how these proteins module the cellular response to IGF-I. Nothing to Disclose: XS, GX, LAM, CJR, DRC

Pub #	OR13-1
Session Information	ORAL SESSION: TRANSLATIONAL - Adrenal Cortical Carcinoma, Adrenal Adenomas & Pheochromocytoma (11:15 AM-12:45 PM)
Title	Sunitinib in Refractory Adrenocortical Carcinoma: Results of a Phase II Trial
Author String	M Quinkler, M Kroiss, S Hahner, C Strasburger, B Allolio, M Fassnacht Charité Campus Mitte, Charité University Medicine Berlin, Berlin, Germany; University Hospital Wuerzburg Wuerzburg, Germany
Body	Background: Adrenocortical carcinoma (ACC) is a rare solid tumor with poor prognosis in advanced stages. The adrenolytic drug mitotane and cytotoxic chemotherapies are current treatment options with limited clinical efficacy. Animal experiments pointed to an adrenotoxic effect of sunitinib suggesting potential antineoplastic activity in ACC. Study population: 38 patients with advanced ACC progressing after mitotane and 1-3 cytotoxic chemotherapies were included. Mitotane treatment was ongoing in 20 patients. Primary endpoint: Response defined as progression free survival of [ge]12 weeks Results: Three patients were unevaluable for response due to withdrawal of consent, noncompliance with the study procedures or a serious adverse event. Of the 35 patients analyzed for response, 5 patients (14%) experienced stable disease (median time to progression 5.8 months, range 5.6-11.2), 24 had progressive disease according to RECIST criteria and 6 patients died of the disease before the first evaluation. Median time to progression was 2.8 months (0.3-5.7). In total, 36 serious adverse events were recorded of which 10 were possibly treatment related. Only 41 treatment related adverse events were documented (mostly CTC grade 1+2). Surprisingly, the response rate was higher in patients not receiving mitotane (4 out of 15 vs. 1 out of 20) suggesting that concomitant mitotane treatment may negatively affect clinical outcome (HR for progressive disease 6.9 (95% CI 0.7-69.9). Therefore, we hypothesize that mitotane may lead to decreased sunitinib plasma concentrations, probably by induction of metabolizing enzymes. This could also explain the low frequency of adverse events which was much lower than expected from other sunitinib trials. Conclusion: Sunitinib has a modest single-agent activity in ACC. Drug interaction with mitotane may abrogate a more impressive anti-tumor effect of sunitinib. Therefore, a clinical trial of sunitinib in mitotane na [iuml]ve patients might be warranted. Disclosures: CS: Speaker, Novartis Pharmaceuticals; Pfizer, Inc.; Novo Nordisk; Scientific Board Member, Lilly USA, LLC; Advisory Group Member, Pfizer, Inc.; Clinical Researcher, Ipsen. Nothing to Disclose: MQ MK, SH, BA, MF

Pub #	OR13-2
Session Information	ORAL SESSION: TRANSLATIONAL - Adrenal Cortical Carcinoma, Adrenal Adenomas & Pheochromocytoma (11:15 AM-12:45 PM)
Title	Mitotane Levels Affect the Outcome of Patients Treated Adjuvantly Following Radical Resection of Adrenocortical Cancer (ACC)
Author String	A Ardito, A Al Ghuzian, M Fassnacht, F Daffara, S Leboulleux, S Wortmann, B Zaggia, S De Francia, G Peraga, IG Hermsen, A Berruti, HR Haak, B Allolio, E Baudin, M Terzolo Faculty of Medicine S Luigi Gonzaga, University of Turin, Orbassano, Italy; Institut Gustave Roussy, Villejuif, France; University Hospital of Wurzburg, Wurzburg, Germany; Faculty of Medicine S Luigi Gonzaga, University of Turin, Orbassano, Italy; Maxima Medical Centre, Eindhoven, Netherlands
Body	We have previously demonstrated that adjuvant mitotane prolong recurrence free survival (RFS) in patients with radically resected ACC (1). Aim of the present study was to assess retrospectively whether mitotane levels are correlated with patient outcome in an adjuvant setting reviewing experience in 3 different referral centres in Europe. There were 120 patients (45 W, 75 M, median age 44 years, range 16-76) radically resectec for ACC who were treated adjuvantly with mitotane from 1996 to 2010. Diagnosis of ACC was made by experienced pathologists using Weiss score (median 6, range 3-9). ACC was stage I in 10 cases, II in 73, III ir 31, IV in 6; 62 patients had secreting ACC. Forty patients were treated with 8-10 grams daily and 80 patients with 1-6 grams daily. Target mitotane concentrations of 14-20 mg/l were attained within 3 months from treatment start in 49 patients (41%), within 6 months in additional 61 patients (51%), while 10 patients (8%) never got them. Mitotane levels >14 mg/l were maintained in more than 75% of determinations in 64 patients (53%). Median duration of treatment was 25.5 months (range: 4-165) and median duration of follow-up was 36 months (6-165). Only 11 patients (9%) experienced severe adverse events (CTC 3), but none of the patient discontinued mitotane definitively for toxicity. At the last follow-up, 58 patients (48%) relapsed and 33 patients (27%) died. RFS was significantly longer in patients who attained target mitotane concentrations within 3 months ($p=0.01$) and in patients who maintained consistently levels >14 mg/l during follow-up ($p=0.004$). In a multivariate analysis (including sex, age, stage, number of mitoses or Ki-67 value) maintenance of target mitotane concentrations was an independent factor influencing RFS (HR=0.41, 95%CI 0.21-0.77; $p=0.006$). The effect on overall survival was significant only in univariate but not in multivariate analysis (HR=0.61, 0.26-1.43). The present data demonstrate for the first time that attainment of mitotane concentrations >14 mg/l is of benefit in an adjuvant setting thus giving indirect evidence of the efficacy of adjuvant mitotane treatment, a controversial issue in the management of patients with ACC. Previously, the importance of getting elevated mitotane concentrations has been demonstrated only for treatment of advanced disease (2). (1) Terzolo M et al., NEJM 2007; 356: 2372 (2) Baudin E et al., Cancer 2001; 92: 1385 Nothing to Disclose: AA, AAG, MF, FD, SL, SW, BZ, SDF, GP, IGH, AB, HRH, BA, EB, MT

Pub #	OR13-3
Session Information	ORAL SESSION: TRANSLATIONAL - Adrenal Cortical Carcinoma, Adrenal Adenomas & Pheochromocytoma (11:15 AM-12:45 PM)
Title	Combination of Sorafenib and Everolimus Impacts Therapeutically on Adrenal Tumor Models
Author String	B Mariniello, A Rosato, G Zuccolotto, B Rubin, R Pezzani, M-V Cicala, M Iacobone, A Fassin, F Mantero University of Padua, Padua, Italy; University of Padua, Padua, Italy; Azienda Ospedaliera Padova, Padua, Italy; University of Padua, Padua, Italy; University of Padua, Padua, Italy
Body	Adrenocortical carcinoma (ACC) is a rare and aggressive tumor with an overall poor prognosis. Medical treatment of ACC is currently based on the use of mitotane, but this therapy does not significantly alter survival. Sorafenib, a tyrosine kinase inhibitor involved in tumor growth and angiogenesis with effects on the serine/threonine kinase Raf and several RTKs (receptor tyrosine kinase) and Everolimus, an inhibitor of mammalian target of rapamycin (mTOR) with antiproliferative effects on a wide spectrum of tumors were tested in ACC models. The expression of VEGF and its receptors (VEGFR1-2) was studied in 18 AC, 33 aldosterone-producing adenomas (APA), 12 cortisol-producing adenomas (CPA) and 6 normal adrenals (NA) by real-time PCR, immunohistochemistry and by immunoblotting in SW13 and H295R AC cancer cell lines. The effects of Sorafenib and Everolimus, alone or in combination, were tested on SW13 and H295R cells and on primary adrenal cultures by evaluating cell viability and apoptosis *in vitro* and tumor growth inhibition of xenografts in immunodeficient mice *in vivo*. VEGF and VEGFR1-2 expressions were detected in all samples. A dose-dependent inhibition of cell viability was observed particularly in SW13 cells after a 24h treatment with either drugs. In addition, a marked synergistic growth inhibition activity was obtained with drug combination. Increasing apoptosis and cell cycle alterations were observed in AC cells treated with the drugs. Finally, a significant mass reduction and increased survival were observed in xenograft models undergoing treatment with the drug combination. Our data suggest that an autocrine VEGF loop may exist within ACC. Furthermore, a combination of molecularly targeted agents may have both antiangiogenic and direct antitumor effects and could represent a new therapeutic tool for the treatment of ACC. Nothing to Disclose: BM, AR, GZ, BR, RP, M-VC, MI, AF, FM

Pub #	OR13-4
Session Information	ORAL SESSION: TRANSLATIONAL - Adrenal Cortical Carcinoma, Adrenal Adenomas & Pheochromocytoma (11:15 AM-12:45 PM)
Title	Transcriptome Analysis Revealed Different Pathway Alterations in Adrenocortical Hyperplasias Caused by PRKAR1A Defects and Adrenal Tumors Harboring Somatic GNAS1 Mutations
Author String	MQ Almeida, EI Bimpaki, A Horvath, C Cheadle, T Watkins, M Nesterova, CA Stratakis National Institutes of Health, Bethesda, MD; School of Medicine, Johns Hopkins University, Baltimore, MD

Body

The overwhelming majority of benign lesions of the adrenal cortex leading to Cushing syndrome are linked to one or another abnormality of the cAMP signaling pathway. Inactivating mutations of the PRKAR1A gene coding for the regulatory 1-α (RIα) subunit of protein kinase A (PKA) are responsible for Carney complex (CNC) in the majority of patients. Primary pigmented nodular adrenocortical disease (PPNAD) is the most common endocrine tumor associated with CNC, occurring in 60% of the patients. A small number of both massive macronodular adrenocortical disease and cortisol-producing adenomas harbor somatic GNAS mutations. In this study, we performed a transcriptome analysis to characterize the basis for PKA-associated tumorigenesis in PPNAD caused by PRKAR1A mutations and in adrenal adenomas harboring somatic GNAS mutations; two different adrenal lesions, both associated with distinct genetic defects in cAMP/PKA signaling. Whole-genome expression profiling was analyzed in normal adrenal glands, 3 PPNAD samples and 3 adrenal tumors harboring somatic GNAS mutations (one sporadic and two associated with McCune-Albright syndrome). Quantitative RT-PCR and Western blot were employed to validate the mRNA array data. Unsupervised hierarchical clustering revealed two distinct subgroups of adrenal tumors; one caused by PRKAR1A defects and another associated with GNAS mutations. Genes related to Wnt signaling pathway (CCND1, CTNNB1, LEF1, LRP5, WISP1 and WNT3) were over-expressed in PPNAD samples, consistent with studies in human and mouse neoplasms carrying PRKAR1A defects. Adrenal tumors with GNAS mutations were significantly enriched with extra-cellular matrix (ECM)-receptor interaction and focal adhesion pathways when compared to PPNAD (fold-enrichment 3.5, $p < 0.0001$ and 2.1, $p < 0.002$, respectively). MAPK and p53 signaling pathways were highly over-expressed in both PPNAD and adrenal tumors with GNAS mutations. Interestingly, NFKB, NFKBIA and TNFRSF1A expression were higher in Gsα mutant tumors than in PPNAD samples at messenger and/or protein levels ($p < 0.05$). EPAC-2 and RAB13, which are activated by a cAMP-dependent but PKA-independent mechanism, were highly expressed in adrenal tumors with GNAS mutations at mRNA and protein levels. In conclusion, transcriptome analysis revealed different pathway alterations in PPNAD and adrenal tumors harboring GNAS mutations, providing new insights into the molecular mechanism of tumorigenesis driven by cAMP/PKA dysregulation.

Sources of Research Support: U.S. National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development intramural project Z01-HD-000642-04 (to Dr. C.A. Stratakis).

Nothing to Disclose: MQA, EIB, AH, CC, TW, MN, CAS

Pub #	OR13-5
Session Information	ORAL SESSION: TRANSLATIONAL - Adrenal Cortical Carcinoma, Adrenal Adenomas & Pheochromocytoma (11:15 AM-12:45 PM)
Title	Staging and Functional Characterization of Pheochromocytoma and Paraganglioma by ^{18}F-FDG PET: A Large Prospective NIH Study
Author String	HJLM Timmers, CC Chen, JA Carrasquillo, M Whatley, A Ling, G Eisenhofer, KS King, JU Rao, KT Adams, K Pacak Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; National Institutes of Health, Bethesda, MD; National Institutes of Health, Bethesda, MD; National Institutes of Health, Bethesda, MD; Memorial Sloan Kettering Cancer Center, New York, NY; University of Dresden, Dresden, Germany
Body	**Background:** In patients with a biochemically established diagnosis of pheochromocytoma or paraganglioma (PPGL), functional imaging is critical for tumor localization and detection of metastases. The aim of this study was to establish the diagnostic sensitivity and specificity of 2-[18F]-Fluoro-2-deoxy-D-glucose positron emission tomography (18F-FDG PET) for tumor localization and staging of PPGL as compared to conventional imaging by [123I]-metaiodobenzylguanidine single photon emission computed tomography (123I-MIBG SPECT), CT and MRI. We also investigated whether clues for a genetic diagnosis underlying PPGL can be gained from functional characterization by 18F-FDG PET. **Methods:** 216 patients (106 males, 110 females, mean±SD age 45,2±14.9 years) with (suspected) PPGL were consecutively studied. There were 60 cases of non-metastatic PPGL, 95 cases of metastatic PPGL and 61 PPGL-negative patients. Besides CT or MRI, patients underwent 18F-FDG PET/CT and 123I-MIBG SPECT/(CT). Standard uptake values (SUVs) were compared between PPGL and normal adrenal glands and across genotypes. **Results:** For non-metastatic tumors, the sensitivity was 95.7% for CT/MRI, 76.8% for 18F-FDG ($p<0.001$ versus CT/MRI) and 77% for 123I-MIBG ($p=0.002$ versus CT/MRI, ns versus 18F-FDG). The specificity of 18F-FDG was 90.2% and for 123I-MIBG it was 77.0%. Cut-off values for 18F-FDG SUVs to distinguish between PPGL and normal adrenal glands were established at 1.1 and 4.6, corresponding with 100% sensitivity and specificity, respectively. 18F-FDG uptake was higher in SDH- and VHL-related tumors than in MEN2 and NF1-related tumors. For metastases, region-based sensitivities were 73.1% for CT/MRI, 80.4% for 18F-FDG ($p<0.001$ versus 123I-MIBG), and 48.5% for 123I-MIBG ($p<0.001$ versus CT/MRI). For bone metastases, the highest sensitivity was reached by 18F-FDG: 93.7%, versus 78.6% for CT/MRI ($p=0.039$. **Conclusion:** As compared to 123I-MIBG SPECT, 18F-FDG PET allows better detection of metastases and provides a higher specificity. Quantification of 18F-FDG uptake allows distinction between PPGL and normal adrenal glands and can provide important clues for a hereditary syndrome underlying PPGL. Sources of Research Support: Intramural Research Program of the NICHD/NIH and Pheo Para Alliance. Nothing to Disclose: HJLMT, CCC, JAC, MW, AL, GE, KSK, JUR, KTA, KP

Pub #	OR13-6
Session Information	ORAL SESSION: TRANSLATIONAL - Adrenal Cortical Carcinoma, Adrenal Adenomas & Pheochromocytoma (11:15 AM-12:45 PM)
Title	Insulin-Like Growth Factor-I (IGF-I) Regulates Pheochromocytoma Cellular Proliferation *In Vitro* and *In Vivo*
Author String	MC Fernandez, MC Venara, S Nowicki, C Alvarez Sedo, HE Chemes, MB Barontini, PA Pennisi Hospital de Ni[ntilde]os Ricardo Gutierrez, Buenos Aires, Argentina
Body	We have previously shown that IGF-1R expression is increased in malignant compared to benign human pheochromocytoma, and that the mouse pheochromocytoma cell line MPC4/30 expresses a functional IGF-1R. Herein, we demonstrate that MPC4/30 cellular proliferation and BrdU incorporation after 5 days in culture increased significantly with rhIGF-1 stimulation (15±4.5 vs 30.3±2.6 x10^4cells $p<0.05$ tTest; 25.7±5.8% vs 34.7±6.0% positive nuclei $p<0.01$ M.Whitney Test, respectively) while the percentage of apoptotic cells was significantly lower (35.8±2.7% vs 10.8±1.3% cleaved caspase 3 positive cells; 21.3±2.7 vs 6.8±1.3 % of positive cells for TUNEL assay, $p<0.01$ M.Whitney Test). MPC4/30 cells migration was also stimulated by rhIGF-1 (12±1 vs 18±1 $p<0.01$, M.Whitney Test). Additionally, MPC4/30 cells formed colonies in soft agar and stimulation with rhIGF-1 increased the number and size of colonies (11±3 vs 26± 3 $p<0.01$; 217±13[mu]m vs 288±69[mu]m $p<0.01$ tTest). To evaluate the impact of IGF-1 on pheochromocytoma cellular proliferation *in vivo*, we generated a mouse model of pheochromocytoma by subcutaneous injection of MPC4/30 cells to control and liver IGF-1 deficient (LID) mice that exhibit 75% reduction in serum IGF-1 levels. We found that six weeks after MPC4/30 cells injection all control mice developed subcutaneous tumors, while only 40% of LID mice showed noticeable tumors. LID mice harboring MPC4/30 and treated daily with 2 mg/kg rhIGF-1 developed tumors as controls. The latency period for tumor growth increased in LID mice as compared to both control and LID mice treated with rhIGF-1 [Median Survival Ratio 2.2 95%CI 1.59-2.81, Chi^2 136.5; 1.83 95%CI 1.35-2.32, Chi^2 53.17 respectively, $p<0.0001$ Logrank Test]. LID mice had 10 times lower probability for developing tumors as compared to controls (Hazard Ratio 0.1 95%CI 0.02-0.27), and rhIGF-1 treatment increased this risk 8 times (Hazard Ratio 7.98 95%CI 1.60-67.06). Our data clearly show that cellular proliferation of pheochromocytoma is regulated by IGF-1 both *in vitro* and *in vivo*. IGF-1 enhanced proliferation, migration and cell's ability to grow unattached. Sources of Research Support: Mincyt Grant PICT 06-01002 awarded to PP. National Council of Research CONICET-Argentina Grant PIP2008-1905 awarded to PP. Nothing to Disclose: MCF, MCV, SN, CAS, HEC, MBB, PAP

Pub #	OR14-1
Session Information	ORAL SESSION: TRANSLATIONAL - Nuclear Receptors: Genome Binding to Novel Functions (11:15 AM-12:45 PM)
Title	Molecular Characterization of Estrogen Action in the Anterior Pituitary
Author String	JD Cook, M Brown Dana Farber Cancer Institute, Boston, MA
Body	Regulation of normal female sexual maturation and reproductive function requires the precise orchestration of hormonal signals between the hypothalamus, anterior pituitary and the ovaries. Estrogens exert both positive and negative feedback effects on the hypothalamus and anterior pituitary. In the anterior pituitary, estrogen (E) regulates reproduction via activation of estrogen receptor alpha (ERα) in pituitary gonadotrope cells. Combining gene expression profiling with genome-wide analysis of ERα binding sites (ie the ER cistrome), we have compared the mechanisms of E action between the mouse pituitary gonadotrope-like cell line alpha-T3-1 and whole pituitaries harvested from E-treated ovariectomized female mice. Our studies have identified novel estrogen-regulated gonadotrope genes and their functional DNA estrogen response elements. Genes include 18 early response genes (such as N-Myc, Ier2, Socs3, Tob1 and Nhsl1) that are transcriptionally induced as early as 30 minutes post-estrogen exposure as well as several genes known to contribute to regulation of reproduction such as kisspeptin and androgen and progesterone receptors. Our analysis has also shown that the most highly enriched transcription factor binding motifs in the ER cistrome of alpha T3-1 and anterior pituitary are ER, AP-1 and the cyclic AMP-binding protein (CREB) furthering support of CREB as a cooperating ER transcription factor regulating gonadotrope function. Finally, as an initial investigation into the tissue-specific action of E, we have compared the global E-regulated temporal gene expression profiles and ER cistromes of mouse pituitary cells, mouse liver and the human MCF7 breast cancer cell line and have discovered that there is minimal overlap - only 80 shared ER DNA binding sites - between ER cistromes of E-treated pituitary cells (n = 8727 ER binding sites), mouse liver (n=5568) and MCF-7 cells (n=5540). Further, while a subset of E-regulated genes including PR, Greb1 and prolactin receptor are induced in all cell types, key cell cycle regulators such as cyclin D1 and c-myc are only upregulated in MCF-7 cells.Together, these data provide important insights into the tissue-specific molecular action of E and the ER cooperating transcription factors that regulate gonadotrope function. Nothing to Disclose: JDC, MB

Pub #	OR14-2
Session Information	ORAL SESSION: TRANSLATIONAL - Nuclear Receptors: Genome Binding to Novel Functions (11:15 AM-12:45 PM)
Title	Characterization of Estrogen-Regulated Non-Coding RNAs
Author String	M Sun, S Gadad, CG Danko, N Hah, WL Kraus University of Texas Southwestern Medical Center, Dallas, TX; University of Texas Southwestern Medical Center, Dallas, TX; Cornell University, Ithaca, NY; Cornell University, Ithaca, NY; Cornell University, Ithaca, NY
Body	Estrogen signaling controls a wide array of physiological and pathological responses in a variety of tissues of the reproductive tract, bone, brain, and cardiovascular system. Many of these effects are mediated by direct actions of estrogen on the genome, acting through nuclear estrogen receptors (ERs). The genomic effects of ligand-activated ERs have typically been examined with respect to protein coding transcripts, but emerging evidence indicates that estrogen signaling affects the expression of a wide variety of non-coding functional RNA transcripts as well, including rRNAs, tRNAs, enhancer RNAs, microRNAs (miRNAs), and long non-coding RNAs (lncRNAs). The diverse actions of estrogen are likely to be manifested, at least in part, by its effects on the expression of these transcripts. miRNAs are short RNA molecules (~22 nucleotides long), which are transcribed as part of longer primary transcripts (pri-miRNAs). They function as post-transcriptional regulators that bind to complementary sequences on target mRNAs resulting in translational repression, transcript degradation, and gene silencing. lncRNAs are generally poorly characterized longer RNAs (200 to >10,000 nt long) that are transcribed from genic or intergenic regions of the genome. They have been implicated in transcriptional regulation and epigenetic control. Although the effects of estrogen signaling on protein coding and miRNA transcripts have been studied in detail, the effects on lncRNAs have not addressed. We used Global Run-On and sequencing (GRO-seq) to determine the immediate effects of estrogen signaling on the transcription of genes for ncRNAs in the ER-positive MCF-7 human breast cancer cells in response to short treatment with estradiol (E2; 0, 10, 40, and 160 minutes). GRO-seq is a direct sequencing method that provides a [ldquo]map[rdquo] of the position and orientation of all engaged RNA polymerases across the genome at high resolution. We used a novel bioinformatic approach to call pri-mRNA and lncRNA transcripts from the GRO-seq data. Many of the lncRNAs represent novel transcripts that have not been previously annotated. We then examined the regulation of the pri-miRNA and lncRNA transcription in response to E2. Our results indicate a rapid, robust, and transient mode of regulation for many of these transcripts. We are now examining the mechanisms by which these transcripts are regulated, as well as their functions in the estrogen-dependent cellular physiology. Sources of Research Support: Grants from the NIH/NIDDK to W.L.K., a predoctoral fellowship from the AHA to M.S., and a postdoctoral fellowship from the PhRMA Foundation to C.G.D. Nothing to Disclose: MS, SG, CGD, NH, WLK

Pub #	OR14-3
Session Information	ORAL SESSION: TRANSLATIONAL - Nuclear Receptors: Genome Binding to Novel Functions (11:15 AM-12:45 PM)
Title	Estrogen Blocks Cholesterol Synthesis in Mouse Liver Exclusively Via Membrane Estrogen Receptors
Author String	F O'Mahony, A Pedram, M Razandi, BJ Harvey, ER Levin Department of Veterans Affairs Medical Center, Long Beach, CA; University of California, Irvine, Irvine, CA; Royal College of Surgeons in Ireland, Dublin, Ireland
Body	In addition to the actions at nuclear estrogen receptors (ER), estrogen signals via ER at the plasma membrane. We have developed a mouse expressing the E domain of classical ERα exclusively at the plasma membrane (MOER - Membrane Only Estrogen Receptor). Estrogen induced rapid ERK and PI3 kinase activation comparably in wild type (WT) and MOER mice but not in knockout mice (ERKO). From the work of others, some gene transcription is regulated by membrane ER in collaboration with classical nuclear receptors but gene regulation and biological functions originating exclusively from membrane ER have not been described. Here, we carried out DNA analysis on isolated liver RNA from ovexed WT, MOER and ERKO mice following 3 days of injections with oil or the ERα agonist PPT. Data were analyzed by Baysian (Cyber T) approach using a confidence interval of 99.9% and a 2-fold change. Lists of increased/decreased genes comparably modulated in WT and MOER (implicating membrane ER action) but not seen in ERKO were compiled. 32 mRNAs were decreased from membrane only ER action. Importantly, 7 of the transcripts were in the cholesterol biosynthesis pathway, including the critical transcription factor SREBP1 and its' pathway targets. Our array data was validated in liver RNA extracts by qRT-PCR and in liver cells isolated for primary culture from the three mice types. The validation carried out *in vitro* also demonstrated a direct affect of PPT on the liver cells. Using primary liver cell cultures from both WT and MOER mice we found that PPT (10 nM) comparably blocked insulin-induced cholesterol synthesis. We investigated the signaling pathway involved and found that short pre-treatment with an AMP-Kinase inhibitor (Compound C) and an ERK inhibitor (PD98059) blocked the suppression by PPT of cholesterol synthesis. PPT activation of ERK by *in vitro* kinase assay was inhibited by pre-treatment with the AMPK inhibitor establishing the order of activation. In addition, SREBP1 was found to be predominantly nuclear following insulin treatment and this nuclear translocation was blocked by PPT pre-treatment. We establish that membrane ERα/PPT signaling suppresses the genes and hence the function of the cholesterol synthesis pathway in liver, not requiring nuclear ER action. Nothing to Disclose: FO, AP, MR, BJH, ERL

Pub #	OR14-4
Session Information	ORAL SESSION: TRANSLATIONAL - Nuclear Receptors: Genome Binding to Novel Functions (11:15 AM-12:45 PM)
Title	Liver X Receptor Activation by a Primary Cholesterol Metabolite Negatively Impacts Bone
Author String	ER Nelson, X Wang, MK Howe, CD DuSell, G Evans, RD Michalek, JC Rathmell, D Gesty-Palmer, S Khosla, DP McDonnell Duke University Medical Center, Durham, NC; Duke University Medical Center, Durham, NC; Mayo Clinic College of Medicine, Rochester, MN
Body	Osteoporosis and age related bone loss are important public health concerns. Therefore, there is interest in the development of medical interventions and lifestyle changes that result in improved bone quality. We have recently demonstrated that 27-hydroxycholesterol (27HC) is a selective estrogen receptor modulator (SERM), elevation of which leads to an osteoporosis-like phenotype in mice. Here, we describe studies that define the mechanisms by which 27HC impacts bone biology. Although 27HC behaved as a partial ER agonist in osteoblasts, it was apparent from our studies *in vitro and in vivo* that 27HC may also act on additional targets. In this regard, it has been shown that 27HC can function as a Liver X Receptor (LXR) agonist. Indeed we determined that *in vitro* treatment with 27HC or synthetic LXR agonists (a) decreases pre-osteoblast proliferation, (b) represses the expression of genes associated with osteoblast differentiation, and (c) decreases osteoblast differentiation and mineral deposition activity. Furthermore, 27HC, acting through the LXRs, leads to increased TNFα production in pre-osteoblasts, resulting in a commensurate increase in RANKL, and a subsequent increase in osteoclastogenesis. In order to confirm that the negative effects of 27HC observed in bone are in part due to its activation of LXRs, we treated female mice with the 'pure' LXR agonist, GW3965. MicroCT analysis revealed that femora from mice treated with GW3965 had a decreased trabecular bone volume fraction, decreased trabecular thickness, and decreased cortical thickness. Importantly, histomorphometric analysis revealed that the number of osteoblasts per perimeter and percent of bone surface covered by osteoblasts were reduced, a finding that corroborates our *in vitro* studies showing that LXR activation decreases osteoblast differentiation. Urine deoxypyridinoline was elevated compared to control animals, indicating increased osteoclast activity. Therefore, LXR activation by synthetic or endogenous agonists decreases bone deposition and increases bone resorption. Finally, we show that estradiol can mitigate the effects of LXR activity by inducing the short heterodimer partner, which then inhibits the LXRs. These data establish a firm association between 27HC and bone quality, a conclusion that has far-reaching medical implications given the established link between metabolic disease and osteoporosis, and that statin use is associated with increased BMD in some patients. Sources of Research Support: NIHR37DK48807 (DPM); DOD postdoctoral award BC085585 (ERN). Nothing to Disclose: ERN, XW, MKH, CDD, GE, RDM, JCR, DG-P, SK, DPM

Pub #	OR14-5
Session Information	ORAL SESSION: TRANSLATIONAL - Nuclear Receptors: Genome Binding to Novel Functions (11:15 AM-12:45 PM)
Title	Retinoid X Receptor Alpha Has Distinct Roles in the Liver of Calorie-Restricted and Insulin-Resistant Mice
Author String	S Motola, CW Ng, C Chouinard, NJ Kennedy, E Fraenkel Massachusetts Institute of Technology, Cambridge, MA; University of Massachusetts Medical School, Worcester, MA
Body	Longevity and insulin resistance induced by calorie restricted (CR) and high fat diets (HFD), respectively, represent two distinct metabolic conditions that affect insulin sensitivity in mammals. Because Retinoic X receptor alpha (RXRα) plays a major role in regulating physiological processes such as cholesterol, fatty acid and steroid metabolism (1), we examined its role in the liver under CR or HFD conditions. We used genome wide analysis techniques coupled with computational analysis to identify upregulated genes and their associated RXRα binding events. Here we show that RXRα regulates oxidative reduction in the CR condition whereas in the HFD condition it shifts to regulate apoptosis. RNA-sequencing was used to discover 2514 significantly differentially expressed genes in both conditions ($p<1e-5$). 1416 genes were upregulated in CR and 1101 genes were upregulated in the HFD condition. Using ChIP-sequencing, we found that in the CR condition, RXRα binds to 571 upregulated genes ($p<9.07e-121$); exclusive to this condition, 94 of them were annotated to the oxidative reduction category ($p<4.8e-22$). However, in the HFD condition, RXRα binds to 326 upregulated genes ($p<2.2e-33$) and 36 of them were annotated to the regulation of apoptosis category, found only in this condition ($p<2.97e-4$). RXRα of the nuclear receptor family can homodimerize or heterodimerize with other class II members while binding to DNA (2). We used motif discovery tools to identify potential RXRα partners based on sequences under binding events associated with the upregulated genes. We found that in both conditions, the RXR:Liver X Receptor (LXR) motif is among the significant sequences associated with binding events. Further computational analysis of the different direct repeats enabled identification of partners that dimerize with RXRα while binding to the upregulated genes in each condition. In the CR condition, RXRα binds to 16 genes involved in oxidative reduction using the direct repeat 4 motif sequence. Surprisingly, the same sequence was found in the HFD condition under 11 binding events associated with genes that regulate apoptosis. This study has revealed the differentially expressed genes in models of longevity and insulin resistance and demonstrated the role of RXRα as a master regulator of distinct biological processes in those conditions. These results will enable better understanding of the transcriptional regulation of longevity and type II diabetes mellitus. (1) Wan YJY et al., MCB, 2000, 20:4436-4444 (2) Mangelsdorf DJ et al., Cell, 2000, 83:841-850 Nothing to Disclose: SM, CWN, CC, NJK, EF

Pub #	OR14-6
Session Information	ORAL SESSION: TRANSLATIONAL - Nuclear Receptors: Genome Binding to Novel Functions (11:15 AM-12:45 PM)
Title	Genome-Wide Analysis of Glucocorticoid Receptor Binding Regions in Muscle Cells Reveals Gene Network Involved in Insulin Signaling
Author String	T Kuo, J New, OS Mayba, TP Speed, J-c Wang UC Berkeley, Berkeley, CA; UC Berkeley, Berkeley, CA
Body	Glucocorticoids are steroid hormones involved in diverse cell type-specific physiological processes, including glucose, lipid, and protein metabolism, and are powerful anti-inflammatory agent. Extensive exposure to glucocorticoids can cause muscle atrophy and insulin resistance. Although these side effects are well documented, mechanisms governing glucocorticoid-induced muscle atrophy and insulin resistance are largely unknown. Our goal is to identify glucocorticoid-regulated primary genes that are responsible for inducing muscle atrophy and insulin resistance. Using chromatin immunoprecipitation-high throughput DNA sequencing (ChIPseq), we have identified 2252 glucocorticoid receptor (GR) binding regions, corresponding to 1604 genes, in mouse C2C12 myotubes. Microarray confirms that 142 of these 1604 genes are regulated by glucocorticoids. Among these potential primary GR targets, FoxO3A and PIK3R1 are two genes with known connection to muscle atrophy and insulin signaling pathway, respectively. Reported by others, constitutively active FoxO3A alone can cause muscle atrophy. Here, we show that glucocorticoids induce FoxO3A transcript and active protein levels. The expression of luciferase reporter that harbors GR binding regions of FoxO3A gene is significantly induced by glucocorticoids. Intriguingly, mutating a single nucleotide in one of the GR binding regions, glucocorticoid-dependent reporter gene expression is abolished entirely, confirming the validity of FoxO3A GRE. Moreover, chromatin conformation capture (3C) analysis suggests interaction between FoxO3A GRE and promoter regions. Overexpressing PIK3R1, the regulatory subunit of PI3K, has been reported to decrease insulin sensitivity; however, its role in muscle atrophy has not been tested. Here, we show that PIK3R1 overexpression in C2C12 myotubes leads to a 35% decrease in cell diameter, which strongly suggests its role in glucocorticoid-induced muscle atrophy. We are in the process of identifying protein domain(s) responsible for overexpressing PIK3R1-induced muscle atrophy. Overall, we have identified several genes, activated by GR concertedly, modulate protein metabolism. These results shall benefit future therapeutic development for better pharmaceutical interventions. Nothing to Disclose: TK, JN, OSM, TPS, J-CW

Pub #	OR15-1
Session Information	ORAL SESSION: CLINICAL - Thyroid Disease & Autoimmunity (11:15 AM-12:45 PM)
Title	Circulating CXCL9 Is Increased in Aggressive Chronic Autoimmune Thyroiditis, in Association with CXCL10
Author String	A Antonelli, SM Ferrari, S Frascerra, A Di Domenicantonio, I Ruffilli, E Ferrannini, P Fallahi University of Pisa, School of Medicine, Pisa, Italy
Body	CXC motif ligand 9 (CXCL9) has been shown to be involved in autoimmune thyroid disorders, however, unti now, no data are present about CXCL9 circulating levels in chronic autoimmune thyroiditis (AT). Serum CXCL9 (and for comparison CXCL10) has been measured in 189 consecutive patients with newly diagnosed AT with respect to 63 euthyroid controls and 30 patients with nontoxic multinodular goiter, and thi parameter has been related to the clinical phenotype. The three groups were similar in gender distribution and age; 26% of AT patients had subclinical hypothyroidism. Serum CXCL9 was significantly higher in AT (148±110 pg/mL) than in controls (71±34 pg/mL) or patients with multinodular goiter (87±35 pg/mL) ($p<0.0001$). Among AT patients, CXCL9 levels were significantly higher in patients older than 50 years, those with a hypoechoic ultrasonographic pattern or with hypothyroidism. In a multiple linear regression model including age, thyroid volume, hypoechogenicity, hypervascularity, TSH, and AbTPO, only age ($p=0.008$) and TSH ($p=0.009$) were significantly related to serum CXCL9 levels. Also CXCL10 was confirmed to be associated with AT, overall in presence of hypothyroidism. In a multiple linear regression model of CXCL9 vs age, TSH, and CXCL10, TSH (standardized coefficient=0.335; $p=0.021$) and CXCL10 (standardized coefficient=0.476; $p<0.001$) were significantly and independently related to CXCL9. In conclusion, high circulating levels of CXCL9 have been first shown in autoimmune thyroiditis patients, overall in presence of markers of a more aggressive immune attack, such hypothyroidism. Furthermore, to the best of our knowledge, this is the first demonstration of a strong relation between circulating CXCL9 and CXCL10, in a human autoimmune pathology. This finding strongly supports the role of a Th1 immune response in the pathogenesis of AT. Longitudinal observations in large patients cohorts will be needed to evaluate if circulating CXCL9 levels may serve as clinical prognostic marker in AT. Nothing to Disclose: AA, SMF, SF, ADD, IR, EF, PF

Pub #	OR15-2
Session Information	ORAL SESSION: CLINICAL - Thyroid Disease & Autoimmunity (11:15 AM-12:45 PM)
Title	Evidence Supporting the Concept of Two Conformational Forms of TSH Receptors on the Cell Surface, Only One of Which Is Recognized by Thyroid-Stimulating Autoantibodies
Author String	S Hamidi, C-R Chen, S McLachlan, B Rapoport Cedars-Sinai Medical Center and UCLA, Los Angeles, CA
Body	TSH receptor (TSHR) N-terminus residues 22-41 (after signal peptide removal) contain a cysteine knot comprising disulfide-bonds (C24-C29 and C31-C41) forming two loops. Chimeric substitution of residues 25-30 reduces the binding and function of polyclonal and monoclonal thyroid stimulating antibodies (TSAb). Nevertheless, the contribution of this region to TSAb epitope(s) remains controversial. We studied the effect of deleting the TSHR upstream loop (residues 22-30) on human monoclonal TSAb M22 binding and function. The M22 epitope lies entirely within the TSHR ectodomain (ECD). However, because of partial steric hindrance, the flow cytometric signal is very weak for TSAb binding to the TSH holoreceptor on the cell surface. In contrast, TSAb binding is readily detected with the ECD tethered to the membrane by a glycosylphosphatidyl inositol (GPI) anchor. Therefore, to study M22 binding we used the TSHR-ECD-GPI, either wild-type or with residues 22-30 deleted (Del-22-30). Quantitative standards were non-functional mouse mAb with downstream epitopes. Del-22-30 ECD-GPI expressed less well on the cell surface, ~50% of the wild-type ECD-GPI. However, relative to the standards, M22 binding to the Del-22-30 ECD-GPI was even lower, ~30% of the wild-type ECD-GPI. Reduced M22 binding to Del-22-30 could be due to a reduction in M22 affinity or recognition by M22 of fewer receptors. Saturation analysis of M22 binding to the wild-type and Del-22-30 ECDs revealed no decrease in M22 affinity. Therefore, residues 22-30 do not contribute to the M22 epitope but their deletion leads to M22 'seeing' fewer receptors relative to the antibody standards. Because the GPI-ECD cannot signal, we studied M22 function with CHO cells expressing the wild-type or Del22-30 holoreceptor (unsuitable for M22 binding studies). With TSH as a standard, deletion of residues 22-30 did not diminish the TSH receptor sensitivity to M22 stimulation of cAMP. In summary, deletion of the upstream Cys-loop (residues 22-30) does not affect TSAb M22 binding affinity to the TSHR-ECD, but does alter the ratio between two conformational forms of receptor, only one of which is recognized by M22. With the holoreceptor, this N-terminal deletion does not alter the sensitivity to M22 stimulation, suggesting that the ECD-GPI data also apply to the holoreceptor in vivo. Our findings support the concept that the TSHR N-terminal domain is important in TSAb activation of the TSHR in Graves' disease. Sources of Research Support: NIH Grant RO1 DK19289-35. Nothing to Disclose: SH, C-RC, SM, BR

Pub #	OR15-3
Session Information	ORAL SESSION: CLINICAL - Thyroid Disease & Autoimmunity (11:15 AM-12:45 PM)
Title	Thyroidal CD40 Expression Plays a Major Role in the Pathogenesis of Graves Disease: Analyses Using a Novel Mouse Model
Author String	AK Huber, E Concepcion, EP Smith, E Jacobson, Y Tomer Mount Sinai School of Medicine, New York, NY; James J Peters VA Medical Center, Bronx, NY; University of Cincinnati, Cincinnati, OH
Body	Graves' disease (GD) is characterized by the production of high levels of thyroid stimulating hormone receptor (TSHR) auto-antibodies which stimulate the TSHR and cause goiter and thyrotoxicosis. A C/T single nucleotide polymorphism (SNP) in the CD40 gene at the -1 position of the 5'UTR, in the Kozak sequence, was shown to be associated with GD, increasing the risk for GD by 30-80%. Functionally, the C allele of this SNP has been shown to result in an increased CD40 gene translation, and cellular CD40 protein expression. Additionally, previous data from our lab have suggested that tissue specific thyroidal CD40 expression plays a role in the pathogenesis of Graves' disease. To determine the effect of thyroidal CD40 expression in Graves' disease, we created transgenic mice that over-expressed CD40 specifically in the thyroid. We then induced the currently accepted Nagayama experimental autoimmune Graves' disease (EAGD) model in these TG mice and compared them to WT littermates. While the frequency of EAGD in wild type and transgenic mice was not significantly different ($p=0.32$), transgenic mice that developed disease had significantly more severe disease, as shown by higher titers of TSHR antibodies ($p=0.004$), and T4 levels ($p=0.005$). Cytokine analysis demonstrated that transgenic mice had significantly higher levels of IL-6, and TNFalpha in their thyroid, suggesting that CD40 triggers thyroid inflammation by a bystander mechanism. In conclusion, our data suggested that while CD40 thyroidal expression is not necessary for the development of EAGD, its over-expression in the thyroid, driven by the C allele of the Kozak SNP, augments the production of pathogenic antibodies. Nothing to Disclose: AKH, EC, EPS, EJ, YT

Pub #	OR15-4
Session Information	ORAL SESSION: CLINICAL - Thyroid Disease & Autoimmunity (11:15 AM-12:45 PM)
Title	Obstetrical and Neonatal Outcomes in Pregnant Women with Serum Thyroid-Stimulating Hormone (TSH) Levels >2.0 mU/L *vs.* Those with TSH <2.0 in the First Trimester of Pregnancy
Author String	S Achit, T Dhar, K Awasthi, B Uppal, JJ Jacob Christian Medical College and Hospital, Ludhiana, India; Christian Medical College and Hospital, Ludhiana, India; Christian Medical College and Hospital, Ludhiana, India
Body	Objective; To determine obstetrical and neonatal outcomes in antenatal women with serum TSH levels [ge] 2.0mU/L vs. those with TSH <2.0 mU/L in the first trimester of pregnancy. Methods: Two hundred consecutive women in the first trimester of pregnancy attending regular antenatal clinic and planning subsequently to deliver at the study hospital were recruited after informed consent. Subjects with clinically obvious thyroid disease or those with previously diagnosed thyroid disease were excluded. Blood samples were evaluated for serum TSH, Free Thyroxine (FT4) levels and anti-Thyroid peroxidase antibody (anti-TPO ab) titres. Patients with undiagnosed primary or subclinical hypothyroidism (TSH levels [ge] 4.0mU/L with either normal or low levels of FT4) were treated and included in the study. Patient with undiagnosed hyperthyroidism were excluded from analysis. Pregnancy related maternal complications (Pregnancy induced hypertension (PIH), Abruption, Preterm Delivery, IUGR and IUD), obstetrical outcomes (Mode of Delivery, foetal distress and meconium staining of amniotic fluid), birth weights and neonatal outcomes (birth asphyxia, Septicaemia, Hypoglycemia and neonatal Hyperbilirubinemia) were recorded. Results: 197 patients completed the study (2 delivered elsewhere and 1 had undiagnosed hyperthyroidism). 107 (53.5%) patients had serum TSH levels [ge] 2.0mU/L (Group A). 18 of these 107(16.8%) patients had significant titres of anti-TPO ab compared to 3 (3.3%) among the 90 with TSH <2.0 mU/L (Group B) (p-0.008). 33 patients (16.5%) had TSH levels [ge] 4.0mU/L and were treated with levothyroxine. There was no difference in baseline values except a history of increased still births in Group A (9.3% vs 2.2% p-0.04). Among maternal complications there was a significant increase in PIH in Group A (9.3% vs 2.2% p-0.04). Average birth weight in Group A was 2740gms vs. 2920gms in Group B (p-0.009). Neonates born to mothers in Group A had 19.6% chances of developing Neonatal Hyperbilirubinemia vs 4.4% Group B (p-0.008). Othe maternal complications, obstetrical outcomes and neonatal complications were not statistically different in both groups. Conclusions: Pregnant women with serum TSH levels [ge] 2.0mU/L in the first Trimester had a fourfold increased risk of developing PIH. Neonates born to these mothers were approximately 200gms lighter and hac a fourfold increased risk of developing neonatal Hyperbilirubinemia. Nothing to Disclose: SA, TD, KA, BU, JJJ

Pub #	OR15-5
Session Information	ORAL SESSION: CLINICAL - Thyroid Disease & Autoimmunity (11:15 AM-12:45 PM)
Title	Subclinical Hyperthyroidism Is Associated with Increased All-Cause Mortality in Elderly Subjects
Author String	G Ceresini, F Lauretani, M Maggio, S Bandinelli, S Morganti, E Usberti, G Valenti, L Ferrucci, GP Ceda University of Parma, Parma, Italy; University Hospital, Parma, Italy; Azienda Sanitaria di Firenze, Florence, Italy; National Institute on Aging, Baltimore, MD

Body

Introduction.

Altered thyroid function, especially subclinical, represents one of the most frequent endocrine abnormality that occur in older individuals. Literature reports demonstrate that even subtle thyroid dysfunctions may be accompanied by important consequences, such as metabolic disorders, abnormality of heart function and cardiac rhythm, altered bone metabolism and cognitive disturbances. Contrasting results have been reported on the relationship between thyroid function abnormalities and mortality in older individuals. Using data from the Aging in the Chianti Area (InCHIANTI) study, an epidemiological study conducted on a population-based sample of persons living in the Chianti geographical area (Tuscany, Italy), we evaluated the relationship between thyroid dysfunction and all-cause mortality in older persons.

Materials and Methods.

Plasma concentrations of thyrotropin, free triiodothyronine, and free thyroxine were evaluated in all subject at the enrolment. Data were available for 950 participants (N.541 female and N. 409 male) aged 65 years or older. Five groups were defined according to thryoid function test as follows: overt hypothyroidism, subclinical hypothyroidism, euthyroidism, subclinical hyperthyroidism, and overt hyperthyroidism. Subjects were followed-up prospectively for all-cause mortality for 6 years. Mortality was compared between the euthyroid group and each of the thyroid dysfunction groups after adjusting data for age and sex.

Results.

Individuals with both overt hypothyroidism (N. 5, 0.52%) and overt hyperthyroidism (N. 14, 1.4%) were excluded because of small numbers. Eighty-six percent of participants (N. 819) had normal thyroid function, 3% (N. 29) had subclinical hypothyroidism, 8.7% (N. 83) had subclinical hyperthyroidism. When data were adjusted for confounders, such as Congestive heart failure, Body Mass Index, Cancer and Stroke, all-cause mortality was significantly increased in subclinically hyperthyroid subjects as compared to euthyroid subjects (H.R., 1.65 [95% C.I., 1.021-2.696], P= 0.04). No significant differences in survival curves were found between subclinically hypothyroid subjects and euthyroid subjects.

Conclusions.

In our population-based sample of older persons, subclinical hyperthyroidism, but not subclinical hypothyroidism, was associated with an increase in all-cause mortality.

Nothing to Disclose: GC, FL, MM, SB, SM, EU, GV, LF, GC

Pub #	OR15-6
Session Information	ORAL SESSION: CLINICAL - Thyroid Disease & Autoimmunity (11:15 AM-12:45 PM)
Title	Age-Related Changes in Thyroid Function: A Longitudinal Study of a Community-Based Cohort
Author String	JP Walsh, AP Bremner, SJ Brown, P Feddema, PJ Leedman, JP Beilby, P O'Leary Sir Charles Gairdner Hospital, Nedlands, Australia; University of Western Australia, Crawley, Australia; University of Western Australia, Crawley, Australia; Diagnostica Stago, Doncaster, Australia; PathWest Laboratory Medicine, Nedlands, Australia; Western Australian Department of Health, Perth, Australia
Body	Background. Cross-sectional studies suggest that serum TSH concentrations increase with age in healthy individuals, leading to recommendations for age-related reference ranges for TSH (1). It is, however, uncertain whether the TSH increase reflects an adaptive response to aging, a higher prevalence of occult thyroid failure in older people, or both (2,3). This question has not been examined in longitudinal studies, and it is also uncertain whether the influence of common genetic variants on thyroid function remains constant with aging. Methods. We measured TSH, free T4, thyroid peroxidase antibodies (TPOAbs), and thyroglobulin antibodies (TgAbs) using the Immulite platform on sera from 1184 participants in the 1981 and 1994 Busselton Health Surveys (4). We derived a reference group of 908 individuals who had no history of thyroid disease and did not have overt hypothyroidism, hyperthyroidism or positive thyroid antibodies. The mean age (SD) at baseline was 45.5 y (14.5); 423 (47%) were female. In 823 participants, genotype data was available for the recently described *CAPZB* polymorphism *rs10917469*, which is associated with differences in serum TSH concentrations (5). Results. At 13 years follow-up, mean serum TSH in the cohort had increased from 1.49 to 1.81 mU/L, a change in mean TSH ([Delta]TSH) of 0.32 mU/L (95% CI 0.27, 0.38, P<0.001). There was no significant change in mean free T4 concentrations (16.6 vs. 16.6 pmol/L, P=0.7). The TSH increase was greater in those who were older at baseline: after adjustment for sex, each 10 year increase in baseline age was associated with an increase in [Delta]TSH of 0.08 mU/L (95% CI 0.04-0.11). People with higher baseline TSH values had proportionally smaller changes in TSH: after adjustment for age and sex, each additional 1 mU/L of baseline TSH was associated with a 0.28 mU/L decrease in [Delta]TSH (95% CI .22, 0.34). The change in TSH did not differ significantly by *CAPZB* genotype (mean [Delta]TSH (SD) 0.37 (0.82) for genotype AA, 0.27 (0.71) for AG and 0.24 (0.42) mU/L for GG; P=0.27). Conclusions. In healthy individuals with no evidence of thyroid disease, aging is associated with a significant increase in TSH concentrations. The largest TSH increase is seen in those with the lowest TSH concentrations at baseline, and there is no accompanying fall in free T4 concentrations. This suggests strongly that the TSH increase arises from an age-related alteration in TSH setpoint or altered TSH bioactivity, and not from occult thyroid disease. (1) Surks MI et al., J Clin Endocrinol Metab 2010; 95:496 (2) Surks MI et al., J Clin Endocrinol Metab 2007; 92:4575 (3) Spencer CA et al., J Clin Endocrinol Metab 2007; 92:4236 (4) Walsh JP et al., J Clin Endocrinol Metab 2010; 95:1095 (5) Panicker V et al., Am J Hum Genet 2010; 87:430 Nothing to Disclose: JPW, APB, SJB, PF, PJL, JPB, PO

Pub #	OR16-1
Session Information	ORAL SESSION: CLINICAL - Evaluation & New Mechanisms of Pituitary Deficiency (11:15 AM-12:45 PM)
Title	Novel Autoimmune Polyendocrine Syndrome: Adult Combined GH, Prolactin and TSH Deficiency Associated with Circulating PIT-1 Antibody
Author String	Y Takahashi, M Yamamoto, G Iguchi, R Takaeno, Y Okimura, T Sano, M Takahashi, H Nishizawa, AE Handayaningshi, K Tojo, A Mokubo, K Iida, H Kaji, K Chihara Kobe University Graduate School of Medicine, Kobe, Japan; Kobe Woman's University, Kobe, Japan; Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan; The Jikei University School of Medicine, Tokyo, Japan; Mokubo Clinic, Kawasaki, Japan; Kakogawa Prefectural Hospital, Kakogawa, Japan; University of Hyogo, Akashi, Japan
Body	The pituitary-specific transcriptional factor-1, POU1F1 also known as PIT-1, is an essential factor for somatotrophs, lactotrophs, and thyrotrophs. A genetic defect in PIT-1 gene results in congenital growth hormone (GH), prolactin (PRL), and thyroid stimulating hormone (TSH) deficiency. Here, we report 3 cases of adult-onset combined GH, PRL, and TSH deficiencies, for which the endocrinological findings were attributed to autoimmunity directed at the PIT-1 protein. We detected anti-PIT-1 antibody along with various autoantibodies in the patients' sera. An ELISA-based screening showed that this antibody was highly specific to the disease. Immunohistochemical analysis revealed that PIT-1-, GH-, PRL-, and TSH-positive cells were absent in the pituitary of patient 2, who also had a range of autoimmune endocrinopathies. These clinical manifestations were compatible with the definition of autoimmune polyendocrine syndrome (APS). However, the main manifestations of APS-I--hypoparathyroidism and Candida infection--were not observed and the pituitary abnormalities were obviously different from the hypophysitis associated with APS. In this study, we describe a novel syndrome, 'anti-PIT-1 antibody syndrome' related to APS. To further elucidate the pathophysiology, we analyzed genes that are involved in the regulation of the immune tolerance. Intriguingly, we found that the same mutation in all 3 patients in a gene, which plays an essential role in the regulation of B cell function. Although the functional analysis of the mutant is under investigation, it is speculated that this mutation may be causally associated with the pathogenesis of 'anti-PIT-1 antibody syndrome'. Nothing to Disclose: YT, MY, GI, RT, YO, TS, MT, HN, AEH, KT, AM, KI, HK, KC

Pub #	OR16-2
Session Information	ORAL SESSION: CLINICAL - Evaluation & New Mechanisms of Pituitary Deficiency (11:15 AM-12:45 PM)
Title	IGF-I Bioactivity More Accurately Reflects Growth Hormone Deficiency Than Total-IGF-I
Author String	AJ Varewijck, SWJ Lamberts, P Uitterlinden, LJ Hofland, JAMJL Janssen Erasmus MC, Rotterdam, Netherlands
Body	*Context* Growth hormone (GH) is considered the main regulator of circulating insulin like growth factor-I (IGF-I). Total (extractable) IGF-I is therefore routinely used for diagnosis of Growth Hormone Deficiency (GHD) and for monitoring treatment. Methods currently used for measurement of circulating total IGF-I may be hampered by interferences of IGF-binding proteins (IGF-BPs). Recently, a Kinase Receptor Activation (KIRA) assay was developed to determine IGF-I bioactivity in human serum. The principle of this assay is based on quantification of IGF-I receptor (IGF-IR) activation after stimulation with serum in vitro. *Objective* To investigate the diagnostic potential of IGF-I bioactivity in adults with GHD. *Design* Single centre observational study. *Study Participants* 94 GH-untreated patients diagnosed with GHD by GH-provocative-tests were included. *Main Outcome Measures* IGF-I bioactivity (determined by the IGF-I KIRA) and total IGF-I (determined by immunoassay) in fasting blood samples. *Results* IGF-I bioactivity was more frequently below the normal range (<-2SD) in untreated GH deficient patients than total IGF-I levels (81.9% vs. 61.7%, respectively), especially in patients >40 years of age. IGF-I bioactivity decreased with the duration of GHD, whereas total IGF-I did not. With a decreasing number of additional pituitary deficits, total IGF-I levels more frequently remained within the normal range, whereas the percentage below the normal range was high for IGF-I bioactivity, independent of additional deficits. *Conclusion* Determination of IGF-I bioactivity may offer advantages in the evaluation of adult GHD compared to total IGF-I as bioactivity more accurately reflects GH action, especially in subjects >40 years of age. Sources of Research Support: Grant from Novo Nordisk A/S (Alphen aan de Rijn, the Netherlands). Novo Nordisk A/S had no involvement in the study design, in the collection, analysis, and interpretation of data. Nothing to Disclose: AJV, SWJL, PU, LJH, JAMJLJ

Pub #	OR16-3
Session Information	ORAL SESSION: CLINICAL - Evaluation & New Mechanisms of Pituitary Deficiency (11:15 AM-12:45 PM)
Title	Clinical Characteristics of Two Dosing Regimens of the Glucagon Stimulation Test (GST) in the Evaluation of Growth Hormone (GH) Reserve and Hypothalamic-Pituitary-Adrenal (HPA) Axis in Adults
Author String	KCJ Yuen, SA Rhoads, K Spiller, MB Gordon Oregon Health & Science University, Portland, OR; Allegheny General Hospital, Pittsburgh, PA

Body

Rationale: Administration of IM glucagon has been shown to be effective in releasing GH and cortisol (1). We previously described experience using a fixed-dose (FD) regime of the GST, and found that high BMIs induced lower peak GH levels, whereas lower cortisol cut-points were needed to reliably evaluate the HPA axis (2). In this study, we compared the FD to a weight-based (WB) regime of the GST in assessing GH reserve and HPA axis.

Methods: Patients referred to the Endocrine Testing Unit suspected of GH deficiency and adrenal insufficiency were studied. Subjects were matched for age, gender and BMI. FD regime is defined as the administration of 1 mg and 1.5 mg IM glucagon for patients who weighed [le] 90 kg and > 90 kg respectively whereas WB regime is defined as the administration 0.03 mg/kg IM glucagon. During the GST, blood was sampled at baseline and every 30 min for 240 min. Peak GH < 3 ng/mL and peak cortisol < 18 g/dL defined GH and cortisol deficiencies.

Results: Forty one patients underwent the FD [11 M, age 48.8 ± 0.7 yrs, BMI 33.6 ± 1.4 kg/m^2], and 44 patients underwent the WB regime [11 M, age 49.4 ± 1.9 yrs, BMI 32.2 ± 1.2 kg/m^2]. Peak glucose (WB: 84% between 60-150 min vs FD: 90% between 30-60 min), peak GH (WB: 98% between 180-240 min vs FD 71% between 90-150 min) and peak cortisol (WB: 82% between 210-240 min vs FD: 69% between 150-240 min) levels occurred later in the WB group. Peak (WB: 166 ± 6 vs FD: 160 ± 7 mg/dL) and nadir (WB: 90 ± 4 vs FD: 75 ± 5 mg/dL) glucose levels, and peak GH (WB: 11.5 ± 2.3 vs FD: 8.0 ± 1.8 ng/mL) levels were comparable in both groups, but the WB group had higher peak cortisol levels (21.0 ± 1.6 vs 13.6 ± 1.5 g/dL, $P < 0.001$). The main side-effect was mild nausea in both groups (WB: 50% vs FD: 39%). In the FD group, 25 (62%) and 23 (56%) patients were GH and cortisol-deficient respectively, while 12 (27%) and 16 (36%) patients were GH and cortisol-deficient respectively in the WB group.

Conclusion: Both dosing regimes of the GST were safe and well-tolerated. While peak glucose, GH and cortisol levels occurred later with the WB regime, the WB regime demonstrates that using higher doses of glucagon does not necessarily induce higher peak and nadir glucose responses, but may be more effective in inducing cortisol release than the FD regime. Our data thus suggests that the WB may be more reliable than the FD regime in evaluating the HPA axis, but requires further clarification with a prospective comparative trial.

1. Leong KS, et al. An audit of 500 subcutaneous glucagon stimulation tests to assess growth hormone and ACTH secretion in patients with hypothalamic-pituitary disease. Clinical Endocrinology 2001; 54: 463-468.
2. Yuen KCJ, et al. Clinical characteristics of the glucagon stimulation test (GST) in the evaluation of growth hormone (GH) reserve and hypothalamic-pituitary-adrenal (HPA) axis in adults: a multi-centered US experience. 92nd Endocrine Society Annual Meeting, San Diego, CA, June 2010.

Sources of Research Support: KCJY has received research grants from Novo Nordisk.

Nothing to Disclose: KCJY, SAR, KS, MBG

Pub #	OR16-4
Session Information	ORAL SESSION: CLINICAL - Evaluation & New Mechanisms of Pituitary Deficiency (11:15 AM-12:45 PM)
Title	Chronic Hypopituitarism after Blast Concussion Mild Traumatic Brain Injury in Iraq/Afghanistan Combat Veterans
Author String	CW Wilkinson, ER Peskind, EA Colasurdo, JB Shofer Veterans Affairs Puget Sound Health Care System, Seattle, WA; Veterans Affairs Puget Sound Health Care System, Seattle, WA; University of Washington, Seattle, WA

Body

Studies of civilian traumatic brain injury (TBI) from all causes have found evidence of chronic hypopituitarism, as defined by deficient production of one or more pituitary hormones measured at least one year after injury, in 33-50% of cases (1). Its occurrence has not been found to be related to trauma severity (1,2). Hypopituitarism is associated with non-specific behavioral symptoms that can be mistaken for PTSD, including fatigue, anxiety, depression, irritability, insomnia, poor concentration and memory, and decreased quality of life (1). Despite these findings, the prevalence of hypopituitarism after blast concussion mild TBI, the signature injury of combat in Iraq and Afghanistan, has not yet been investigated. Mild TBI (mTBI) is characterized by brief loss of consciousness or loss of memory for events surrounding the trauma or any alteration of mental state (disorientation, confusion). In order to determine the frequency of pituitary dysfunction after blast concussion mTBI, we are measuring pituitary and target organ hormones in blood samples from Iraq/Afghanistan Veterans with mTBI taken at least one year subsequent to their last blast exposure. Most have experienced multiple blast exposures. Criteria for identifying abnormal circulating levels of LH, FSH, total testosterone, prolactin, ACTH, cortisol, TSH, free thyroxine, GH, IGF-I, and arginine vasopressin (AVP) were derived from RIA or EIA measurement of basal morning concentrations in a large group of male non-Veteran control subjects. In general, values below the 5th percentile or above the 95th percentile were defined as abnormal. When both pituitary and target organ hormones were measured for a given axis, a specific combination of criteria signaled dysfunction of that axis. Using the criteria defined in controls, 10 of 26 Veterans with blast mTBI were found to have abnormal hormone levels in one or more pituitary axes. Seven mTBI subjects exhibited deviant plasma AVP concentrations, either above or below the 5th-95th percentile normal range. The frequency of abnormally low or abnormally elevated AVP levels has been found to be relatively high in the acute stage of non-blast TBI, but it tends to decline with time. These preliminary findings suggest that the prevalence of hypopituitarism after blast concussion mTBI is similar to that in other forms of TBI, and that recovery and rehabilitation of blast-exposed Veterans may be facilitated by comprehensive screening for pituitary dysfunction.

(1) Ghigo E et al., Brain Inj, 2005; 19:711
(2) Lieberman SA et al., J Clin Endocrinol Metab 2001; 86:2752

Sources of Research Support: Department of Defense Congressionally Directed Medical Research Programs Concept Award PT090753 to CWW; Geriatric Research, Education and Clinical Center (GRECC), Northwest Network VISN-20 Mental Illness Research, Education and Clinical Center (MIRECC), and Research and Development Service, VA Puget Sound Health Care System.

Nothing to Disclose: CWW, ERP, EAC, JBS

Pub #	OR16-5
Session Information	ORAL SESSION: CLINICAL - Evaluation & New Mechanisms of Pituitary Deficiency (11:15 AM-12:45 PM)
Title	Hyponatremia in Aneurysmal Subarachnoid Hemorrhage Is Due to the Syndrome of Inappropriate Antidiuresis and Acute Glucocorticoid Deficiency
Author String	MJ Hannon, LA Behan, B Rogers, M Sherlock, DM Smith, A Agha, CJ Thompson Beaumont Hospital/RCSI Medical School, Dublin, Ireland; Centre for Endocrinology, School of Clinical and Experimental Medicine, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK
Body	Hyponatraemia is the most common electrolyte abnormality following subarachnoid haemorrhage (SAH) and contributes to increased morbidity and mortality. Retrospective data suggests that the syndrome of inappropriate diuresis (SIAD) is the most common cause of hyponatraemia in SAH, though cerebral salt wasting has been postulated by some workers to be the predominant abnormality. Data which has shown acut glucocorticoid deficiency following SAH has suggested that some cases of euvolaemic hyponatraemia may also be caused by this mechanism. We prospectively studied the hormonal and haemodynamic influences involved in the development of hyponatraemia in 100 patients (61% female, median age 53 (range 16-82)) with non-traumatic aneurysmal SAH. Each patient had plasma sodium (pNa), urea, osmolality, glucose and 0900h cortisol (PC), and urinary sodium and osmolality measured on days 1, 2, 3, 4, 6, 8, 10 and 12 following SAH. Fluid balance and haemodynamic parameters were recorded daily. Results were compared with 15 patients admitted to ITU following vascular surgery. A PC<300nmol/L in a patient in ITU was regarded clinically as inappropriately low. 49% of patients developed hyponatraemia (pNa<135 mmol/L), including 14% who developed clinically significantly hyponatraemia (pNa<130 mmol/L). 36/49 (73.4%) developed hyponatraemia between days 1 an 3 post SAH. The median duration of hyponatraemia was 3 days (range 1-10 days). In 35/49 (71.4%), hyponatraemia was due to SIAD as defined by standard diagnostic criteria. 14% of SAH patients had at least one PC<300nmol/L; 5 of these (35.7%) developed hyponatraemia. In 4 patients hyponatraemia was preceded by acute cortisol deficiency and responded to hydrocortisone treatment. In contrast, all controls had PC>500 nmol/L on day 1, and >300 nmol on days 3-12. There were no cases of cerebral salt wasting. There was no relationship between the incidence of hyponatraemia and the defined anatomical territory or severity of SAH. There was no difference in the incidence of hyponatraemia between those patients who had an intervention and those who did not (p=0.11), or in the incidence of hyponatraemia between those patients who had clipping and those who had coiling. In the first prospective study of its kind, hyponatraemia occurs in over half of aneurysmal SAH cases, predominantly due to SIAD. Acute glucocorticoid deficiency is a treatable cause of a minority of cases of hyponatraemia. We found no evidence of cerebral salt wasting. Nothing to Disclose: MJH, LAB, BR, MS, DMS, AA, CJT

Pub #	OR16-6
Session Information	ORAL SESSION: CLINICAL - Evaluation & New Mechanisms of Pituitary Deficiency (11:15 AM-12:45 PM)
Title	Is the 250 [mu]g ACTH Test a Useful Tool for the Diagnosis of Central Hypoadrenalism in Adult Patients with Pituitary Disorders?
Author String	E Ferrante, V Morelli, G Mantovani, C Giavoli, E Verrua, E Sala, S Bergamaschi, E Profka, I Chiodini, A Lania, A Spada, P Beck-Peccoz University of Milan, Milan, Italy; Fondazione IRCCS C[agrave] Granda Ospedale Maggiore Policlinico, Milan, Italy; Istituto Clinico Humanitas, Rozzano, Milan, Italy
Body	Introduction The diagnosis of central hypoadrenalism (HPAI) in patients with hypothalamic-pituitary disorders is a major clinical pitfall. The gold standard procedure for the diagnosis remains the insulin tolerance test (ITT), while recent studies confirmed that standard-dose corticotropin stimulation test (SDCT, 250 [micro]g ACTH test) has a wide variability in the cortisol thresholds, with a grey area between 16 and 30 [micro]g/dl. Aim to assessed HPA axis function by ITT test in patients with pituitary disorders and uncertain diagnosis of HPAI by SDCT. Patients and Methods. Insulin tolerance test (ITT) was performed in a cohort of 50 consecutive patients (34F & 16M, mean age 40.7±10.8 yrs) referred to our centre for pituitary disorders. In 43 patients 30-minutes serum cortisol levels at SDCT was between 16 and 30 [micro]g/dl (GROUP A) while in 7 cortisol levels were below 16 [micro]g/dl, in the absence of other biochemical data and clinical symptoms of HPAI and any other pituitary deficiency (GROUP B). Results A normal response to ITT (peak cortisol levels ≥ 18 [micro]g/dl) was found in the great majority of GROUP A (37/43) and in about a half of GROUP B patients (4/7). Therefore, despite the choice of a cut-off as low as 16 [micro]g/dl, in this study SDCT showed a very low sensitivity (Se=33%, Sp=90%). With regard to basal cortisol levels, no patient with confirmed diagnosis of HPAI by both tests showed levels higher than 10.6 [micro]g/dl. Conversely, no patient with normal cortisol response to ITT showed levels lower than 3.7 [micro]g/dl. Conclusions SDCT is not a reliable tool to identify patients with HPAI. Additional studies in large cohorts should be undertaken to revise the cut-off values of the test and to avoid unnecessary substitution therapies in patients with pituitary disorders. Nothing to Disclose: EF, VM, GM, CG, EV, ES, SB, EP, IC, AL, AS, PB-P

Pub #	OR17-1
Session Information	ORAL SESSION: CLINICAL - Female Reproductive Endocrinology: Effects of Genes & Environment on Reproductive Hormones & Health Outcomes (11:15 AM-12:45 PM)
Title	Fasting Regulates the Hypothalamic-Pituitary-Gonadal Axis by Decreasing Activin A and Increasing Inhibin B and Follistatin Levels in a Leptin-Independent Manner
Author String	VA Moragianni, KN Aronis, JP Chamberland, CS Mantzoros Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; Boston VA Healthcare System, Harvard Medical School, Boston, MA

Body

Objective: Activin, inhibin and follistatin (FST) are members of the TGF-β superfamily of hormones that regulate the hypothalamic-pituitary-gonadal (HPG) axis in humans. Activin stimulates pituitary FSH release, whereas inhibin and FST bind and neutralize activin, leading to downregulation of FSH secretion. Leptin is another potent modulator of the HPG axis. In fasting-induced hypoleptinemic states, leptin replacement restores LH pulsatility and ovulatory menstruation. Currently, no evidence exists on the interplay of these hormones, or the influence of fasting/feeding on their levels. The objectives of our study were to elucidate (i) the secretion pattern of activin, inhibin and FST,(ii)whether their levels are affected by energy deprivation, and (iii)if such an effect exists, whether it is mediated by leptin.

Methods: We conducted 2 separate studies of healthy, lean, regularly-menstruating females;(1)study A, consisting of 3 separate 72hr admissions, during which 6 women were either maintained on an isocaloric diet or fasted for 72hr and were administered metreleptin or placebo replacement (IV q6hr),(2)study B, consisting of 3 separate fasting 72hr admissions, during which 5 women were administered either 1 of 3 metreleptin doses (0.01,0.1,or 0.3mg/kg IV qd). We measured activin A, inhibin B and FST levels in serum samples collected every 15min for 24hr on the third day of study A and the third fasting day of study B, using commercially-available ELISAs. We utilized PulseXP software for the secretion pattern analysis, and STATAv.11 for hierarchical mixed-effects linear modeling (study A) and repeated measurements ANOVA (study B).

Results: Neither activin A, inhibin B or FST were found to have a pulsatile or day-night pattern of secretion. When compared to the fed state, fasting inhibin B and FST ($p<0.001$) levels increased,whereas activin A ($p=0.008$) levels decreased significantly. Metreleptin in replacement doses was not found to have an effect on altering fasting-state values (all $p>0.05$). Acute metreleptin administration in increasing doses (physiologic and pharmacologic) did not affect these hormone levels (all $p>0.05$).

Conclusions: Our findings are consistent with the teleologic theory of decreased reproductive capacity during hypocaloric states. During periods of energy deprivation activin A levels are decreased, whereas inhibin B and FST levels are increased, acting in concert to down-regulate the HPG. These effects of fasting are not mediated by leptin.

Sources of Research Support: National Institutes of Health - National Center for Research Resources grant M01-RR-01032 (Harvard Clinical and Translational Science Center) and grant number UL1 RR025758. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health. Funding was also received from the National Institute of Diabetes and Digestive and Kidney Diseases grants 58785, 79929 and 81913, and AG032030. Amylin Pharmaceuticals, Inc. supplied metreleptin for this study but had no role in the study design; conduct of the study; collection, management, analysis, and interpretation of the data; or the preparation, review, or approval of the manuscript.

Nothing to Disclose: VAM, KNA, JPC, CSM

Pub # OR17-2

Session Information ORAL SESSION: CLINICAL - Female Reproductive Endocrinology: Effects of Genes & Environment on Reproductive Hormones & Health Outcomes (11:15 AM-12:45 PM)

Title Obesity: Attenuated Pituitary Responsiveness Following GnRH Stimulation

Author String AJ Polotsky, A Kucherov, AP Bradford, J Lesh, B Babbs, J Chosich, N Santoro
Albert Einstein College of Medicine, Bronx, NY; University of Colorado Denver, Aurora, CO

Body

Female adult obesity is associated with menstrual cycle irregularities, ovulatory dysfunction and obstetrical complications. This reproductive phenotype is progressively worsened by further increases in body mass and is not solely due to anovulation. Prior studies of ovulatory, obese women indicate significantly reduced LH pulse amplitude with unchanged LH pulse frequency compared to normal weight controls(1).

Objective: To examine the pituitary response to GnRH stimulation in eumenorrheic (non-PCOS) obese women in the absence of estradiol feedback. To eliminate endogenous estradiol feedback, we administered a 7-day, weight-adjusted dose of an aromatase inhibitor (letrozole) and conducted testing in the follicular phase at completion of the course of letrozole.

Methods: 6 obese (BMI, 34.1±1.1 kg/m^2) and 9 normal weight (BMI, 21.0±0.5 kg/m^2) women underwent 8-hour, q10 min blood sampling sessions, with a 75 ng/kg bolus of GnRH given at 4 hours. LH & FSH were assayed by a well-characterized immunofluorometric assay (DELFIA, PerkinElmer). LH pulsatility was evaluated using a modified Santen-Bardin method(2). Group means were compared using t tests.

Results: Obese and normal weight women were of similar age (29.5±1.6 years vs. 29.2±1.2 years, respectively, p=0.91). Prior to administration of GnRH, there was a trend for reduced pituitary LH secretion in obese women compared to controls (mean LH, 4.9±0.7 IU/L vs. 7.9±1.4 IU/L, respectively, p=0.07; LH pulse amplitude, 2.3±0.4 IU/L vs. 4.5±1.1 IU/L, respectively, p=0.14), but no difference in LH pulse frequency (obese, 0.5±0.1 pulses/4 hrs vs. normal weight, 0.6±0.1 pulses/4 hrs, p=0.69).

Following GnRH stimulation, obese women exhibited significantly reduced mean LH as compared with controls (7.2±1.1 IU/L vs. 12.5±1.5 IU/L, respectively, p=0.03). Similarly, there was a trend for overall attenuation in pituitary response with obesity (delta LH: obese, 8.9±2.0 IU/L vs. control, 14.7±2.0 IU/L, p=0.07; peak LH: obese, 11.4±2.6 IU/L vs. control, 20.0±2.5 IU/L, p=0.06). FSH values demonstrated trends similar to LH.

Conclusions: Our results indicate attenuated pituitary responsiveness to GnRH in obese women following the release of negative feedback inhibition of estradiol on the hypothalamic-pituitary axis. These findings suggest a possible pituitary site of action for obesity-mediated alterations of reproductive hormones and argue against estradiol feedback as a mechanism for the reduced LH secretion in obesity.

(1) Jain A, Polotsky AJ, Rochester D, Berga SL, Loucks T, Zeitlian G, Gibbs K, Polotsky HN, Feng S, Isaac B, Santoro N. Pulsatile luteinizing hormone amplitude and progesterone metabolite excretion are reduced in obese women. J Clin Endocrinol Metab. 2007;92:2468-73.
(2) Santen RJ, Bardin CW 1973 Episodic luteinizing hormone secretion in man. Pulse analysis, clinical interpretation, physiologic mechanisms. J Clin Invest 52:2617-2628

Sources of Research Support: NIH U54 HD058155 Center for the Study of Reproductive Biology; K24 HD041978 to NS; 1UL1 RR025780 (University of Colorado CTRC).

Nothing to Disclose: AJP, AK, APB, JL, BB, JC, NS

Pub #	OR17-3
Session Information	ORAL SESSION: CLINICAL - Female Reproductive Endocrinology: Effects of Genes & Environment on Reproductive Hormones & Health Outcomes (11:15 AM-12:45 PM)
Title	Bone Microarchitecture Is Impaired in Adolescent Amenorrheic Athletes Compared with Eumenorrheic Athletes and Non-Athletic Controls
Author String	T Nazem, KE Ackerman, M Russell, M Tappen, N Mendes, ML Bouxsein, M Misra Massachusetts General Hospital and Harvard Medical School, Boston, MA; Children's Hospital and Harvard Medical School, Boston, MA; Massachusetts General Hospital and Harvard Medical School, Boston, MA; Mass General Hospital for Children and Harvard Medical School, Boston, MA
Body	**BACKGROUND:** The female athlete triad is characterized by low energy availability, menstrual dysfunction and low bone mineral density (BMD). BMD is lower in young amenorrheic athletes (AA) compared to eumenorrheic athletes (EA) and non-athletic controls (C), and may contribute to fracture risk during a critical time of bone accrual. Data also indicate that abnormal bone microarchitecture (BMa) is an important and independent determinant of fracture risk. However, BMa has not been assessed in adolescent AA vs. EA and C. **OBJECTIVE:** To determine if BMa is altered in AA vs. EA and C, and evaluate determinants of BMa in adolescent athletes and non-athletes. We hypothesized that BMa is impaired in AA compared to EA and C despite weight bearing exercise. **METHODS:** We assessed BMD and BMa in 48 subjects; 13 AA, 22 EA and 13 C (14-21y) using DXA and high resolution peripheral QCT. Exercise and diet history, and vitamin D levels were obtained. **RESULTS:** The groups did not differ in chronological age (CA), bone age (BA), BMI, fat mass, lean mass, o vitamin D levels. Athletes had higher activity levels than C. AA had later age at menarche, longer duration of amenorrhea and lower BA for CA compared with EA and C. Lumbar, hip and total body BMD were lower in AA vs. EA and C, and lumbar bone mineral apparent density (LBMAD) Z-scores were -0.975, 0.126 and -0.309 respectively (p=0.02). For BMa at the radius, trabecular bone volume (BV/TV) was lower in AA vs. EA and C (0.131 vs. 0.148 and 0.159 AU; p=0.04). At the tibia, trabecular number (TbN) was reduced in AA vs. EA and C (1.78 vs. 2.01 and 2.04 mm^{-1}; p=0.01), while trabecular separation (TbSp) was increased (0.484 vs. 0.410 and 0.414 mm; p=0.003). At the radius and tibia, age at menarche was inversely associated with cortical thickness (CtTh) and BV/TV, and positively with TbSp. At the radius, age at menarche was inversely associated with TbN and TbTh. There were modest associations of BMD with BMa. LBMAD Z predicted all BMa measures except tibial TbTh. Total body BMD Z predicted all BMa measures except radial TbN and tibial TbTh. Hip BMD Z predicted radial BV/TV and TbTh, and tibial CtTh, BV/TV, TbN and TbSp. **CONCLUSION:** We demonstrate that in addition to low BMD, adolescents with AA have impaired bone microarchitecture at the radius and tibia compared with EA and C. Amenorrhea and later age at menarche are important determinants of impaired BMa. BMa may provide information independent of BMD regarding bon health. Sources of Research Support: In part by NIH grants 1 UL1 RR025758-01 and 1 R01 HD060827-01A1. Nothing to Disclose: TN, KEA, MR, MT, NM, MLB, MM

Pub #	OR17-4
Session Information	ORAL SESSION: CLINICAL - Female Reproductive Endocrinology: Effects of Genes & Environment on Reproductive Hormones & Health Outcomes (11:15 AM-12:45 PM)
Title	Genome-Wide Bivariate Association Analysis Identifies Novel Candidate Genes for Ages at Menarche and Natural Menopause and for Bone Mineral Density: The ReproGen and GEFOS Consortia
Author String	Y-H Hsu, X Chen, C Elks, JM Murabito, K Estrada, TB Harris, KL Lunetta, A Murray, KK Ong, JRB Perry, FR Rivadeneira, L Stolk, AG Uitterlinden, CM Zillikens, DP Kiel, D Karasik Hebrew SeniorLife, Boston, MA; Boston University, Boston, MA; Erasmus University, Rotterdam, Netherlands; Boston University, Boston, MA; University of Exeter, Exeter, UK; University of Cambridge, Cambridge, UK; National Institute on Aging, Bethesda, MD; Addenbrooke's Hospital, Cambridge, UK
Body	Age at menarche (AAM) and age at natural menopause (ANM) are associated with the risk for osteoporosis. Early menarche is associated with higher peak bone mass whereas menopause is associated with a rapid phase of bone loss. Univariate genome-wide association studies (GWAS) have identified multiple loci associated with variation in AAM, ANM, and bone mineral density (BMD). Multivariate analysis of correlated phenotypes may increase the statistical power to identify genes associated with one or several phenotypes. The aim of this study was to identify novel genes for AAM, ANM, and/or BMD (lumbar spine, LS and femoral neck, FN) phenotypes using multivariate analysis in women of European descent. We performed bivariate analysis for AAM and BMD as well as ANM and BMD using an empirical-weighted linear-combined test statistics (eLC) approach. Results from a univariate meta-analysis of 2.5 million SNPs .was obtained from the GEFOS consortium for BMD (23,678 women, aged ~60.5 yrs on average) and from the ReproGen consortium for AAM and ANM (87,802 women and 38,968 women, respectively). The eLC combines test statistics from each univariate meta-analysis while accounting for the correlation among phenotypes, providing an unbiased estimation of multivariate test statistics. Bivariate p-values [le] $5x10^{-8}$ were considered genome-wide significant (GWS). Several GWS loci were found in bivariate analysis including previously reported loci discovered by each univariate meta-analysis. Novel loci that achieved GWS by bivariate analysis (not found if only univariate analysis is performed) included SNPs located in/near *GRB10* (on 7p12.2), *C6orf173/RSPO3* (6q22.3) and *LIN7C/LGR4/BDNF* (11p14) for AAM-BMD phenotype pairs; and *DMC1* for ANM-BMD pairs. *GRB10* codes for growth factor receptor-bound protein, an adapter protein that negatively regulates Wnt signaling; *RSPO3* is a thrombospondin that activates the beta-catenin signaling cascade; variants in this gene were recently found to be strongly associated with waist-hip ratio, and this effect was more evident on women; *BDNF* (brain-derived neurotrophic factor) is associated with body mass and smoking behavior. Using this bivariate approach on correlated phenotypes, we discovered novel loci that would not be considered GWS in univariate GWAS for AAM, ANM or BMD. Some of the newly discovered genes are involved in pathways influencing skeletal and reproductive endocrine metabolism and may have pleiotropic effects on both systems. Nothing to Disclose: Y-HH, XC, CE, JMM, KE, TBH, KLL, AM, KKO, JRBP, FRR, LS, AGU, CMZ, DPK, DK

Pub #	OR17-5
Session Information	ORAL SESSION: CLINICAL - Female Reproductive Endocrinology: Effects of Genes & Environment on Reproductive Hormones & Health Outcomes (11:15 AM-12:45 PM)
Title	Endogenous Estrogen Levels and Coronary Heart Disease Outcomes in the Women's Health Initiative Randomized Trials
Author String	DC Bauer, GN Farhat, JA Cauley, A Huang, A LaCroix, J Lee, D Grady, K Yaffe, J Manson, N Parimi, E Vittinghof, SR Cummings University of California, San Francisco, San Francisco, CA; California Pacific Medical Center, San Francisco CA; University of Pittsburgh, Pittsburgh, PA; Fred Hutchinson Cancer Research Center, Seattle, WA; University of California, Davis, Davis, CA; University of California, San Francisco, San Francisco, CA; University of California, San Francisco, San Francisco, CA; Harvard Medical School, Boston, MA
Body	The relationship between endogenous sex hormone levels and coronary heart disease (CHD) in postmenopausal women is uncertain. The WHI trials found that estrogen plus progestin (E+P) treatment was associated with a 29% increased risk of CHD, while estrogen alone (E-alone) had no effect on CHD risk. We used data from the WHI trials to determine if pre-treatment serum estradiol (E2) levels: 1) were independently associated with CHD events and 2) modified the effect of either E+P or E-alone treatment on CHD risk. In the E-alone trial, 10,739 women (average age 63.6±7.3 yrs) were randomized to conjugated equine estrogens (CEE, Premarin, 0.625/d) or placebo and followed for an average of 7.1 years. In the E+P trial, 16,608 women (average age 63.3±7.1 yrs) were randomized to CEE plus medroxyprogesterone acetate (Prempro, 0.625/2.5 per day) or placebo and followed for an average of 5.6 yr. CHD events (acute myocardial infarction, silent MI identified on serial electrocardiography, or CHD death) during the E-alone (N=418) and E+P trial (N=335) were adjudicated centrally without knowledge of treatment status. Pre-randomization archived serum was assayed for total E2 using a radioimmunoassay (University of Southern California, CA) from the 748 women with CHD events and 400 randomly selected women. Cox models that accounted for the case-cohort sampling were used to analyze associations between E2 quartiles and CHD risk and interactions between baseline E2 levels and treatment effect. In both trials CHD risk was lower among women with higher baseline endogenous E2 levels, regardless of treatment (p-trend<0.05). For example, in the E+P trial, women in the highest quartile of total E2 ([ge]15.5 pg/dl) had a 70% lower risk of CHD events compared to those in the lowest quartile ([le]9.02 pg/ml) (HR=0.3 95% CI: 0.1-0.5 adjusted for age, waist circumference, medical history and medication use). Results comparing lowest to highest quartile of E2 were similar in the E-alone trial (HR=0.5, 95% CI: 0.3-1.0). Bioavailable-E2 associations were similar to those of total E2 in both trials. Baseline levels of total E2 did not modify the relationship between treatment and CHD risk in either the E-alone (interaction p-value= 0.18) or the E+P (interaction p-value =0.73) trial. We conclude that in the WHI trials, higher endogenous E2 levels are associated with substantially lower CHD risk. Neither total nor bioavailable-E2 alter the effect of E-alone or E+P treatment on the risk of CHD. Nothing to Disclose: DCB, GNF, JAC, AH, AL, JL, DG, KY, JM, NP, EV, SRC

Pub #	OR17-6
Session Information	ORAL SESSION: CLINICAL - Female Reproductive Endocrinology: Effects of Genes & Environment on Reproductive Hormones & Health Outcomes (11:15 AM-12:45 PM)
Title	Pre-Chemotherapy Anti-Müllerian Hormone Is Predictive of Long-Term Ovarian Function in Women with Breast Cancer
Author String	RA Anderson, DA Cameron University of Edinburgh, Edinburgh, UK; University of Edinburgh, Edinburgh, UK
Body	Future reproductive function is a concern for many young women with cancer as some chemotherapy regimens carry a substantial risk of premature ovarian failure. Younger women are at reduced risk, but there are few data allowing individualization based on endocrine measures. AMH is produced by granulosa cells of preantral/small antral follicles, and is a marker of the ovarian reserve. We investigated whether AMH measured at cancer diagnosis predicts ovarian activity up to 2 years following chemotherapy. Fifty premenopausal women with early breast cancer were recruited at diagnosis. Mean age at recruitment wa 42.5 years, range 29.1 to 51.1. All had regular spontaneous menstrual cycles. Chemotherapy regimens were anthracycline/cyclophosphamide based, with approximately half receiving a taxane and 64% subsequently treated with tamoxifen. Blood samples were taken before starting chemotherapy and women kept a daily menstrual diary throughout 2 years of followup as an index of ovarian activity. AMH was measured using the GenII assay (Beckman Coulter). Eleven women withdrew from the study, mostly due to disease recurrence. Menstrual data were available on 40 women at 12 months followup, and 29 at 24 months. Pretreament AMH was strongly negatively correlated with age (Spearman r=-0.55, $p<0.0001$), with a median value of 0.40ng/ml. AMH fell very rapidly with treatment, becoming undetectable (<0.16ng/ml) after 1 cycle of chemotherapy in 68% of women. At 12 months, 20/21 women with AMH below the median were amenorrheic, vs 14/19 who retained menses ($p=0.08$). However at 24 months, 16/17 women with a low pretreatment AMH were amenorrheic, vs 6/12 witl a high AMH ($p=0.01$, odds ratio 16.0) and this relationship remained significant after adjustment for age ($p=0.03$). ROC analysis showed significant predictive value of pretreatment AMH (AUC 0.75, $p<0.05$) with peak likelihood ratio for ongoing menses at 24 months of 4.7 at AMH >0.92ng/ml, specificity 91% and sensitivity 43%. Similar proportions of women in the two AMH groups were treated with tamoxifen. Conclusion. These prospective data confirm the markedly accelerated loss of AMH with chemotherapy. Women with higher pretreatment AMH were more likely to retain ovarian function at 2 years after breast cancer diagnosis, independent of age. This may be generalisable to other cancers to aid fertility-related decision-making. Sources of Research Support: MRC. Nothing to Disclose: RAA, DAC

Pub #	OR18-1
Session Information	ORAL SESSION: CLINICAL - Parathyroid Hormone (11:15 AM-12:45 PM)
Title	Quantitative Analysis of Methylation Defects and Correlation with Clinical Characteristics in Patients with Pseudohypoparathyroidism and *GNAS* Epigenetic Alterations
Author String	G Mantovani, FM Elli, AM Barbieri, L de Sanctis, AG Lania, P Beck-Peccoz, A Spada Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Universit[agrave] degli Studi di Milano, Milan, Italy; Universit[agrave] di Torino, Torino, Italy
Body	The two main subtypes of pseudohypoparathyroidism (PHP), PHP-Ia and -Ib, are caused by mutations in *GNAS* exons 1-13 and methylation defects in the imprinted *GNAS* cluster, respectively. PHP-Ia patients show Albright hereditary osteodystrophy (AHO) and resistance toward multiple hormones (primarily PTH, TSH, GHRH and gonadotropins), whereas PHP-Ib patients classically display hormone resistance limited to PTH and TSH. Incoming data suggest that the two diseases share more genetic and clinical similarities than previously thought. In particular, methylation defects have been detected in a subset of patients with PHP and AHO, but the correlation between molecular findings and the severity of the disease has not been investigated yet. In the present study, we included 30 patients with either classical PHP-Ib (N=16) or PHP with different degrees of AHO (N=14), all found to have broad *GNAS* methylation defects in the absence of mutations. Differential methylation of *GNAS* DMRs A/B, AS, XL, NESP was assessed using highly quantitative analysis based on PCR-pyrosequencing. The data obtained were correlated with the following clinical characteristics: age at diagnosis, endocrine function (PTH, TSH, FT4, calcium, phosphorus levels) and the presence or absence of AHO signs (short stature, brachydactyly, round face, ectopic ossifications, mental retardation). No statistical difference was observed between the group of patients with classical PHP-Ib and the group with PHP plus AHO. In particular, the degree of the imprinting defect (percentage of methylation at each DMR expressed in respect with a pool of 20 normal subjects age- and gender-related) did not correlate with the onset of the disease, the severity of endocrine resistances, nor with the presence/absence of specific AHO signs. In conclusion, similar molecular alterations may lead to a broad spectrum of diseases, from isolated PTH resistance to complete PHP-Ia. Our study further confirms the existence of an overlap between molecular and clinical features of PHP-Ia/Ib, highlighting the need of an updated classification that takes into account the recent knowledge on the molecular basis underlying these defects. Nothing to Disclose: GM, FME, AMB, LdS, AGL, PB-P, AS

Pub #	OR18-2
Session Information	ORAL SESSION: CLINICAL - Parathyroid Hormone (11:15 AM-12:45 PM)
Title	A Family of Pseudohypoparathyroidism Type Ia with an 850-Kb Deletion Encompassing the Whole *GNAS1* Locus: Variable Clinical Phenotypes in Dizygotic Twins
Author String	T Mitsui, K Nagasaki, M Takagi, S Narumi, T Ishii, T Hasegawa Keio University School of Medicine, Tokyo, Japan; Niigata University Graduate School of Medicine and Dental Sciences, Niigata, Japan
Body	**Background**: Pseudohypoparathyroidism type Ia (PHP-Ia) is characterized by PTH resistance and Albright's hereditary osteodystropy (AHO), which includes short stature, obesity, round face, brachydactyly and subcutaneous calcification. About 70% of patients with PHP-Ia have a point mutation in *GNAS1* (encoding Gsα). To date, no case with total deletion of *GNAS1* has been reported. **Patients**: Detailed clinical information has been published (Ref.1). In brief, the proband, a 10-year-old girl, had typical AHO: obesity (BMI, > 97th centile), round face, brachydactyly and subcutaneous calcification on her left ankle and back. Biochemical tests revealed compensated PTH resistance (Ca, 8.9 mg/dl Pi, 5.0 mg/dl, and intact PTH 152 pg/ml (ref 10-60)). A dizygotic twin brother of the proband also had mild manifestations of AHO: overweight (BMI, 90-97th centile) with subcutaneous calcification on his left hand. He had hypocalcemia (Ca, 7.0 mg/dl) with a high PTH level (intact PTH, 377 pg/ml). The mother of the proband presented AHO (short stature (-2.0 SD), round face and mild obesity) without PTH resistance. The proband and her brother were diagnosed as having PHP-Ia. **Methods & Results**: To clarify the molecular mechanism of PHP-Ia of the siblings, we first searched a point mutation in the coding exons of *GNAS1* by PCR-based direct sequencing, but found no mutation in the two affected children and their mother. Next, we screened copy number changes (deletion or duplication) by the multiplex ligation-dependent probe amplification method, and found that the whole *GNAS* locus was likely to be deleted. This putative deletion was confirmed by oligonucleotide array comparative genomic hybridization (SurePrintG3 180K, Agilent), which revealed an 850-kb deletion at 20q13.32 encompassing known 11 genes including *NESP55, NESPAS, GNASXL*, *GNAS1* exon1-13 in all three subjects. **Conclusions**: We identified the first PHP-Ia patients with a total *GNAS1* deletion inherited from mother. Clinical expression of hypocalcemia and AHO were variable even in a family with the complete *GNAS1* haploinsufficiency, indicating additional factor(s) influencing the PHP-Ia phenotype. Moreover, we could find no recognizable phenotypic difference between our patients and previously reported *GNAS1* mutation-positive ones. This implies that a subset of patients with '*GNAS1* point mutation-negative' PHP-Ia might have a *GNAS* deletion, because such deletions cannot be detected by PCR-based sequence analyses. (1) Nagasaki K et al. Clin Pediatr Endocrinol 2005; 14(2), 39-44 Nothing to Disclose: TM, KN, MT, SN, TI, TH

Pub #	OR18-3
Session Information	ORAL SESSION: CLINICAL - Parathyroid Hormone (11:15 AM-12:45 PM)
Title	A Case of Reversible Cardiomyopathy Due to Idiopathic Primary Hypoparathyroidism
Author String	D Sanyal KPC Medical College, Kolkata, India
Body	***Introduction:*** Calcium has a direct effect on the strength of myocardial contraction through excitation-contraction coupling. Hypocalcemia reduces myocardial contractility, but the incidence of congestive cardiac failure(CCF) and cardiomyopathy due to hypocalcemia is very rare. ***Clinical Case:*** A 30 years old man presented with progressive dyspnoea, orthopnoea for 3 weeks. Clinically he had CCF(NYHA IV) with no history of coronary artery disease, valvulopathy, hypertension, diabetes mellitus, alcohol abuse, viral fever. He had a 3 years past history of intermittent tingling, cramps of hand and feet with positive Chvostek's and Trousseau's sign. His complete blood count, urea, creatinine, liver function tests were normal. Troponin-I and CPK-MB was normal, ECHO with Doppler showed global hypokinesia, dilated Left Ventricular cavity and severe systolic dysfunction with Ejection Fraction 24%, coronary angiogram was normal. He was diagnosed as a case of dilated cardiomyopathy(DCM). His serum calcium-5.4mg/dl(Normal:8.5-10.5), phosphate-9.4mg/dl(Normal:2.5-4.9), magnesium-2.2mg/dl (Normal:1.8-3), iPTH -6.6pg/ml(Normal:15-68.3), 25 hydroxy Vitamin D was 58ng/dl(Normal: 30-80). CT scan of brain showed extensive basal ganglia and thalamic calcification. TSH-1.27mIU/ml(Normal:0.5-5), FT4:1.24ng/dl(Normal:0.9-1.7), post Synacthen Cortisol-22.1 micro gm/dl(Normal >18) was normal. Treatment with diuretics, digitalis & ACE inhibitors resulted in slight clinical improvement. Correction of hypocalcemia, initially with intravenous calcum followed by oral calcium with calcitriol, led to further improvement and he reached functional class NYHA I. The left ventricle gradually returned to normal size and systolic function recovered completely in two months. ***Conclusion:*** This is a case of a young man with reversible hypocalcemic DCM due to idiopathic primary hypoparathyroidism. Hypocalcaemia is an important and rare reversible cause of DCM. Routine measurements of serum calcium should be considered in whom the etiology of cardiomyopathy is unknown. Hypocalcemic cardiomyopathy is usually refractory to conventional treatment for cardiac failure but responds favorably to restoration of normocalcemia. Few cases of hypocalcemic cardiomyopathy have been reported, in these cases, correction of hypocalcemia was associated with complete or partial resolution of CCF and in some cases the left ventricular geometry and systolic function recovered completely. Nothing to Disclose: DS

Pub #	OR18-4
Session Information	ORAL SESSION: CLINICAL - Parathyroid Hormone (11:15 AM-12:45 PM)
Title	Should Bisphosphonates Be Started in Patients with Bone Disease after Parathyroidectomy?
Author String	K Wood, R Sippel, H Chen, H Mazeh University of Wisconsin School of Medicine and Public Health, Madison, WI; University of Wisconsin School of Medicine and Public Health, Madison, WI
Body	**Background:** Parathyroidectomy increases Bone Mineral Density (BMD) in patients with primary hyperparathyroidism (PHPT) (1). This increase is not matched by bisphosphonate therapy alone, (2) and as such surgery remains the treatment of choice for osteoporotic patients with PHPT (3). However, it is not known whether addition of a bisphosphonate after parathyroidectomy leads to even greater increases in BMD **Methods:** We retrospectively reviewed a prospective database of patients undergoing parathyroidectomy for PHPT between November 2000 and January 2009. BMD was recorded before and at least 6 months after surgery. We excluded patients with persistent disease and those with DXA scans performed on different machines. **Results:** 966 patients underwent parathyroidectomy during this period of which 120 (12%) met inclusion/exclusion criteria. 112 patients had available total hip data with a mean T score of -1.26 and a mean follow up time of 20.2 months. 6 patients (23%) were on a bisphosphonate after surgery and 87 (77%) had parathyroidectomy alone. There was a 2.15% increase in total hip BMD the patients who received bisphosphonates post-operatively vs. 2.04% in the surgery alone group (p=0.93). 83 patients had available spine data with a mean T Score of -1.5 and a mean follow up time of 20.6 months. 23 (28%) were on a bisphosphonate after surgery and 60 (72%) had parathyroidectomy alone. There was a 4.78% increase in spine BMD in the patients who received bisphosphonates post-operatively vs 0.82 % in the surgery alone group (p=0.007). **Conclusion:** The initiation of bisphosphonate therapy after parathyroidectomy lead to a significant increase ir spine BMD when compared to surgery alone. Randomized, prospective studies are needed but this suggests there is an additional benefit of bisphosphonate therapy after surgery in osteoporotic patients with primary hyperparathyroidism. (1) Silverberg SJ et al.N Engl J Med 1999 Oct 21;341(17):1249-55. (2) Khan AA et al. J Clin Endocrinol Metab 2004 Jul;89(7):3319-25. (3) Bilezikian JP et al., J Clin Endocrinol Metab. 2009 Feb;94(2):335-9. Nothing to Disclose: KW, RS, HC, HM

Pub #	OR18-5
Session Information	ORAL SESSION: CLINICAL - Parathyroid Hormone (11:15 AM-12:45 PM)
Title	Anabolic Effect of Teriparatide Was Undermined by Low HDL Cholesterol and High Total Cholesterol Levels
Author String	YK Jeon, KM Kim, KJ Kim, IJ Kim, S-K Lim, Y Rhee Pusan National University School of Medicine, Busan, Korea; Severance Hospital, Yonsei University College of Medicine, Seoul, Korea

Body

It is well known that administration of intermittent parathyroid hormone (PTH) has potent ability to increase bone mass regardless of the underlying conditions or species. Recent animal study using $LDLR^{-/-}$ mice showed that anabolic effect of PTH was blunted by hyperlipidemic status (1), meanwhile PTH anabolism was rescued by enhancing high density lipoprotein-cholesterol (HDL-C) function (2). The objective of this study was to find whether lipid profiles also affect anabolic effect of intermittent PTH in human. We studied 40 patients diagnosed with severe osteoporosis who had been treated with recombinant human PTH (1-34), teriparatide (TPTD), for 12 months at Severance Hospital, Yonsei University College of Medicine. These patients received daily subcutaneous injection of 20 [mu]g TPTD for 12 months (age: 33-85 years, 33 females and 7 males). Bone turnover markers and routine chemistry including total cholesterol (TC), triglyceride and HDL-C were performed at 0,3 and 12 months, and bone mineral density (BMD) by dual X-ray absorptiometry at 0 and 12 months, of TPTD treatment. In this study, we analyzed the relationship between percent changes of BMD at the lumbar spine (LS), femoral neck (FN) and total hip (TH) versus basal clinical parameters such as age, body mass index, glucose, and lipid profiles. In addition, the relations between percent changes of BMD were compared with initial 3 month-changes of serum collagen type 1 cross-linked C-telopeptide (CTX) and osteocalcin (OCN). The percent change of BMD was significantly increased more at LS ($9.5 \pm 1.6\%$, $p<0.001$) compared to FN (0.5 ± 1.1 %, $p=0.797$) and TH ($2.9 \pm 1.0\%$, $p=0.022$) after 12 months treatment of TPTD. The percent changes of BMD at LS showed negative correlation with TC levels ($r=-0.464$, $p=0.009$) and positive correlation with HDL-C ($r=0.627$, $p=0.004$), and the 3 month-changes of CTX and OCN ($r=0.568$, $p=0.002$ and $r=0.497$, $p=0.012$, respectively). Low plasma HDL-cholesterol was significantly associated with lesser response of lumbar BMD to TPTD even after the adjustment for age, lipid profiles, and initial changes of bone turnover markers (standardized $\beta = 0.715$, $t=3.687$, $p=0.003$). The results of this study support the hypothesis that enhanced oxidized lipid by low HDL-C negatively impinge on bone density as well as response to PTH. In conclusion, circulating plasma lipid levels, especially HDL-C seem to influence osteoanabolic effect of TPTD in human.

(1) Huang MS, Lu J, Ivanov Y, Sage AP, Tseng W, Demer LL, Tintut Y. Hyperlipidemia impairs osteoanabolic effects of PTH. J Bone Miner Res 23:1672-1679, 2008
(2) Sage AP, Lu J, Atti E, Tetradis S, Ascenzi MG, Adams DJ, Demer LL, Tintut Y. Hyperlipidemia induces resistance to PTH bone anabolism in mice via oxidized lipids. J Bone Miner Res 2010

Nothing to Disclose: YKJ, KMK, KJK, IJK, S-KL, YR

Pub #	OR18-6
Session Information	ORAL SESSION: CLINICAL - Parathyroid Hormone (11:15 AM-12:45 PM)
Title	Hyperparathyroidism after Low Neck Irradiation for Extracranial Head and Neck Cancers
Author String	N Bhandare, L Kennedy, CG Morris, WM Mendenhall University of Florida, Gainesville, FL; Cleveland Clinic, Cleveland, OH

Body

Purpose: To investigate the incidence of hyperparathyroidism (HPTr) after radiation therapy (RT) in patients treated for extra-cranial head and neck tumors who received low anterior neck(LAN) RT, and its association with RT.

Methods and Materials: Records of 343 patients with no known pre-existing sporadic hyperparathyropidism (HPTs) who were treated with RT for extra-cranial head and neck tumors between 1970 and 2000 were retrospectively reviewed. Of these, 204 patients received LAN RT. The age at the time of treatment ranged from 5 to 66 years. Median follow-up was 7.6 years (1.0-34.5 years). The two groups (with and without LAN RT) were subjected to a Chi square test to examine the significance of radiation to the incidence of HPT.

Results: Of patients with LAN RT, 14 had clinically confirmed parathyroid dysfunction (high serum calcium, normal or high PTH). The median period of detection of HPTr after RT was 20.2 years (range 7-31 years). Of the 7 patients who had surgery for symptomatic HPTr, 5 had single parathyroid adenomas that were histologically confirmed. Of these patients, 1 had history of renal calculi 9 years prior to parathyroid surgery, 1 had history of peptic ulcers and 1 had history of chronic gastric problems and pancreatitis. Two of these patients also had thyroid tumors. Besides these, 1 patient had multiglandular parathyroid hyperplasia, and another 1 had surgery, but pathologic findings were nonspecific whether it was an adenoma or hyperplasia. Two other patients with confirmed HPTr but asymptomatic at the time of detection, were revealed to have parathyroid adenomas after surgery for thyroid cancer. Five patients had confirmed HPTr but were asymptomatic at the time of detection though their records indicated that one had history of renal calculi 7 years prior, one had history of gastric ulcer, and one was later diagnosed with osteoporosis. None of these 5 patients underwent surgery. Of the patients without LAN RT, only one was diagnosed with HPTs and had an adenoma. Statistical analysis using Chi square test indicated that LAN radiation was significant for incidence of HPT($p = 0.0058$).

Conclusion: HPT is significantly increased in patients with head and neck cancer who received radiation to the LAN compared to those who did not - 6.9% v 0.7%. This suggests LAN RT may play a causative role in HPTr.

Nothing to Disclose: NB, LK, CGM, WMM

Pub #	OR19-1
Session Information	ORAL SESSION: CLINICAL - Pediatric Endocrinology: Bone & Obesity (11:15 AM-12:45 PM)
Title	Intra- and Postoperative PTH Measurements Predict Hypocalcemia after Total Thyroidectomy in Children
Author String	AV Freire, A Chiesa, O Acha, D Braslavsky, R Grinspon, M Troiano, M Morini, MG Ballerini, MG Ropelato I Bergada, L Gruneiro de Papendieck Ricardo Gutiérrez Children's Hospital, Buenos Aires, Argentina; Ricardo Gutiérrez Children's Hospital, Buenos Aires, Argentina
Body	**Introduction:** Hypocalcemia after thyroidectomy develops because of hypoparathyroidism secondary to parathyroid trauma or their inadvertent removal during thyroid resection. Due to its short half-life, PTH was reported as a useful monitor of postsurgical hypoparathyroidism in adults but there are no studies within a pediatric population. **Objective:** To evaluate the diagnostic accuracy of intra and postoperative PTH to predict the risk of hypocalcemia in children undergoing total thyroidectomy. **Methods:** We performed a prospective blind longitudinal cohort study. Twenty patients (3-17y) undergoing total thyroidectomy were included. Intact PTH was measured by ICMA (IMMULITE, Siemens, CVs <5.4 %, functional sensitivity=8pg/ml). Peripheral PTH measurements at 5 min after thyroid removal (intraoperative) and 60 min after thyroid removal (postoperative) were considered the predicting variables. The end-point variable was the postsurgical outcome: hypocalcemia was defined as total calcium (TCa)<8 mg/dL and/or ionized calcium (iCa)<0.8 nmol/L. Surgeons and endocrinologists ignored PTH levels. Signs or symptoms of hypocalcemia TCa and/or iCa were monitored regularly for 48 h after surgery. Treatment (calcium and active vitamin D3) were given to hypocalcemic patients. ROC curves were used to identify the PTH level that provided the best prediction of postsurgical hypocalcemia according to their sensitivity (S), specificity (Sp) diagnostic efficiency (DEf) and positive predicted value (PPV). Relative risk (RR) was calculated by Fisher's test. **Results:** 10/20 patients developed hypocalcemia whereas 3/10 were symptomatic. Serum calcium dropped throughout the first 6 h in 40% of patients and at 24 h in 40%. None of the patients presented hypocalcemia after 36 h post surgery. Intraoperative PTH<14 pg/mL predicted hypocalcemia with S:80%, Sp:100%, DEf:90% (95%CI, 72-100) and PPV:100%, while postoperative PTH<14 pg/mL showed S:80%, Sp:90%, DEf:82% (95%CI, 63-100) and PPV:80%. An intra or postoperative serum PTH<14 pg/mL bear a RR of 9 for developing hypocalcemia after thyroidectomy. **Conclusion:** Intra and postoperative PTH is an accurate tool for predicting hypocalcemia after thyroidectomy in children. Routine use of it would avoid unnecessary controls in patients identified as low-risk of hypocalcemia and allow the introduction of timely prophylactic calcium treatment in high-risk patients. This strategy would reduce morbidity as well as length and costs of hospitalization. Nothing to Disclose: AVF, AC, OA, DB, RG, MT, MM, MGB, MGR, IB, LGdP

Pub #	OR19-2
Session Information	ORAL SESSION: CLINICAL - Pediatric Endocrinology: Bone & Obesity (11:15 AM-12:45 PM)
Title	Low Bone Turnover May Explain Low Bone Mass in Down Syndrome
Author String	TW Fowler, NS Akel, J Vander Schilden, RA Skinner, WR Hogue, FL Swain, D Gaddy, GR Wenger, D LeBlanc, KG McKelvey, LJ Suva UAMS, Little Rock, AR; UAMS, Little Rock, AR; UAMS, Little Rock, AR; UAMS, Little Rock, AR
Body	Down Syndrome (DS) is a human aneuploidy resulting from the presence of a third chromosome 21 that occurs in approximately 1 per 1,000 live births. DS is associated with a number of physical characteristics and clinical conditions, including a degree of mental retardation, thrombocytosis, thyroid dysfunction, and a 500-fold increased risk of megakaryocytic leukemia. Peak bone-mass accrual in these patients is also impaired, likely due to hypotonia, as well as nutritional and hormonal deficiencies during infancy and adolescence. As life expectancy of individuals with DS has increased to a survival rate of >50% to age 50, the incidence of age-related skeletal problems, such as osteopenia and bone fragility has also increased. DS has recently been associated with an increased rate of fracture in the expanding DS patient populations. However, the specific effects of trisomy 21 on the skeleton remain poorly defined. To study this question, we evaluated bone mass and bone turnover markers in a series of DS patients and determined the skeletal phenotype of the Ts65Dn mouse model of DS. We measured BMD in 30 euthyroid DS patients (15 males and 15 females (ages 19-52)). DS patients demonstrated low BMD (T scores <-1.5) at multiple sites including lumbar spine, distal radius, femoral neck and proximal femur, compared with age- and sex-matched subjects without DS. Low BMDs were associated with measured serum biochemical markers (P1NP and CTx) at the lower end of normal, suggesting low bone turnover. Similarly, Ts65Dn mice that are characterized by segmental trisomy for the region of mouse chromosome 16 that contains approximately 75% of the chromosome 21-homologous genes, display a profound low bone mass phenotype. We examined the tibia and femur of adult Ts65Dn mice (3 months and 24 months of age), and found significant decreases in bone volume fraction, and significant changes in trabecular bone architecture and cortical bone geometry. These skeletal changes correlated with significantly decreased osteoblast and osteoclast development in *ex vivo* bone marrow cultures, a significant reduction in bone biochemical markers (~50% decrease in serum P1NP and TRAP5b levels) and with a significant decrease in bone formation rate. Our studies demonstrate the potential of DS mouse models to improve our understanding of chromosome 21 gene dosage effects in bone and implicate decreasing bone turnover as the cause of the extraordinary bone fragility in DS patients. Sources of Research Support: DK74024 (to DG); HD047656 (to GRW); Carl L. Nelson Chair in Orthopaedic Creativity (to LJS). Nothing to Disclose: TWF, NSA, JVS, RAS, WRH, FLS, DG, GRW, DL, KGM, LJS

Pub #	OR19-3
Session Information	ORAL SESSION: CLINICAL - Pediatric Endocrinology: Bone & Obesity (11:15 AM-12:45 PM)
Title	25-Hydroxyvitamin D Levels and *In Vivo* Insulin Sensitivity in Black and White Children: Is There a Relationship?
Author String	K Rajakumar, J de las Heras, S Lee, F Bacha, MF Holick, SA Arslanian University of Pittsburgh School of Medicine, Pittsburgh, PA; Cruces Hospital, Barakaldo, Spain; University of Pittsburgh School of Medicine, Pittsburgh, PA; University of Pittsburgh School of Medicine, Pittsburgh, PA; Boston University School of Medicine, Boston, MA
Body	Adult studies report a positive relationship between 25-hydroxyvitamin D [25(OH)D] concentrations and insulin sensitivity, and a negative one with fasting hyperglycemia and type 2 diabetes. However, data are sparse in pediatrics. Obesity is a risk factor for vitamin D deficiency and insulin resistance, and adiposity may confound and modify the relationship between 25(OH)D and insulin sensitivity. Therefore, this investigation aimed to (1) examine the relationship between plasma 25(OH)D concentrations and *in vivo* insulin sensitivity (IS) in black and white children, and (2) assess if this relationship is modified by total and abdominal adiposity. Plasma 25(OH)D concentrations were analyzed in 331 children (177 white, 144 male) aged 8 to <20 yrs, who had undergone a hyperinsulinemic-euglycemic clamp to assess IS, with measurements of total body adiposity (DXA), and abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). In blacks and whites, children in the lowest plasma 25(OH)D quartile had significantly lower IS than those in the highest quartile, however after adjusting for adiposity differences the difference in IS disappeared. Plasma 25(OH)D was positively associated with insulin sensitivity (all subjects: $r=0.252$, $p<0.001$; blacks: $r=0.245$, $p=0.002$; whites: $r=0.269$, $p<0.001$). However, in a multiple regression analysis, the relationship between 25 (OH)D and IS (partial $r=0.166$, $p=0.003$) disappeared when adjusted for BMI (partial $r=0.043$, $p=0.434$), fat mass (partial $r=0.062$, $p=0.272$), VAT (partial $r=0.052$, $p=0.361$) or SAT (partial $r=0.013$, $p=0.814$). In summary, our data in a large number of youth, using state-of-the- art methods, show no independent relationship between vitamin D levels and *in vivo* insulin sensitivity, but rather one mediated through adiposity which is inversely related to insulin sensitivity and vitamin D levels. Therefore, direct measures of total body and abdominal adiposity should be carefully evaluated when examining the relationship between vitamin D status and insulin sensitivity.

Sources of Research Support: This work was supported by United States Public Health Service grant RO1 HD27503 (SA), K24 HD01357 (SA), Richard L. Day Endowed Chair (SA), K23 HD052550 (KR), Rio Hortega contract from the Instituto de Salud Carlos III of the Spanish Ministry of Health (CM07/00211) (JH), 1UL1RR025771 CTSI and UL1 RR024153 CTSA (previously MO1 RR00084 GCRC).

Nothing to Disclose: KR, JdlH, SL, FB, MFH, SAA

Pub #	OR19-4
Session Information	ORAL SESSION: CLINICAL - Pediatric Endocrinology: Bone & Obesity (11:15 AM-12:45 PM)
Title	Fruit and Vegetable Juice Concentrate (FVJC) Improves Glucose Disposition Index (GDI) and Serum Retinol (SR) in Obese Children. A 6-Month Pilot, Randomized, Double-Blind Placebo-Controlled Study
Author String	JA Canas, L Damaso, A Altomare, K Killen, B Balagopal Nemours Children's Clinic, Jacksonville, FL; Nemours Children's Clinic, Jacksonville, FL
Body	Background and Objectives: Obesity is associated with alterations in pancreatic β-cell function and insulin resistance (IR), independent risl factors for development of type 2 diabetes mellitus (T2DM) in children. Obese children also have reduced SR presumably from reduced intake of fruits and vegetables. Its effect on pancreatic β-cell function and insulin sensitivity has not been studied in children. SR circulates bound to retinol binding protein 4 (RBP4) and thyroxin-binding transthyretin (TTR) in a 1:1:1 molar ratio. Vitamin A deficiency (VAD) is suspect when SR levels are < 0.7 [mu]mol/L and RBP4:TTR molar ratio is < 0.36. VAD in laboratory animals impairs glucose-induced acute insulin response (AIR) and glucose tolerance and are reversible with supplementation. FVJC consumption in lean and obese adults results in significant increase in serum carotenoids and tocopherols, but without a concomitant increase in SR levels. The effects of FVJC supplementation on insulin sensitivity and SR in children have not been documented. Method and Design: A total of 39 age-matched males (age: 6-10 and Tanner stageResults: VAD was present in 10% and SR < 0.7 [mu]mol/L in 31% of the cohort at baseline. Significant differences between the NW, OW and O-groups existed at baseline for BMI-Z score, AIR, QUICKI, HOMA-IR, GIR, FMI (p< 0.05) but not SR, RBP4, TTR, GDI or vitamin A consumption. SR was negatively correlated with AIR (r= -0.358, p=0.03) when adjusted for age, BMI and serum cholesterol. There was a modest increase in SR and a significant improvement in GDI (p= 0.037) in the supplement group *vs*. placebo. Conclusion: A 6-month FVJC vs. placebo supplementation along with MNT without significant weight loss appears to improve GDI but not insulin sensitivity in children. The modest increases in SR may be responsible for this effect. The data suggest that FVJC has a beneficial effect on β-cell function. Future studies should confirm these findings. Sources of Research Support: Nemours Research Foundation. Disclosures: JAC: Principal Investigator, NSA International. Nothing to Disclose: LD, AA, KK, BB

Pub #	OR19-5
Session Information	ORAL SESSION: CLINICAL - Pediatric Endocrinology: Bone & Obesity (11:15 AM-12:45 PM)
Title	Predictors and Outcomes of Weight Loss in Morbidly Obese Adolescents Undergoing Laparoscopic Gastric Banding (LAGB)
Author String	SE Lerner, R Conroy, M Censani, SE Oberfield, J Zitsman, I Fennoy Columbia University Medical Center, New York, NY; Columbia University Medical Center, New York, NY
Body	Background: Obesity in childhood and adolescence has been shown to track into adult life, associated with childhood and early adult onset of cardiovascular disease and diabetes. Dietary interventions typically result i limited weight loss compared to surgical interventions, typically not surpassing 10% of total body weight. Objective: To determine preoperative anthropometric and metabolic parameters predicting significant weight loss following LAGB. To report metabolic outcomes associated with significant weight loss. Methods: The Center for Adolescent Bariatric Surgery is a multidisciplinary program of nutrition, medical care, and laparoscopic adjustable gastric banding (LAGB). Data were evaluated from a patient's initial visit, and at the post-operative visit with greatest weight loss, at least 12 months following LAGB. Thirty-eight of the 63 adolescents achieved 20 % excess body weight (EBW) loss, averaging 18.7 months post-op, compared to the other 25 patients, evaluated at an average of 16.8 months post-op. T-tests comparing these groups were used, with cut-offs of $p < 0.01$ and <0.05 noted. Results: 63 patients completed at least one year of medical follow-up following LAGB. 38 patients (9 M/ 29 F) averaged 50.5% EBW (29.7 kg) loss vs. 25 (12 M/13 F) who averaged 2.9% EBW (3.2 kg) loss. Predictors of weight loss were drawn from data collected at patients' initial visits. Older age, taller height, lower BMI% and BMI-Z, waist circumference (WC)/height, 30-minute glucose (Glu-30) and 120-minute insulin (Ins-120) on oral glucose tolerance test, 1,25 dihydroxy Vitamin D, and absent history of PCOS, characterized the grou who achieved >20% EBW loss ($0.01 < p < 0.05$). Post-operative differences demonstrated that the 20% EBW loss cohort had ($p < 0.01$) decreased weight, BMI, BMI%, and BMI-Z, diastolic BP (DBP) %, WC, WC/height, Glu-30, Glu-60, Ins-60, Ins-120, and greater interval change in weight. At $p< 0.05$, this cohort ha lower systolic BP (SBP) and SBP%, DBP, Ins-0, HDL, alkaline phosphatase, uric acid, SHBG, less acanthosi nigricans, but fewer Metformin Rx, and higher total cholesterol. Conclusions: These data suggest that significant weight loss is achievable in a large percentage of adolescents following LAGB, but that initial younger age, higher BMI-Z and higher glucose and insulin levels in response to a glucose load predict less weight loss. Of those who achieve at least 20% EBW loss, significant metabolic improvement is likely. Sources of Research Support: Empire Clinical Research Investigator Program. Nothing to Disclose: SEL, RC, MC, SEO, JZ, IF

Pub #	OR19-6
Session Information	ORAL SESSION: CLINICAL - Pediatric Endocrinology: Bone & Obesity (11:15 AM-12:45 PM)
Title	Predictors of Adipose Tissue Insulin Resistance in Obese Adolescents with and without Type 2 Diabetes
Author String	MM Kelsey, HEF Jeri, REB Jane, NJ Kristen University of Colorado Denver, Aurora, CO; University of Colorado Denver, Aurora, CO

Body

Background: We previously reported that adolescents who are obese and have type diabetes (T2D) have progressively worsening whole-body insulin resistance (IR) compared with lean controls. It is unclear whethe this IR is primarily originating in muscle, fat or liver. Non-esterified fatty acids (NEFA) are associated with IR. Furthermore, decreased insulin suppression of NEFAs is associated with adipose tissue IR.

Objectives: To compare fasting NEFA levels and insulin-induced NEFA suppression in obese adolescents with and without T2D, and with lean controls. To determine predictors of NEFA suppression in these youth.

Methods: 48 lean (n=10), obese (n=12), and T2D (n=26) adolescents had insulin sensitivity measured by a $80mU/m^2/min^{-1}$ hyperinsulinemic-euglycemic clamp, reported as glucose disposal rate (GDR). NEFAs were measured pre- and post-clamp and percent NEFA suppression was calculated. Regression analysis was used t assess between group differences and predictors of fasting NEFAs and NEFA suppression.

Results: Lean subjects had lower BMI z-scores (BMIz) and fat mass; however, obese and T2DM subjects hac similar BMIz by design. Mean GDR ($mg/kg/min^{-1}$) was significantly lower in obese than controls and in T2I subjects than in obese or lean controls. There were no significant differences in fasting NEFA between the groups. Although not statistically significant, there is a trend toward greater mean percent NEFA suppression in lean (88$\pm$13%) than in obese (73$\pm$32%) or T2D (65$\pm$31). There was significant variability in ability to suppress NEFA with high-dose insulin in obese and T2D subjects, with a subset displaying complete failure o NEFA suppression. NEFA suppression was significantly inversely associated with triglycerides (TG) ($p=0.0007$), alanine aminotransaminase (ALT) ($p=0.004$), and systolic blood pressure (SBP) ($p=0.007$), after controlling for confounding variables. There was a trend toward association between NEFA suppression and GDR ($p=0.07$).

Conclusions: There is a subset of obese adolescents with and without T2D who fail to suppress lipolysis ever with high-dose insulin, indicating that these youth have marked adipose tissue IR, of concern due to affects of adipose IR on inflammation and development of cardiovascular disease. In this study of adolescents, adipose tissue IR was associated with higher TG, ALT, and SBP. Future studies will assess NEFA suppression at lower doses of insulin, to determine more subtle adipose IR in obese and T2D youth.

Nothing to Disclose: MMK, HEFJ, REBJ, NJK

Pub #	Y2
Session Information	ENDOCRINE YEAR IN BASIC/CLINICAL SCIENCE SESSION: CLINICAL - The Year in Obesity (11:15 AM - 12:00 PM)
Title	The Year in Obesity
Author String	MW Schwartz University of Washington, Seattle, WA
Body	Obesity continues to be a leading public health concern in the US and other developed countries, taking an ever-increasing toll on adults and children alike. Public awareness of the problem has benefitted from First Lady Michelle Obama's identification of childhood obesity as a cause worthy of both concern and action. Despite unhelpful and seemingly ludicrous efforts to politicize it, this campaign offers a meaningful and welcome boost to efforts aimed at curbing further increases of childhood obesity prevalence. Meanwhile, the Food and Drug Administration appears, in the wake of criticism over approved drugs rosiglitazone and sibutramine, to have established a new policy mandating that drugs must be proven free of cardiovascular toxicity in large clinical trials before approval for obesity treatment can be considered. While no one wishes to see toxic drugs enter the marketplace, this new policy carries a price tag in the tens of millions that is piled atop already substantial drug development costs. Combined with a continued, global failure to translate basic science advances into effective new therapies, drug manufacturers that have not already done so now seem poised to abandon their obesity programs. Consequently, hopes are dim that effective new obesity drugs will hit the market anytime soon. News is more positive on the basic science front, where evidence that tissue inflammation mediates obesity-induced insulin resistance continues to mount. Interestingly, hypothalamic areas involved in energy homeostasis also become inflamed in rodent models of obesity and, unlike inflammation in peripheral tissues, this inflammation has the potential to promote weight gain, rather than simply being a consequence of obesity. Research in this area ultimately seeks to clarify mechanisms underlying the biological defense of elevated body weight -- a vexing phenomenon that explains why weight lost through dieting is usually regained in full and, as such, is the leading obstacle to effective obesity treatment. As its underlying mechanisms come to light, we will learn whether hypothalamic inflammation plays a causal role or is instead a biomarker for as yet undiscovered processes contributing to obesity pathogenesis. Answers to these questions may ultimately identify new approaches to obesity treatment and prevention. Nothing to Disclose: MWS

Pub #	W2-1
Session Information	WORKSHOP: CLINICAL - Testosterone Assay Workshop: Coalition for Quality Testing: Improving Accuracy of Testosterone Assays (11:15 AM - 12:00 PM)
Title	Leading the Charge for Excellence in Hormone Testing
Author String	W Rosner St Luke's/Roosevelt Hospital Center, New York, NY
Body	Nothing to Disclose: WR

Pub #	W2-2
Session Information	WORKSHOP: CLINICAL - Testosterone Assay Workshop: Coalition for Quality Testing: Improving Accuracy of Testosterone Assays (11:15 AM - 12:00 PM)
Title	The CDC's Testosterone Standardization Project & the Coalition's Progress towards Performance Criteria
Author String	HW Vesper Centers for Disease Control and Prevention, Atlanta, GA
Body	Nothing to Disclose: HWV

Pub #	W2-3
Session Information	WORKSHOP: CLINICAL - Testosterone Assay Workshop: Coalition for Quality Testing: Improving Accuracy of Testosterone Assays (11:15 AM - 12:00 PM)
Title	Advocating for Higher Standards in Testosterone Testing
Author String	RM Carey University of Virginia Health System, Charlottesville, VA
Body	Nothing to Disclose: RMC

Pub #	CDW3-1
Session Information	CAREER DEVELOPMENT WORKSHOP: Career Development Workshop: Careers in Industry (11:30 AM - 12:45 PM)
Title	Panelist
Author String	LJ Suva Merck & Co/University of Arkansas, Little Rock, AR
Body	Disclosure Incomplete: LJS

Pub #	CDW3-2
Session Information	CAREER DEVELOPMENT WORKSHOP: Career Development Workshop: Careers in Industry (11:30 AM - 12:45 PM)
Title	Panelist
Author String	JF Poyer Novo Nordisk Inc., Grandview, TX
Body	Disclosure Incomplete: JFP

Pub #	CDW3-3
Session Information	CAREER DEVELOPMENT WORKSHOP: Career Development Workshop: Careers in Industry (11:30 AM - 12:45 PM)
Title	Panelist
Author String	AA Chines Pfizer, Inc, Collegeville, PA
Body	Disclosure Incomplete: AAC

Pub #	CDW3-4
Session Information	CAREER DEVELOPMENT WORKSHOP: Career Development Workshop: Careers in Industry (11:30 AM - 12:45 PM)
Title	Panelist
Author String	JA Magner Genzyme Corp, Cambridge, MA
Body	Disclosure Incomplete: JAM

Pub #	CDW3-5
Session Information	CAREER DEVELOPMENT WORKSHOP: Career Development Workshop: Careers in Industry (11:30 AM - 12:45 PM)
Title	Panelist
Author String	A Deshmukh Abbott, Boston, MA
Body	Disclosure Incomplete: AD

Pub #	MC1-1
Session Information	MASTER CLINICIANS: CLINICAL - Difficult Thyroid Cancer Cases (12:15 PM - 1:45 PM)
Title	Difficult Thyroid Cancer Cases
Author String	R Tuttle Memorial Sloan Kettering Cancer Center, New York, NY
Body	Disclosures: RMT: Research Funding, Genzyme Corporation; Consultant, Abbott Laboratories, Veracyte, Inc., Novo Nordisk.

Pub #	MC1-2
Session Information	MASTER CLINICIANS: CLINICAL - Difficult Thyroid Cancer Cases (12:15 PM - 1:45 PM)
Title	Difficult Thyroid Cancer Cases
Author String	BR Haugen University of Colorado, Denver School of Medicine, Aurora, CO
Body	Disclosures: BRH: Speaker, Genzyme Corporation; Study Investigator, Veracyte, Inc.

Pub #	NS5-1
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Breakout Sessions (1:00 PM - 2:00 PM)
Title	Endocrine Stim Testing 101
Author String	S Carpenter Mayo Clinic, Rochester, MN
Body	Disclosure Incomplete: SC

Pub #	NS5-2
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Breakout Sessions (1:00 PM - 2:00 PM)
Title	Endocrine Stim Testing 101
Author String	RM Hower Lehigh Valley Hospital, Allentown, PA
Body	Endocrine Tests are dynamic tests used to detect hormone imbalances in children and adults. Tests are designed to check the pituitary gland and the hormones it makes. Endocrine tests may also check growth hormone, adrenal gland function, kidney stones, bone health, blood sugar and reproductive issues. Endocrine testing nurses schedule, coordinate and conduct tests to determine excess or deficiencies in any one or more of these hormones. Endocrine tests can be divided into categories examining the function of certain organs such as the adrenal glands and thyroid, or certain diseases such as primary hyperaldosteronism, pheochromocytomas, diabetes insipidus, growth hormone deficiency or excess, hypogonadism, premature puberty, hypo- and hyperglycemia, intestinal malabsorption, kidney stone production and calcium metabolism. Tests are backed by protocols and are subject to review. Patients are scheduled for tests and receive instructions regarding necessary preparations needed for the test. Collaboration in research studies is also an important part of the role as well as mentoring medical students. Nothing to Disclose: RMH

Pub #	NS5-3
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Breakout Sessions (1:00 PM - 2:00 PM)
Title	Practical Application of Yoga in Endocrine Nursing Practice
Author String	B Lucasey University of Kansas Medical Center, Kansas City, KS
Body	Medicine is now becoming more and more aware of the importance of the balance between mind and body, realizing that many physical ailments have their origins in poor mental functioning as well as poor physical conditioning. Alternative therapies are fast finding a niche in the overall health management field. The reason for this are simplicity and lack of side effects. But how do you safely advise a specific type of exercise program? Take that first step and becoming familiar with what yoga can do for you and the people that look to you for advice. This session will provide an overview of The Hatha branch of yoga with examples of balance, strength and flexibility poses. Yoga is simple (no equipment), efficient (10 - 60 minutes sessions) and affordable (no gym membership required). Yoga can be practiced within a group setting or simply alone in the comfort and privacy of your home. Yoga is a method to come back to yourself - expand your boundaries and relax. How simple is that? Nothing to Disclose: BL

Pub #	NS5-4
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Breakout Sessions (1:00 PM - 2:00 PM)
Title	IV Bisphosphonates: The Role of the Endocrine Nurse
Author String	L Coppolo Basset Medical Center, Cooperstown, NY
Body	Disclosure Incomplete: LC

Pub #	NS5-5
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Breakout Sessions (1:00 PM - 2:00 PM)
Title	The Endocrine Nurse's Role in Post-Operative Pituitary Surgery Care
Author String	MH Gurel Massachusetts General Hospital, Boston, MA
Body	Disclosure Incomplete: MHG

Pub #	NS5-6
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Breakout Sessions (1:00 PM - 2:00 PM)
Title	A Review of Emerging Diabetes Therapies or It's Time To Stop Flogging the Beta Cells
Author String	S Hendricks The Methodist Hospital, Houston, TX
Body	Recent government reports predict that the number of adults with diabetes could double or triple by the year 2050. The vast majority of this increase is expected to be in type 2 diabetes. In an effort to meet this challenge scientists are exploring new targets for diabetes therapeutics. Older therapies that primarily target the failing beta cells have been limited by ineffectiveness, weight gain, poor durability and hypoglycemia. New therapies have emerged that leverage gut hormones, or incretins, for control of post- prandial glucoses without the burden of weight gain and hypoglycemia. In fact some are associated with weight loss. These agents are available in oral and injectable options. Another hormone , amylin, normally co-secreted with insulin in healthy beta cells is deficient in diabetes. When administered before meals in insulin-deficient individuals amylin has been useful in post-prandial glucose control through delaying of gastric emptying with early satiety and inhibition of glucagon secretion An old therapy, bromocriptine, long used for treatment of hyperprolactinemia has recently been approved for treatment of type 2 diabetes. This represents a new class of antidiabetic therapy that may lower blood glucose without increasing insulin or cause significant weight gain. The exact mechanism of action is not fully understood, it is known to mimic the action of dopamin, a neurochemical messenger, at specific dopamine receptors in the brain. We have at least three new classes of agents on the horizon. The first and closest to market is the SGLT-2 inhibitors which work in the kidney to lower the glucose threshold causing glycosuria. This loss of glucose in the urine lowers serum glucose and causes weight reduction as a result of loss of calories in the urine. Another exciting therapy in the pipeline is the glucagon receptor antagonists. This class of therapies offer the potential to decrease hepatic glucose production and lower blood glucose in patients with type 2 diabetes. A third class of therapy in the offing is the glucokinase activators (GKAs). In the liver, glucokinase mediates glucose utilization and glycogen synthesis. In the pancreas glucokinase is involved in glucose-stimulated insulin release and may have an effect in antagonizing apoptosis in B cells. As with some of our other new weapons in our glucose fighting armamentarium GKAs may have dual mechanisms of action in the pancreas and liver. Nothing to Disclose: SH

Pub #	NS5-7
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Breakout Sessions (1:00 PM - 2:00 PM)
Title	Developing a Clinical Research Project in Endocrine Nursing Practice
Author String	ME Eckert-Norton State University of New York Downstate, Brooklyn, NY
Body	Endocrine nursing practice presents many opportunities for clinical research. However, it is often the case tha nurses working in endocrinology may need support in developing research questions and identifying appropriate methods to address these questions. In this workshop, participants will initiate a reflective process to identify potential research topics in their practice, get tips for searching for relevant literature and suggestions for methods that could be applied to the topics identified. A bibliography of tools to support the development of a research proposal will be included. Coauthor: Patricia S. Via, NP, MS; Division of Endocrinology, McGuire Veterans Affairs Medical Center, Richmond, VA. Disclosure Incomplete: MEE-N

Pub #	NS5-8
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Breakout Sessions (1:00 PM - 2:00 PM)
Title	Developing a Clinical Research Project in Endocrine Nursing Practice
Author String	PS Via McGuire Veterans Affairs Medical Center, Richmond, VA
Body	Nothing to Disclose: PSV

Pub #	SBS1-1
Session Information	SPECIAL BASIC SCIENCE SESSION: BASIC - Special Basic Science Session: Nuclear Receptors: Past, Present & Future (1:00 PM - 1:45 PM)
Title	Discovery of Nuclear Receptors, Functions & Structures of Their Domains & Their Superfamily Nature
Author String	J-A Gustafsson University of Houston, Houston, TX
Body	The nuclear receptor (NR) superfamily has enormous importance in physiology and disease and NRs are common pharmaceutical targets. Studies on NR function has had a massive impact on medicine. These talks will highlight both early and recent of the many contributions to the NR field over the past 50 years. One initial breakthrough was discovery of specific retention of radiolabeled estrogen in target tissues around 1960, and demonstration of their proteinaceous and macromolecular character with high affinity and low capacity, implying the molecules were receptors. It was shown in the late 1960s and early 1970s that receptors localized in the nucleus and chromatin. Shortly thereafter, nuclear receptors for other ligands were found and the concept grew that these were highly related. Receptors were found to be abnormal in hormone resistance syndromes that has been relevant to comprehending pathogenesis of disease and better understanding drug therapy. The first NR purified to homogeneity was the glucocorticoid receptor (GR), that was shown to be a single protein composed of three domains, a ligand binding domain (LBD), a DNA-binding domain (DBD) and a third domain later shown to be the N-terminal domain. Purified GR was shown to bind specifically to DNA sites on a glucocorticoid sensitive gene. These sites proved to be glucocorticoid response elements, able to confer glucocorticoid regulation onto originally hormone insensitive genes. These findings helped open a new, molecular phase in nuclear receptor research, and use of the antibodies facilitated the first cloning of NR gene sequences and the obtaining of receptor domain 3-dimensional structures. Ultimately full length receptors were cloned that led to demonstration that the NRs comprised a superfamily of receptors, resulting in discovery of many additional receptors and unexpected ligands, expanding the field especially in the area o metabolic control. Use of cloned gene sequences allowed for obtaining much larger quantities of receptors an to determinations of the X-ray crystal structures of the ligand bound LBDs. These structures provided important insights into how the ligand can perturb the receptor to induce post receptor effects of agonists and antagonists and for pharmaceutical design. Disclosures: J-AG: Consultant, KaroBio AB, BioNovo Inc.

Pub #	SBS1-2
Session Information	SPECIAL BASIC SCIENCE SESSION: BASIC - Special Basic Science Session: Nuclear Receptors: Past, Present & Future (1:00 PM - 1:45 PM)
Title	Actions of Nuclear Receptors: Transcription Control & Coregulation
Author String	JD Baxter Methodist Hospital Research Institute, Houston, TX
Body	Even though nuclear binding of ligands for NRs was discovered, the field was unsure what was the primary mechanism of NR action. Thus, in the early 1970s, theories involved membrane stabilization, promotion of precursor uptake, post-transcriptional control and transcriptional control. Several researchers showed hormone induced uptake of precursors, or even changes in chromatin appearance, but none of these experiments were conclusive and could have been interpreted in various ways. These issues were resolved initially by experiments with the chick oviduct system where it was shown that estrogen and progesterone induce ovalbumin mRNA to an extent that the effect had to be on transcription. This discovery pointed the field to the nucleus, and interest in and publications in the field increased exponentially. Many studies mentioned in the above Gustafsson abstract solidified the concept of NRs as transcriptional regulators. However, it was later discovered that NRs also have important actions through second messenger signaling systems. There was also great progress in deciphering how various ligands man[pulate the receptors to have varied agonist and antagonist functions to The issue was how did receptors transmit information downstream? In the 1990s, the concept of coregulators was put forward using more indirect data, including use of X-ray structures and binding experiments. Then both corepressor and coactivator gene sequences were cloned and the expressed proteins were shown to have these properties. Many elements of the actions of these coregulatory molecules were then deciphered. It is now clear that there are over 300 different coactivators and corepressors and that their actions are influenced by chromatin remodeling events and that they interact with other coregulators to influence transcription. Coregulators can have many additional functions such as stimulation of RNA splicing They are known to be abnormal in disease states and are now considered as targets for pharmaceutical attack. Nothing to Disclose: JDB

Pub #	CMF3-1
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Risk Assessment for Osteoporotic Fracture (1:00 PM - 1:45 PM)
Title	Risk Assessment for Osteoporotic Fracture
Author String	TJ Weber Duke University Medical Center, Durham, NC
Body	Disclosures: TJW: Speaker, Eli Lilly & Company, Amgen, Genentech, Inc., Novartis Pharmaceuticals.

Pub #	CMF3-2
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Risk Assessment for Osteoporotic Fracture (1:00 PM - 1:45 PM)
Title	Risk Assessment for Osteoporotic Fracture
Author String	R Eastell University of Sheffield, Sheffield, UK
Body	Nothing to Disclose: RE

Pub #	CMF4-1
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Thyroid Nodules & Cancer in Children (1:00 PM - 1:45 PM
Title	Thyroid Nodules & Cancer in Children
Author String	GL Francis Medical College of Virginia, Children's Hospital of Richmond, Richmond, VA
Body	Nothing to Disclose: GLF

Pub #	CMF4-2
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Thyroid Nodules & Cancer in Children (1:00 PM - 1:45 PM
Title	Thyroid Nodules & Cancer in Children
Author String	SG Waguespack University of Texas MD Anderson Cancer Center, Houston, TX
Body	Nothing to Disclose: SGW

Pub #	M14
Session Information	MEET-THE-PROFESSOR: CLINICAL - Amenorrhea (1:00 PM - 1:45 PM)
Title	Amenorrhea
Author String	CK Welt Massachusetts General Hospital, Boston, MA
Body	Nothing to Disclose: CKW

Pub #	M16
Session Information	MEET-THE-PROFESSOR: CLINICAL - Management of Hypogonadism through Puberty (1:00 PM - 1:45 PM)
Title	Management of Hypogonadism through Puberty
Author String	N Pitteloud University of Lausanne, Lausanne, Switzerland
Body	Nothing to Disclose: NP

Pub #	M18
Session Information	MEET-THE-PROFESSOR: CLINICAL - Pheochromocytoma & Paraganglioma Syndromes: What's New? (1:00 PM - 1:45 PM)
Title	Pheochromocytoma & Paraganglioma Syndromes: What's New?
Author String	C Jimenez University of Texas MD Anderson Cancer Center, Houston, TX
Body	Nothing to Disclose: CJ

Pub #	M19
Session Information	MEET-THE-PROFESSOR: CLINICAL - Premature Adrenarche (1:00 PM - 1:45 PM)
Title	Premature Adrenarche
Author String	SE Oberfield Columbia University Medical Center, New York, NY
Body	Nothing to Disclose: SEO

Pub #	M20
Session Information	MEET-THE-PROFESSOR: CLINICAL - The Value of Medical Therapy before Pituitary Surgery (1:00 PM - 1:45 PM)
Title	The Value of Medical Therapy before Pituitary Surgery
Author String	L Katznelson Stanford University, Stanford, CA
Body	Session supported by: Pfizer, Inc. & Ipsen, US (1) Carlsen SM et al., JCEM 2008;93:2984 Disclosures: LK: Researcher, Novartis Pharmaceuticals; Speaker, Ipsen.

Pub #	M21
Session Information	MEET-THE-PROFESSOR: CLINICAL - What To Do for Sweating & Flushing (1:00 PM - 1:45 PM)
Title	What To Do for Sweating & Flushing
Author String	P-MG Bouloux Royal Free Hospital, London, UK
Body	Nothing to Disclose: P-MGB

Pub #	M15
Session Information	MEET-THE-PROFESSOR: CLINICAL - Interpretation of Challenging Thyroid Function Tests (1:00 PM - 1:45 PM)
Title	Interpretation of Challenging Thyroid Function Tests
Author String	VJ Bernet Mayo Clinic Jacksonville, Ponte Vedra Beach, FL
Body	Nothing to Disclose: VJB

Pub #	M17
Session Information	MEET-THE-PROFESSOR: CLINICAL - Managing Cardiovascular Risk in Type 2 Diabetes (1:00 PM - 1:45 PM)
Title	Managing Cardiovascular Risk in Type 2 Diabetes
Author String	S Dagogo-Jack University of Tennessee, Memphis, TN
Body	Session supported by: Kowa Pharmaceuticals America, Inc. and Lilly USA, LLC and Merck & Co., Inc. Disclosures: SD-J: Speaker, Eli Lilly & Company, GlaxoSmithKline; Consultant, Merck & Co., Roche Pharmaceuticals; Principal Investigator, Astra Zeneca, Bristol-Myers Squibb.

Pub #	P2-1
Session Information	POSTER SESSION: BASIC - Regulation of Nuclear Receptors & Gene Expression (1:30 PM-3:30 PM)
Title	Novel Insights into the Molecular Regulation of PTEN by Rapid Estrogen Signaling in the Human Endometrium
Author String	MM Scully, S Wachtel, LK Cabrera, AP Bradford, TA Jackson University of Colorado Denver, Aurora, CO
Body	Each year, approximately 142,000 women worldwide will develop endometrial cancer and an estimated 42,000 women will die from this cancer. E2s, acting through the E2 receptor (ER), provide the primary proliferative signal in the endometrium, and hyper-estrogenicity is thought to be the primary cause of type 1, hormone-dependent, endometrial cancer. While ERs have historically been thought of as ligand-dependent transcription factors that exert their effects through gene regulation, recent observations suggest that E2s and other steroid hormones can elicit rapid activation of cellular signal transduction pathways. The impact of rapid, non-nuclear actions of E2-activated ER on endometrial cancer cell proliferation is not well studied. The PTEN tumor suppressor regulates cell growth by antagonizing the PI3 kinase/Akt pathway. In normal endometrium, PTEN levels are upregulated during the E2-stimulated, proliferative phase of the menstrual cycle and down regulated in the growth-inhibited secretory phase. Furthermore, PTEN activity and stability have been shown to be regulated by C-terminal phosphorylation. PTEN activity is decreased by C-terminal phosphorylation and stability is increased. We hypothesized that non-nuclear E2/ER actions play a key role in PTEN regulation. To test this hypothesis, endometrial cancer cells expressing ER and PTEN were treated with E2 over a short time course. In response to E2 stimulation, rapid PTEN C-terminal phosphorylation, decreased PTEN lipid phosphatase activity, and increased protein were observed. Use of inhibitors identified casein kinase II as an E2-responsive mediator of PTEN phosphorylation, and similar effects were observed in breast and cervical cancer cell lines. We show for the first time that E2 regulates PTEN phosphorylation, activity and stability through rapid signaling actions. Due to the high levels of circulating E2 observed in women at high risk for endometrial cancer, PTEN may be aberrantly maintained in a low activity state, allowing for excess proliferation. Therefore, unopposed E2 stimulation and its consequent inactivation of PTEN may contribute to endometrial hyperplasia, the precursor to endometrial cancer. E2 regulation of PTEN also extends to other hormone responsive cells, suggesting that the non-nuclear effects of E2/ER on PTEN may play a role in a variety of tissues. Our results suggest that misregulation of E2/ER non-genomic signaling could provide a widespread mechanism for tumorigenesis. Sources of Research Support: NIH NCI RO1 CA 125427; University of Colorado, Denver Department of Obstetrics and Gynecology Academic Enrichment Fund. Nothing to Disclose: MMS, SW, LKC, APB, TAJ

Pub #	P2-2
Session Information	POSTER SESSION: BASIC - Regulation of Nuclear Receptors & Gene Expression (1:30 PM-3:30 PM)
Title	DHHC-7 and DHHC-21 Are Palmitoyl Acyltransferases for Estrogen and Progesterone Receptors
Author String	A Pedram, M Razandi, RJ Deschenes, ER Levin University of California, Irvine, Irvine, CA; Department of Veterans Affairs Medical Center, Long Beach, CA; University of South Florida, Tampa, FL
Body	Plasma membrane-localized pools of estrogen, progesterone, and androgen receptors (ER, PR, AR) play important functional roles. Receptor protein monomers traffic to the plasma membrane (PM) upon palmitoylation of a conserved cysteine in the respective E domains, promoting a physical interaction with the transporting protein, caveolin-1. However, the identity of the palmitoylacyltransferase(s) (PAT) that causes palmitoylation is unknown. To investigate this, we expressed in MCF-7 cells a cDNA library encoding all 23 mammalian PATs. Only clones expressing DHHC-7 and DHHC-21 PATs showed significantly enhanced palmitoylation of endogenous ERα. Expressed siRNAs against DHHC-7 or DHHC 21 (but not DHHC-22 as control) each substantially reduced endogenous ERαpalmitoylation, PM localization, and 17-β-estradiol (E2) rapidly activating ERK and PI3/AKT kinases and cAMP generation. E2-induced viability (MTT assay) of MCF-7 cells was also inhibited by DHHC-7 or 21 knockdown. In CHO cells, only expressed DHHC-7 and 21 PATs physically interacted with wild type (WT) ERα but not a palmitoylation site, cysteine 447 to alanine point mutant ERα. Also, C447A ERα was not palmitoylated by the DHHC proteins in contrast to robust palmitoylation of expressed WT ERα. To further support the structure of ERα required for palmitoylation, we expressed DHHC-7 or 21 in CHO cells with plasmids coding for either a green fluorescence protein (GFP) or a GFP/9 amino acid ERα palmitoylation motif fusion protein: Only the fusion protein showed acylation and membrane localization. Expression of DHHC-7 and 21 in MCF-7 cells significantly increased the number of ERα at the PM by 60%, indicating PAT production is probably a limiting factor for endogenous membrane ER trafficking. Similar to ER, WT PR but not an E domain, cysteine point mutant PR was palmitoylated only from expression of DHHC-7 and 21 proteins. From siRNA knockdown, DHHC7/21 PATs significantly contributed to the PM localization of and the rapid signaling by endogenous PR in breast cancer cells. Similar studies are commencing for AR in prostate cancer cells. In summary only the two DHHC proteins stimulate palmitoylation of ER and PR and receptor trafficking to and functioning at the PM. These PATs are targets to selectively disrupt rapid steroid receptor signaling, allowing a clearer understanding of what biological events require these actions. Sources of Research Support: Supported by the VA and NCI. Nothing to Disclose: AP, MR, RJD, ERL

Pub #	P2-3
Session Information	POSTER SESSION: BASIC - Regulation of Nuclear Receptors & Gene Expression (1:30 PM-3:30 PM)
Title	The Involvement of ERαlGαi Interactions in Estrogen- and Xenoestrogen-Induced Signaling in Pituitary Tumor Cells
Author String	CS Watson, G Hu, Y-J Jeng, A Wozniak, N Bulayeva, J Guptarak University of Texas Medical Branch, Galveston, TX
Body	The plasma membrane version of estrogen receptor-α (mERα) is involved in nongenomic estrogenic signaling leading to kinase activation cascades and the generation of a variety of second messengers in many cell types. Included in these signaling paradigms are ones involving G proteins. We used a variety of approaches to confirm this type of signaling pattern in a GH3 rat pituitary tumor cell line (GH3/B6/F10) selected for its robust expression of mERα. A combination of microarray and immunoblot data demonstrated the expression of many G protein subtypes in this cell type (Gαq, Gα11, Gαs, Gαi-o and 1-3, Gα12, Gß1-2, and Gγ). Several types of inhibitor studies confirmed that specific G protein subtypes (Gαi), ERs, and caveolar structures were all involved in the pathways leading to downstream extracellular-regulated kinase (ERK) activation. Ca^{++} signaling showed different G protein involvement parameters. We focused on two subcategories for further analysis, Gαi and Gαs. Immunoblots confirmed that an antibody (Ab) selective for the GTP-bound form of Gαi generated a signal when cells were treated with estradiol (E_2). Adaptation of our quantitative plate immunoassay for use with this Ab confirmed that Gαi was activated at 15 seconds by low concentrations of several estrogens (E_2, bisphenol A, nonylphenol). After 5 min of treatment with a variety of estrogens this response was suppressed (likely a compensatory reduction of GTP-activated Gαi). In contrast, Gαs was not activated by any estrogens tested in this assay. GTP-activation of Gαi was inhibited by pertussis toxin (consistent with the known selectivity of this inhibitor) and enhanced by irreversible cumulative binding to GTPγS. Co-immuno-capture experiments were done under a variety of conditions, some providing evidence that ERα and Gαi can partner in these cells. Therefore, we contribute new evidence and strategies demonstrating that ERα can directly and selectively interact with a specific Gα subtype in initiating estrogenic signaling via both physiologic and nonphysiologic estrogens. Sources of Research Support: NIHR01 ES015292. Nothing to Disclose: CSW, GH, Y-JJ, AW, NB, JG

Pub #	P2-4
Session Information	POSTER SESSION: BASIC - Regulation of Nuclear Receptors & Gene Expression (1:30 PM-3:30 PM)
Title	Identification of the Role of Linker Histone H1 in Estrogen Receptor Binding and Activity
Author String	L Cato, M Brown Dana-Farber Cancer Institute, Boston, MA
Body	The activity of the estrogen receptor (ER) in the nucleus is a highly dynamic process, which is tightly regulated by many factors. Among the molecules proposed to facilitate ER activity are proteasome components, chromatin-remodeling complexes, heat shock proteins and histones. Particularly the linker histone H1 seems important, as it is essential to the stability of the higher-order chromatin structure and has been associated with the regulation of gene expression for many years. Eleven linker histones have been identified in humans to date, differing in their timing of expression, extent of post-translational modification and affinity to DNA in chromatin. However, their exact function and ability to differentially affect gene expression are not as clearly understood. It seems probable that linker histones need to be removed, reduced in amount or somehow modified during transcription in order to allow access to the transcription machinery and that these processes are different for the different linker histone subtypes. Here we show that phosphorylation of linker histone H1 is a prerequisite for the full activation of ER-regulated target genes in ER positive breast cancer cells. This process is mediated through the activity of the cyclin-dependent kinase 2 (CDK2), which modifies H1 in a hormone-dependent manner, and thereby loosens the interaction of the linker histone with DNA in chromatin. The extent of phosphorylation differs significantly between the different subtypes suggesting a differential involvement of the H1s in ER-mediated gene expression. Collectively, our results show that H1 modification is required for ER-mediated gene expression in breast cancer and that the H1 subtypes may play distinct functional roles on the transcriptional regulation of ER target genes. Nothing to Disclose: LC, MB

Pub #	P2-5
Session Information	POSTER SESSION: BASIC - Regulation of Nuclear Receptors & Gene Expression (1:30 PM-3:30 PM)
Title	Effect of Estrogen on Long-Paced Oscillation of ER Activity: A Key for the Identification of More Efficacious Replacement Therapies?
Author String	S Della Torre, A Biserni, G Rando, G Monteleone, P Ciana, B Komm, A Maggi University of Milan, Milan, Italy; University of Milan, Milan, Italy; TOP srl, Lodi, Italy; Wyeth Research, Collegeville, PA
Body	By the use of *in vivo* imaging, we investigated the dynamics of estrogen receptor (ER) activity in intact, ovariectomized, and hormone replaced ERE-Luc reporter mice. The study revealed the existence of a long paced, non circadian, oscillation of ER transcriptional activity. Among the ER expressing organs, this oscillation was asynchronous and its amplitude and period were tissue-dependent. Ovariectomy affected the amplitude, but did not suppress ER oscillations suggesting the presence of tissue endogenous oscillators. Long term administration of Raloxifene, Basedoxifene, combined estrogens alone or with Basedoxifene to ovariectomized ERE-Luc mice showed that each treatment induced a distinct *spatio*-temporal profile of ER activity, demonstrating that the phasing of ER activity among tissues may be regulated by the chemical nature and the concentration of circulating estrogen. This points to the possibility of a hierarchical organization of the tissue-specific pace-makers. Conceivably, the rhythm of ER transcriptional activity translates locally into the activation of specific gene networks enabling ER to significantly change its physiological activity according to circulating estrogens. In reproductive and non-reproductive organs this hierarchical regulation may provide ER with the signaling plasticity necessary to drive the complex metabolic changes occurring at each female reproductive status. We propose that the tissue specific oscillatory activity here described is an important component of ER signaling necessary for the full hormone action including the beneficial effects reported for non reproductive organs. Thus this mechanism needs to be taken in due consideration to develop novel, more efficacious and safer hormone replacement therapies. Sources of Research Support: EU (STREP EWA LSHM-CT-2005-518245); NIH (RO1AG027713); Pharmaceutical Company Pfizer. Nothing to Disclose: SDT, AB, GR, GM, PC, BK, AM

Pub #	P2-6
Session Information	POSTER SESSION: BASIC - Regulation of Nuclear Receptors & Gene Expression (1:30 PM-3:30 PM)
Title	SIRT1 Regulates the Function of Thyroid Hormone Receptor β1 through Direct Deacetylation
Author String	JH Suh, A Cvoro, D Sieglaff, J Baxter, P Webb Methodist Hospital Research Institute, Houston, TX
Body	The SIRT1, NAD^+-dependent deacetylase, is an important modulator in glucose and lipid metabolism. Severa reports have shown that the loss of SIRT1 reduces the expression of gluconeogenic genes in hepatocyte. Because thyroid hormone receptor β1 (TRβ1) also directly upregulates the expression of these genes, we hypothesized that SIRT1 may play a role in TR β1 function. Here, we demonstrate that TRβ1 transactivation is modulated by SIRT1 in human hepatocytes. Over expression of SIRT1 enhances the transcriptional activity of TR β1 in its target gene promoters and SIRT1 knockdown reduces the TRβ1 transcriptional activity and expression of TR β1 target genes, such as phosphoenol pyruvate carboxylase kinase (PEPCK) and Glucose-6-Phosphatase (G6Pase), which are related in Gluconeogenesis. *In vivo* and *in vitro* protein interaction assays revealed that SIRT1 directly interacts with and deacetylates TR β1. Moreover, SIRT1 decreases the stability of TR β1 protein, and the decreased TRβ1 protein level is restored by knockdown of SIRT1 mRNA, overexpression of dominant negative SIRT1 mutant or treatment with SIRT1 inhibitor. Together, these data indicate that SIRT1 is a novel co-regulator of TR, and the regulation of TR transactivation by SIRT1 may pla an important role in hepatocytes. Nothing to Disclose: JHS, AC, DS, JB, PW

Pub #	P2-7
Session Information	POSTER SESSION: BASIC - Regulation of Nuclear Receptors & Gene Expression (1:30 PM-3:30 PM)
Title	Cellular Stress Determines the Transcriptional Response to Glucocorticoids through Hormone-Independent Phosphorylation of Human Glucocorticoid Receptor at Serine 134
Author String	AJ Galliher-Beckley, JG Williams, JA Cidlowski National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC; National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC
Body	P38 MAPKs are kinases directly activated in response to environmental stressors and a variety of inflammatory cytokines. Here we report a novel target for p38 MAPK, Serine 134 of the human glucocorticoid receptor (GR). Unlike most other phosphorylation events that occur on the GR, Ser134 phosphorylation was found to be hormone independent in several different human, mouse, and rat cell lines and in mouse cardiac tissue. Ser134 phosphorylation of the hGR was induced by a wide variety of stress-activated stimuli including serum starvation, ultraviolet irradiation, osmotic shock, and oxidative stress. Pharmacological inhibitors and shRNA-mediated knockdown experiments correlate this phosphorylation with the activation of p38 MAPK. Cells expressing a mutant of the receptor incapable of Ser134 phosphorylation (S134A GR) had a significantly altered hormone-dependent genome-wide transcriptional response to glucocorticoids when compared to wild-type GR. These data suggest that phosphorylation status of Ser134 is critical for modulating GR function. Interestingly, Ser134 phosphorylation did not alter either nuclear translocation of the GR or the stability of the receptor protein in the absence or presence of ligand. Ser134 phosphorylation did however significantly increase the association of the GR with the 14-3-3 class of signaling proteins, suggesting that ability of the GR to associate with 14-3-3 proteins will ultimately impact the transcriptional response to glucocorticoids. Together these data suggest that Ser134 phosphorylation acts as a molecular sensor on the glucocorticoid receptor, monitoring the level of cellular stress and modulating glucocorticoid signaling to result in differential gene expression profiles. Thus, our results also provide a mechanism by which p38 MAPK activity can dictate how cells will ultimately respond to glucocorticoids. Nothing to Disclose: AJG-B, JGW, JAC

Pub #	P2-8
Session Information	POSTER SESSION: BASIC - Regulation of Nuclear Receptors & Gene Expression (1:30 PM-3:30 PM)
Title	Multiple Noncoding Exons 1 of Nuclear Receptors NR4A Family (NGFIB,NURR1,NOR1) and NR5A1 (SF1 in Human Vascular and Adrenal Tissues
Author String	M Demura, F Wang, T Yoneda, S Karashima, S Mori, M Oe, M Kometani, T Sawamura, Y Cheng, Y Maeda, M Namiki, K Ono, Y Nakamura, H Sasano, Y Demura, K Saijoh, Y Takeda Kanazawa University, Kanazawa, Japan; Kanazawa University, Kanazawa, Japan; Tohoku University, Sendai Japan; Kanazawa University, Kanazawa, Japan; Ishikawa Prefectural Central Hospital, Kanazawa, Japan
Body	**Introduction:** Aldosterone is involved in atherosclerosis and cardiovascular disease as well as hypertension. Locally synthesized aldosterone in blood vessels may be associated with the development of atherosclerosis. Nuclear receptor families NR4A (NGFIB, NURR1, NOR1) and NR2F (COUP-TFI, COUP-TFII, NR2F6) activate, whereas NR5A1 (SF1) represses CYP11B2 transcription. **Objective:** To elucidate the mechanism of differential regulation of nuclear receptors between artery and adrenal cortex. **Methods:** 5'-rapid amplification of cDNA ends identified transcription start sites. Multiplex RT-PCR determined use of alternative noncoding exons 1 (ANEs). **Results:** In adrenocortical H295R cells, angiotensin II, KCl or cAMP all stimulated CYP11B2 transcription and NR4A was up-regulated, whereas NR2F and NR5A1 were down-regulated. H295R cells used 4 ANEs of NGFIB (NR4A1), 3 of NURR1 (NR4A2), 2 of NOR1 (NR4A3), and 2 of SF1 (NR5A1). RT-PCR confirmed various mRNA transcripts with an ANE followed by common protein-coding regions in both artery and adrenal cortex. Quantitative multiplex RT-PCR showed NGFIB employed multiple ANEs in a tissue-specific manner: 35.3% and 9.9% of NGFIB transcripts arose from ANE2 in artery and adrenal cortex, respectively. The NR4A mRNA levels in artery were significantly high compared to adrenal cortex, whereas the NR5A1 mRNA level in adrenal cortex was 47 times that seen in artery. **Conclusions:** NR4A and NR5A1 (SF1) employed various ANEs in human artery and adrenal cortex. NR4A1 showed a significant difference in use of ANEs between those tissues. This may lead to novel therapeutic options for atherosclerosis targeting local aldosterone. Sources of Research Support: KAKENHI (#21790889). Nothing to Disclose: MD, FW, TY, SK, SM, MO, MK, TS, YC, YM, MN, KO, YN, HS, YD , KS, YT

Pub #	P2-9
Session Information	POSTER SESSION: BASIC - Regulation of Nuclear Receptors & Gene Expression (1:30 PM-3:30 PM)
Title	Mechanism of Gene Regulation by Cooperative Actions of Progesterone and Prolactin Analyzed in a 3D Primary Mammary Epithelial Cell Culture System
Author String	AE Obr, SL Grimm, JP Lydon, KA Bishop, JW Pike, DP Edwards Baylor College of Medicine, Houston, TX; University of Wisconsin, Madison, WI
Body	Both progesterone (P4) and prolactin (PRL) are required for proliferation of the mammary epithelium and for alveologenesis. Classical endocrine ablation and gene knock out studies show that neither hormone alone is sufficient. Gene microarray experiments with mammary glands of acute P4 treatment of ovariectomized mice and PRLR knockout (KO) mice, have identified a common set of proliferation gene targets regulated by P4 and PRL. Whether these genes are simply redundant targets or whether regulation requires cooperative interactions between P4 and PRL signaling is not known. To study the mechanisms of hormonal regulation of these genes we have developed 3D culture systems of primary mouse mammary epithelial cells (MECs) embedded in Matrigel that form polarized acini composed of luminal epithelial cells and myoepithelial cells and maintain expression of endogenous PR. Similar to the mammary gland in vivo, PR expression is heterogeneous in luminal epithelial cells and is expressed in non-proliferating cells as determined by Ki67 serial section staining. PR is also functional, mediating a proliferative response to P4, and P4 induction of several of the common in vivo identified target genes of P4 and PRL including Wnt4, RANKL, and amphiregulin. Since RANKL is an important paracrine regulator of P4 proliferation in the mammary gland in vivo, we have focused on this gene to analyze potential cooperation between P4 and PRL signaling. P4 and PRL alone each induced RANKL expression and the two hormones together gave an additive induction under certain conditions. To define whether RANKL is a direct target of PR or Stat5 (downstream target of PRL signaling) transient co-transfection experiments in mouse NMuMG cells were done with PR, PRLR, Stat5 and six different RANKL enhancer regions as reporter genes linked to luciferase. Three enhancer regions were activated by R5020 or PRL. By ChIP assay, progesterone-dependent binding of PR and PRL-mediated binding of Stat5 to these same RANKL enhancers was detected as well as hormone-dependent acetylation of histone H4 indicating that PR and Stat5 binding sites are associated with transcriptionally active enhancers. Experiments are ongoing to define the actions of both hormones together and to scale up the 3D MEC system to perform ChIP-Seq analysis to define the genomic PR and Stat5 binding sites on the endogenous RANKL gene in response to single and multiple hormones. Nothing to Disclose: AEO, SLG, JPL, KAB, JWP, DPE

Pub #	P2-10
Session Information	POSTER SESSION: BASIC - Regulation of Nuclear Receptors & Gene Expression (1:30 PM-3:30 PM)
Title	COUP-TFII-Nucleolin Interaction Regulates RARβ2 Expression in Human Breast Cancer Cells
Author String	LM Litchfield, CG Emberts, CM Klinge University of Louisville, Louisville, KY
Body	COUP-TFII is an orphan nuclear receptor that has reduced expression in tamoxifen-resistant breast cancer cells. The role of COUP-TFII in tamoxifen-resistance is not well understood, but may involve its interaction with another protein, nucleolin. The goal of the current study was to examine COUP-TFII-nucleolin interaction in two human breast cancer cell lines: MCF-7 and T47D, and to determine how this interaction regulates expression of a tumor suppressor, retinoic acid receptor β2 (RARβ2). Interaction of COUP-TFII with nucleolin was confirmed in the nuclear extracts of both cell lines by coimmunoprecipitation. Transient transfection of MCF-7 cells with COUP-TFII and nucleolin leads to increased expression of RARβ2. This increase in RARβ2 was blocked by treatment with AS1411, an anticancer aptamer known to target nucleolin. Treatment with all-*trans* retinoic acid (atRA) increases RARβ2 expression by activating COUP-TFII in MCF-7 and T47D cells, which can also be blocked by AS1411 or with siRNA knockdown of nucleolin. To examine whether COUP-TFII and nucleolin act at the promoter of the RARβ2 gene to activate transcription, a luciferase reporter construct containing the promoter of the RARβ2 gene was used in transient transfection experiments. Cotransfection with COUP-TFII leads to an increase in RARβ2-luc activity, which can be blocked by treatment with AS1411. These data indicate a coregulatory role for nucleolin in COUP-TFII-regulated RARβ2 transcription. Sources of Research Support: Susan G. Komen for the Cure grant KG080365 to CMK. LML is supported by a fellowship from NIH T32 ES011564. Nothing to Disclose: LML, CGE, CMK

Pub #	P2-11
Session Information	POSTER SESSION: BASIC - Regulation of Nuclear Receptors & Gene Expression (1:30 PM-3:30 PM)
Title	Glucocorticoid-Dependent Repression of the Glucocorticoid Receptor Gene
Author String	S Ramamoorthy, JA Cidlowski National Institute of Environmental Health Sciences, Research Triangle Park, NC
Body	Glucocorticoids regulate diverse physiological functions ranging from development to metabolism by binding and activating the glucocorticoid receptor (GR). Glucocorticoids also induce homologous down-regulation of GR mRNA and protein (desensitization), a process that may contribute to glucocorticoid resistance. Previous studies in transfected cell systems have shown that one of the mechanisms by which glucocorticoids down regulate GR is by altering GR transcription. This repression of GR mRNA involves activated GR binding to undefined intragenic elements on the coding region of the GR cDNA. In the present study we have investigated the molecular mechanism responsible for the down regulation of endogenous GR mRNA. Time course studies following dexamethasone treatment showed a rapid decrease in steady state level of GR mRNA in several human, mouse and rat cell lines as well as in various mouse tissues. A 50-70% maximal decrease in GR mRNA was observed and the down-regulation was sustained for 8-12 hours after dexamethasone treatment. Furthermore we show that functional GR is required for this rapid repression of GR mRNA. Chromatin immunoprecipitation (ChIP) analysis revealed that GR binds in a ligand dependent manner to sites within exon 5-8 of the GR gene. Ligand induces prolonged receptor occupation at these GR coding regions, which might lead to transcriptional pausing. Thus we show that GR coding region contains regulatory elements sufficient for repression of its own mRNA. Since the biological effects of glucocorticoids are dependent on the presence of functional receptor, this repression of the GR mRNA may be associated with tissue resistance that develop with chronic high dose glucocorticoid treatment. Nothing to Disclose: SR, JAC

Pub #	P2-12
Session Information	POSTER SESSION: BASIC - Regulation of Nuclear Receptors & Gene Expression (1:30 PM-3:30 PM)
Title	Synergistic Regulation of Inflammatory Genes by Glucocorticoids and TNFα: A Potential Role in Long-Term Glucocorticoid Side Effects
Author String	EA Lannan, JA Cidlowski National Institute of Environmental Health Sciences, Research Triangle Park, NC
Body	Synthetic glucocorticoids are widely used for treatment of many inflammatory diseases such as asthma, arthritis, and chronic obstructive pulmonary disorder (COPD), however, glucocorticoid treatment can cause a variety of side effects including weight gain, muscle breakdown, insulin resistance, osteoporosis, and glaucoma. Unexpectedly we have discovered that some genes can be co-regulated by the synthetic glucocorticoid Dexamethasone (Dex) and the pro-inflammatory cytokine tumor necrosis factor alpha (TNF). Based on this finding, we hypothesize that co-regulation of genes by glucocorticoids and inflammatory cytokines might be responsible for some of the negative side effects of glucocorticoid treatment. Genome-wide microarray analysis was employed to identify genes regulated by both Dex and TNF in human lung A549 cells, which contain endogenous glucocorticoid and TNF receptors. We found 7 genes synergistically upregulated by treatment with both Dex and TNF. We evaluated the mechanism of co-regulation of one of these genes, serpinA3 (alpha-1 antichymotrypsin), because it encodes an inflammatory protein associated with COPD. Up-regulation of serpinA3 requires the presence of both the glucocorticoid receptor (GR) and TNF soluble receptor 1 (TNFSR1). Treatment of A549 cells with the naturally occurring glucocorticoid, cortisol, also resulted in a synergistic increase in serpinA3 mRNA levels, indicating that this increase was not an effect specific to Dex. Finally, in vivo treatment of C57/BL6 mice with Dex and TNF resulted in an additive increase in serpinA3 mRNA levels in both lung and liver tissues, suggesting that this gene regulation may have physiological relevance to treatments of inflammatory diseases. Investigation of the serpinA3 promoter region reveals multiple NF-kB binding sites and glucocorticoid responsive elements. These studies demonstrate for the first time that glucocorticoids and pro-inflammatory compounds can co-regulate genes involved in human disease, and this phenomenon may underlie the adverse effects of glucocorticoid therapy. Nothing to Disclose: EAL, JAC

Pub #	P2-13
Session Information	POSTER SESSION: BASIC - Regulation of Nuclear Receptors & Gene Expression (1:30 PM-3:30 PM)
Title	Targeted Inhibition of Telomerase Expression and Human Cancer Cell Growth by miRNA-498 in Response t 1,25-Dihydroxyvitamin D_3
Author String	K Ravi, Z Shen, U Jinwal, SV Nicosia, X Zhang, W Bai USF College of Medicine, Tampa, FL
Body	Telomerase is an essential enzyme that counteracts the telomere attrition accompanying DNA replication during cell division. Regulation of the promoter activity of the gene encoding its catalytic subunit, the telomerase reverse transcriptase, is known to be the dominant mechanism conferring the high telomerase activity in proliferating cells, such as embryonic stem and cancer cells. The present study reveals a new mechanism of telomerase regulation by non-coding small RNA by showing that miR-498 induced by 1,25-dihydroxyvitamin D3 decreases the mRNA expression of the human telomerase reverse transcriptase, i.e. the hTERT. MiR-498 was first identified in a microarray analysis as the miRNA mostly induced by 1,25-dihydroxyvitamin D3 in ovarian cancer cells and subsequently validated by quantitative PCR analyses in multiple cancer cell types. A functional vitamin D response element (VDRE) was established in the 5' regulatory region of miR-498 genome. Further studies showed that miR-498 targeted the 3'-untranslated region (3' UTR) of hTERT mRNA and decreased its expression. More importantly, the levels of miR-498 expression were decreased in malignant human ovarian tumors as well as human ovarian cancer cell lines and the ability of 1,25-dihydroxyvitamin D3 to decrease hTERT mRNA and to suppress ovarian cancer cell growth was compromised when miR-498 was depleted using anti-miR-498 oligonucleotides or [ldquo]sponge [rdquo]. Taken together, our studies define a novel mechanism of telomerase regulation by small non-coding RNAs and identify miR-498 as an important mediator for the anti-tumor activity of 1,25-dihydroxyvitamin D3. Sources of Research Support: R01 grant from NIH/NCI to W.B. (CA111334). Nothing to Disclose: KR, ZS, UJ, SVN, XZ, WB

Pub #	P2-14
Session Information	POSTER SESSION: BASIC - Regulation of Nuclear Receptors & Gene Expression (1:30 PM-3:30 PM)
Title	Nuclear Receptor ERRγ Is a Transcriptional Regulator of LIPIN1 in Primary Hepatocytes
Author String	D-K Kim, D Chanda, H-S Choi Chonnam National University, Gwangju, Republic of Korea
Body	It has been reported that LIPIN acts as a phosphatidic acid phosphatase (PAP) enzyme which catalyzes the conversion of phosphatidate to diacylglycerol (DAG), a key substrate for triacylglycerols (TAGs) and phospholipid biosynthesis. Here, we report that nuclear receptor ERRγ is a novel transcriptional regulator of LIPIN1 and it is controlled by an inverse agonist GSK5182. Overexpression of ERRγ significantly increased LIPIN1 promoter activity and mRNA levels in both hepatoma cells and primary hepatocytes, whereas knockdown of ERRγ reduced expression of LIPIN1 mRNA. Consistent with the changes in transcription levels, LIPIN1 protein levels also induced by ERRγ in primary hepatocytes. Transient transfection and chromatin immunoprecipitation (ChIP) assay demonstrated that ERRγ exerts its effect on the transcriptional regulation of LIPIN1 through ERRE1 of LIPIN1 promoter. We also found that transcriptional regulation of ERRγ on LIPIN1 promoter and mRNA levels is controlled by the competition between PGC-1α and SHP, which was further confirmed by ChIP assay. Finally, GSK5182, an inverse agonist of ERRγ, significantly inhibited ERRγ mediated induction of LIPIN1 promoter activity and mRNA levels. Taken together, our findings suggest that control of ERRγ transcriptional activity by its specific inverse agonist could provide a novel approach for regulation of LIPIN1-mediated lipid metabolism. Nothing to Disclose: D-KK, DC, H-SC

Pub #	P2-15
Session Information	POSTER SESSION: BASIC - Regulation of Nuclear Receptors & Gene Expression (1:30 PM-3:30 PM)
Title	Regulation of the p53 Pathway by Human Estrogen-Related Receptor β in Prostate Cancer Cells
Author String	Y Lu, SK Drenkhahn, GA Jackson, NJE Starkey, DB Lubahn University of Missouri, Columbia, Columbia, MO; University of Missouri, Columbia, Columbia, MO; University of Missouri, Columbia, Columbia, MO; University of Missouri, Columbia, Columbia, MO
Body	Several nuclear receptors, including Estrogen Receptor and Androgen Receptor, have been shown to regulate the tumor suppressor p53. Here we investigate the ability of human Short-Form Estrogen Related Receptor beta (SFhERR β) to interact with the p53 signaling pathway. We **hypothesize** that DY131's growth inhibitory effect on prostate cancer (PCa) cells is due to the interaction between ERRβ and the p53 signaling pathway. To test this we transfected SFhERR β into the human PCa cell line DU145 and the mouse PCa cell line TRAMP-C2 and treated each with the ERRβ agonist, DY131. To determine p53 pathway activity, we monitored the protein concentration of p53, p53 phosphorylation at Ser 393, p53 downstream target p21/WAF1, and mdm2 activator Akt via western blot analysis. We also monitored the growth inhibitory effec of DY131-treated PCa cell lines. **Results**: We found that DY131 is able to increase p53 protein concentration and p53 phosphorylation at Ser393 in several PCa cell lines with an EC50 <1uM for both, and this effect could be regulated by transfection with SFhERRβ. We also found that the p53 target gene, cyclin-dependent kinase inhibitor p21/WAF1, is upregulated by DY131 treatment. In contrast, Akt, which activates mdm2, does not change upon treatment with DY131. In addition, DY131 showed a growth inhibitory effect on both human and mouse PCa cell lines (TRAMPC-2, IC50~5uM; DU145, IC50 ~20uM). These data indicate that DY131 can stimulate p53 signaling pathway activity, at least partially by increasing p53 protein concentration through SFhERRβ. **Conclusion:** Our results suggest a new level of regulation of the p53 pathway via SFhERRβ, which is potentially mdm2 independent. Additionally, our results indicate that modulation of ERRβ may serve as a new target for prostate cancer treatment. Sources of Research Support: NIH R01-AT002978 and NIH 1P50At006273. Nothing to Disclose: YL, SKD, GAJ, NJES, DBL

Pub #	P2-16
Session Information	POSTER SESSION: BASIC - Regulation of Nuclear Receptors & Gene Expression (1:30 PM-3:30 PM)
Title	TR2 Mediates VPA-Enhanced *Oct4* Promoter Activity
Author String	HF Teng, YL Kuo, HS Liu, KH Lin, SL Chen National Central University, Jhongli, Taiwan; Armed Forces Taoyuan General Hospital, Taoyuan, Taiwan; Chang Gung University, Taoyuan, Taiwan
Body	Induced pluripotent stem cells (iPS) are derived from somatic cell through ectopic expression of stem cell-specific transcription factors, including *Oct4, Nanog, Sox2, Lin28,* and *c-Myc.* However, how do these factors work to achieve this reprogramming process is largely unknown. In previous study, we had shown that VPA could enhance the activity of promoter driven reporter, pStable-*Oct4p-1.9k-luc,* in somatic cells. Stable transfection assays of a series of deletion mutants of the *Oct4* promoter shown that VPA probably act through the proximal promoter region (-638~+52). Since a hormone response element (HRE; -46~-27) in the proximal region had been shown to play crucial roles in the regulation of *Oct4* promoter activity, it implied that VPA might enhance *Oct4* promoter activity by recruiting some nuclear receptors to this HRE. Mutation of the HRE site blocked VPA mediated activation, thus proving its importance in this mechanism. To date, a few nuclear hormone receptors targeting this HRE has been identified. Among them, we thought the orphan receptor TR2 may be the most possible candidate participating in this pathway. Because (1) TR2 enhanced VPA instigated Oct4 promoter activity and this increase could be mitigated by retinoic acid, and (2) TR2 is highly expressed in our target cells. It will be interesting to know whether VPA directly binds to TR2 to stimulate its transactivational activity. In the future, a hormone-binding defective mutant of TR2 will be employed to confirm its role in this pathway. Nothing to Disclose: HFT, YLK, HSL, KHL, SLC

Pub #	P2-17
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Cyclin D1b Promotes Metastatic Phenotypes in Models of Prostate Cancer through Manipulation of AR Transcriptional Output
Author String	M Augello, C Burde, D Frigo, D McDonnell, K Knudsen Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA; Thomas Jefferson University, Philadelphia, PA; Cincinnati University, Cincinnati, OH; Duke University, Durham, NC
Body	While D-type cyclins were first characterized based on functions in cell cycle control, it is now clear that a major activity of this cyclin class is to control transcription factor activity. While the transcriptional regulatory functions of D-cyclins had been demonstrated in a number of tumor model systems, in vivo confirmation of this concept emerged through the work of Sicinski and colleagues, who showed that transcription factors comprise the major subset of proteins associated with cyclin D1, and that key phenotypes of the cyclin D1 knockout mouse are a result of altered cyclin D1-mediated transcriptional regulation. Discerning the impact o the cell cycle vs. transcriptional regulatory functions of D-cyclins in cancer is currently a major focus of the cyclin field. Intriguingly, while cyclin D1 has weak oncogenic activity, a variant that arises through alternative splicing (cyclin D1b) has markedly enhanced oncogenic potential, as judged by in vitro and in vivo assays. Consonantly, it was shown that cyclin D1b production is markedly induced in nearly 1/3 of prostatic adenocarcinomas (PCa), while only a small subset exclusively express canonical cyclin D1. Functional studie revealed that cyclin D1b exerts little effects on cell cycle progression, indicating that alternative functions of this variant must underlie the observed oncogenic activities. Here, models were developed to mimic the shift toward cyclin D1b production, and unbiased analysis performed to assess cellular consequence. First, significant effects were observed with regard to androgen receptor (AR) signaling, which is critical for PCa progression; as expected, cyclin D1 suppressed AR activity, whereas cyclin D1b proved deficient in this function. Rather, cyclin D1b promoted AR-dependent expression of genes associated with cellular migration and invasion. Second, cyclin D1b conferred enhanced AR-dependent growth in soft agar and bolstered invasive potential. Third, molecular analyses revealed the mechanisms underpinning cyclin D1b function, wherein cyclin D1b altered AR activity at a previously unexplored target gene associated with migration, invasion, and cancer progression. Together, the study to be presented defines a mechanism to explain cyclin D1b-mediated effects in human disease, provides the first evidence of a direct AR driven gene that is induced exclusively during PCa progression, and identifies cyclin D1b as a major factor associated with metastatic phenotypes. Nothing to Disclose: MA, CB, DF, DM, KK

Pub #	P2-18
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Cyclin D1 Expression Level Is Associated with ER beta (ERβ) Expression in Human Prostate Cancer
Author String	Y Nakamura, F Fujishima, Y Kurotaki, K Ono, S Ishidoya, Y Arai, H Sasano Tohoku University Graduate School of Medicine, Sendai, Japan; Tohoku University Graduate School of Medicine, Sendai, Japan
Body	Estrogen receptor beta (ERβ) is known to be expressed in prostate cancer cells and ERβ acts as a tumor suppressor in human prostate cancer (1). ERβ has also been reported to be involved in the cell cycle of prostate cancer cells by controlling the expression levels of cell cycle regulators including cyclin D1, which plays an important role in proliferation and development of prostate cancer (2). Therefore, it is important to examine the association between ERβ and cyclin D1 expression in order to improve the clinical response to hormonal therapy in patients diagnosed with prostate cancer. In this study, we evaluated their immunoreactivities in human prostate cancer (n=37) and lymph nodes with metastatic carcinoma (n=7) obtained from surgery, and correlated the findings with clinicopathological features of the patients. Cyclin D1 immunoreactivity was detected in 21 cases of prostate cancer (57%), and was significantly correlated with tha of ERβ (P<0.05). In addition, cyclin D1 immunoreactivity was detected in all the cases of lymph nodes with metastatic carcinoma (n=7), and ERβ immunoreactivity in these cases was also marked. The quantitative RT-PCR (qPCR) demonstrated that estradiol (E2) (10nM) increased the expression level of cyclinD1 mRNA in PC-3 prostate cancer cells incubated for 48h. These data all suggest that cyclin D1 expression levels are possibly regulated by estrogen via ERβ, and their association may influence the prostate cancer development. 1. Fujimura T et al., Cancer Sci 2010;101:646 2. Hurtado A et al., Cell Oncol 2008;30:349 Nothing to Disclose: YN, FF, YK, KO, SI, YA, HS

Pub #	P2-19
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	CDK1 Phosphorylates AR on S308 in a Cell-Cycle-Dependent Manner
Author String	Y Koryakina, A Piasecka, CES Comstock, KE Knudsen, DG Gioeli University of Virginia, Charlottesville, VA; Thomas Jeffereson University, Philadelphia, PA; Cancer Center, University of Virginia, Charlottesville, VA; Kimmel Cancer Center, Philadelphia, PA
Body	Androgen Receptor (AR) phosphorylation may play a critical role in disease, regulating gene expression, growth, and survival of cancer cells. In light of the essential role of the AR in prostate development and its role as an oncogene in prostate cancer we sought to determine the effects of the cell cycle on AR phosphorylation and function. Using in vitro kinase assays we determined that CDK1 phosphorylates the AR on S308. The in vitro phosphorylation of S308 was specific to CDK1, as CDK5 and CDK9 did not phosphorylate the AR on S308. LNCaP cells chemically arrested in different stages of the cell cycle revealed that S308 phosphorylation was increased in G2/M when compared to G1 arrested cells whereas no change was observed in S81 or S94 phosphorylation. Additionally, sorting live asynchronous LNCaP cells into different cell cycle compartments using flow cytometry followed by IP/Western blotting showed an increase in S308 phosphorylation in G2/M enriched cells. We then used FACS to further explore the effects of the cell cycle on S308 phosphorylation. Approximately 1.5-3% of asynchronous LNCaP cells are phosphorylated on S308. The G2/M fraction in asynchronous LNCaP cells comprises 7-10% of total cells with up to 15% of cells in G2/M phosphorylated on S308. S308 phosphorylation was dramatically increased in cells chemically enriched in G2/M with 54% of the total cell population phosphorylated on S308. G2/M enriched cells contained 60-64% of cells in G2M, 82-84% of which were phosphorylated on S308. The observation that S308 phosphorylation peaks when CDK1 activity is highest combined with the in vitro kinase data suggests that CDK1 phosphorylates the AR on S308 in cells. Consistent with this, short-term treatment of LNCaP cells with a CDK1 inhibitor reduced S308 phosphorylation in G2/M cells. Interestingly, the increase in S308 phosphorylation parallels changes in AR transcriptional activity. qPCR analysis of flow cytometry sorted LNCaP cells revealed that AR activity on PSA, FKBP51, SGK and other AR target genes decreases throughout the cell cycle with peak hormone induced transcriptional activity occurring in G1 and the lowest activity in G2/M. This raises the possibility that AR S308 phosphorylation negatively regulates AR transcriptional activity on this subset of genes in G2/M. Collectively our results suggest that CDK1 phosphorylates the AR on S308 in cells during G2/M and that this phosphorylation event correlates with decreased AR activity. Sources of Research Support: National Institute of Health Grant R01 CA124706 to DG. Nothing to Disclose: YK, AP, CESC, KEK, DGG

Pub #	P2-20
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	SHLP6: A Novel, Naturally Occurring, Mitochondrial Peptide Is a Potent Suppressor of Angiogenesis in Prostate Cancer
Author String	LJ Cobb, HK Nakamura, P Cohen UCLA, Los Angeles, CA
Body	Mitochondrial DNA (mtDNA) mutations have been demonstrated in prostate cancer, particularly at the 16S rRNA region; and abnormal mRNA transcripts from that locus have been described. We and others showed that a novel open reading frame (ORF) within the mitochondrial 16S rRNA region, encoding for a 24 AA peptide called humanin, is a potent cell-survival and metabolic factor. Hypothesizing that additional bioactive peptides may be encoded from this region we identified an additional six ORFs that encode peptides transcribed from within the 16s rRNA gene in the mtDNA, which we have named small humanin-like peptides, or SHLPs. SHLP1-5 are functionally analogous to humanin, and act as potent survival factors. Intriguingly an additional peptide, SHLP6, functions in a completely opposing manner. We demonstrate that endogenous SHLP6 is expressed in serum and in multiple tissues, including the prostate, and that its expression declines with age. In addition, the addition of exogenous SHLP6 promotes apoptosis in prostate cancer cell lines including 22RV1, LNCaP and DU145. To assess the in vivo potency of SHLP6, we treated 22RV1 xenografts in SCID mice with SHLP6 for 1 week, and observed a potent inhibition of CaP xenograft growth and angiogenesis in vivo. In addition, we show that SHLP6 expression is decreased in preliminary staining of human prostate cancer, compared with normal prostate tissue. Analysis of gene and protein expression of SHLP6 treated cells reveals modulation of cell cycle and apoptosis genes and a dramatic inhibition of VEGF expression. Importantly, treatment of prostate cells with siRNA against SHLP6 leads to a dramatic increase in VEGF expression, suggesting that endogenous SHLP6 plays a role in the regulation of VEGF levels. We therefore propose the revolutionary concept that a previously unrecognized family of peptides is produced from the mitochondria and that one of these, SHLP6, plays a key role in regulating angiogenesis, is altered in prostate cancer, and may serve as a novel therapeutic and diagnostic agent in this disease. Nothing to Disclose: LJC, HKN, PC

Pub #	P2-21
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	New Treatments for Prostate Cancer Bone Metastases That Target Prostatic Acid Phosphatase
Author String	AC Levine, W Yang, V Janout, S Regan, S Yao, A Kirschenbaum Mount Sinai School of Medicine, New York, NY; Lehigh University, Bethlehem, PA
Body	Prostate cancer (PCa) bone metastases are the major cause of the morbidity and mortality associated with the disease. There are no effective therapies that prevent or treat PCa bone metastases. Although PCa cells eventually produce osteoblastic lesions in bone, there is an initial and ongoing osteolytic phase that is essentia for PCa bone metastases. PCa cells secrete soluble factors that enhance osteoclastic activity, thereby stimulating both bone-targeting and growth in bone. We *hypothesized* that prostatic acid phosphatase (PAP) secreted by PCa cells in bone activates osteoclasts and that inhibition of PAP with small molecule inhibitors of PAP such as tartrate and glyceric acid would decrease this effect. We further developed *conjugates* of a bone-targeting bisphosphonate, alendronate, with tartrate (VJ771T) or glyceric acid (VJ772G) to prevent PCa bone-targeting and treat established PCa bone metastases. *Methods:* Human PCa cell lines (VCaP/PAP+ and PC3/PAPneg) were grown alone or in co-culture with pre-osteoclast RAW cells in the presence of RANKL and MCSF on osteologic calcium hydroxyapatite coated discs in the presence or absence of tartrate, VJ771T or VJ772G and the effects of co-culture and the various treatments on the ability of RAW pre-osteoclast cells to resorb bone matrix assessed after 7 days. *Results:* Neither PCa cell line alone could induce resorption pits. RAW cells alone induced some resorption pits but treatment with tartrate or either conjugate had no effect on this parameter. Co-culture of RAW cells with the PAP-negative PC3 cell line increased resorption pits but this was unaffected by treatment with tartrate or either conjugate. In contrast, co-culture of RAW cells with the PAP-positive VCaP cell line significantly enhanced pit formation by RAW cells and this activity was dramatically reduced by the addition of tartrate, the tartrate-alendronate conjugate VJ771T and the glyceric acid-alendronate conjugate VJ772G. *Conclusions:* Prostatic acid phosphatase secreted by PCa in bone activates osteoclastic resorption thereby aiding PCa entry into and growth in bone. Our newly developed compounds consisting of small molecule PAP inhibitors conjugated to a bone-targeting bisphosphonate offer promising new therapy for the prevention and treatment of PCa bone metastases. Nothing to Disclose: ACL, WY, VJ, SR, SY, AK

Pub #	P2-22
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	HOXB13 Promotes Prostate Cancer Cell Invasion by the Decrease of Intracellular Zinc
Author String	Y-R Kim, I-J Kim, R-Y Park, TW Kang, C Jung Chonnam National University Medical School, Gwangju, Korea; Chonnam National University Medical School, Gwangju, Korea
Body	Healthy prostate maintains the highest level of zinc than any other tissues while its loss is obvious during the prostate tumor development and progression into androgen independent tumors. Yet, the function of zinc in prostate cancers is not known. Our previous studies showed that HOXB13 was overexpressed to provide a positive growth signal in androgen-refractory prostate cancers under hormone-deprived condition. In order to clarify the mechanism by which HOXB13 functions in this fatal disease, we performed cDNA microarray analysis to profile HOXB13 target genes in androgen-free environment. Most strikingly regulated genes were ZnTs, a group of zinc output transporters. HOXB13 upregulated expression of ZnT4 but not Zip1, a typical zinc input transporter, and suppression of HOXB13 correspondingly resulted in the intracellular accumulation of zinc. HOXB13-deregulated cells (overexpressed or suppressed) dramatically altered NF-[kappa] B-mediated signaling pathway regardless of TNF-α stimulation. NF-[kappa]B activation by HOXB13 was nearly eliminated by suppression of ZnT4. Further study showed that HOXB13 stimulated IKKα expression to inhibit I[kappa]Bα and stimulated nuclear translocation of RelA/p65. Correspondingly, in androgen-free environment, suppression of HOXB13 inhibited invasive potential of prostate cancer cell which can be negated by the suppression of ZnT4. Taken together, these results demonstrate that HOXB13 seems to be a major stimulator for NF-[kappa]B signaling in the absence of androgen and stimulates NF-[kappa]B-mediated invasive potential by a way of decrease of intracellular zinc concentration in androgen-deprived condition. Overexpressed HOXB13 in hormone refractory prostate cancers may be one of major contributors for these malignant cells biological behavior. Nothing to Disclose: Y-RK, I-JK, R-YP, TWK, CJ

Pub #	P2-23
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Neonatal Mouse Mesenchyme Increases Efficiency of Xenografting Primary Human Prostate Cancer Cells *In Vivo*
Author String	R Toivanen, D Berman, H Wang, J Pedersen, M Frydenberg, S Ellem, G Risbridger, R Taylor Monash University, Clayton, Australia; Johns Hopkins University School of Medicine, Baltimore, MD; TissuPath, Hawthorn, Australia
Body	Rare therapy-resistant cancer stem cells that can regenerate and sustain tumour growth, otherwise known as cancer repopulating cells, are novel therapeutic targets. In prostate cancer, the existence, identification and study of these cells has been hindered by the lack of *in vivo* assays, primarily because xenotransplantation of primary prostate cancer tissues into immune-deficient mice has a low success rate[1]. Thus, the goal of this study was to develop a bioassay for the study of cancer repopulating cells in prostate cancer, based on optimized methods of xenografting primary prostate cancer specimens. We show that enriching the host microenvironment with neonatal mouse mesenchyme significantly increases the survival and proliferation of primary prostate cancer tissues through paracrine signalling, and that this modified technique can be successfully applied to isolated cancer cells. To demonstrate this, we obtained 14 primary specimens with localized prostate cancer and grafted the specimens as pieces or isolated cells (including unfractionated cells, or populations of $\alpha2\beta1integrin^{hi}$ or $\alpha2\beta1integrin^{lo}$ cells) with or without mouse mesenchyme, under the renal capsule of NOD-SCID hosts for 4-14 weeks. The presence of mouse mesenchyme increased the survival of tumour tissue (6/6 patients & 66% of grafts *versus* 4/6 patients & 41% of grafts) and doubled the proliferation index of prostate cancer pieces, whilst maintaining patient pathology. This optimized method can be successfully applied to isolated cells, which show tumor regeneration (4/9 patients; 32% of grafts), active proliferation and maintenance of patient pathology when grafted with mouse mesenchyme; no tumor formation was observed when cells where grafted alone. Similar to previous reports using metastatic specimens, unfractionated, $\alpha2\beta1integrin^{hi}$ and $\alpha2\beta1integrin^{lo}$ cells demonstrated tumor formation at comparable rates, further demonstrating the reliability of this assay[2]. In summary, the inclusion of neonatal mouse mesenchyme enhances the efficacy of grafting localized prostate cancer tissues and cells. This reliable primary xenograft model will have broad applications, furthering our understanding of the biology of prostate cancer progression and creating a valuable tool for pre-clinical testing. Furthermore, the ability to now regenerate tumors from primary cells *in vivo* will allow us to elucidate the cellular hierarchy of prostate cancer and identity novel therapeutic targets. 1. Z. A. Wang and M. M. Shen, Oncogene (2010). 2. L. Patrawala, T. Calhoun-Davis, R. Schneider-Broussard et al., Cancer Res 67 (14), 6796 (2007). Nothing to Disclose: RT, DB, HW, JP, MF, SE, GR, RT

Pub #	P2-24
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	[lsquo]Death-from-Cancer[rsquo] Signature Affects AR Signaling
Author String	RS Schrecengost, RT Sussman, CE Comstock, X-Y Zhang, SB McMahon, KE Knudsen Thomas Jefferson Kimmel Cancer Center, Philadelphia, PA; Thomas Jefferson, Philadelphia, PA; Thomas Jefferson, Philadelphia, PA
Body	Recently, the deubiquitylase Ubiquitin-Specific Protease 22 (USP22) was identified as a member of the 11 gene 'death-from-cancer' signature, which can predict poor response to therapy and/or propensity toward metastasis of multiple tumor types, including breast and prostate (1). However, the mechanism for how USP22 impacts cancer is currently unknown. Our new data implicate a specific role for USP22 in hormone-dependent cancers, especially prostate cancer (PCa) where USP22 expression is elevated in primary and metastatic PCa. USP22 has an established role in regulating oncogenic c-Myc activity as it is recruited by c-Myc for transcriptional activation through deubiquitylation of histones, and is necessary for c-Myc-mediate cell transformation. Similarly, our data in PCa cells demonstrates that USP22 controls c-Myc activity but does not affect expression levels. Additional data has also identified USP22 as a critical effector of androgen receptor (AR) levels and output. This is of great clinical relevance since PCa is intricately dependent on AR signaling for disease initiation and progression to castrate resistant disease (CRPC). First, USP22 overexpression in hormone-dependent and CRPC cell lines increased AR protein expression and activity, but did not perturb transcript. Second, in the absence of ligand, USP22 upregulation resulted in enhanced AR recruitment to target genes, as determined by chromatin immunoprecipitation, which corresponded with significant enhancement of cell proliferation and BrdU incorporation. Third, USP22 depletion resulted in attenuated AR activity and caused a significant reduction in AR protein levels that could be attributed to protein stability. Importantly, these functions appear to have consequence for AR-dependent cell cycle control in prostate cancer. In sum, these data suggest that USP22 upregulation is sufficient for aberrant c-Myc activity, altered AR expression and activity, and establishment of a CRPC phenotype. We propose that modulation of UPS22 expression and/or activity may present a novel platform to achieve combinatorial suppression of AR and c-Myc function, and therefore could be potentially developed as a novel approach for treatment of PCa. Glinsky GV et al., J Clin Invest 2005;115(6):1503-21. Nothing to Disclose: RSS, RTS, CEC, X-YZ, SBM, KEK

Pub #	P2-25
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Simvastatin Inhibits Advanced Prostate Cancer in TRAMP Mice
Author String	SK Drenkhahn, GA Jackson, JL Bogener, CD Huber, JD Browning, KL Fritsche, CE Wiedmeyer, CL Besch-Williford, DB Lubahn University of Missouri, Columbia, MO; University of Missouri, Columbia, MO; University of Missouri, Columbia, MO; University of Missouri, Columbia, MO; University of Missouri, Columbia, MO
Body	Controversy in the literature suggests a potential off-label benefit of statins in the prevention of breast and prostate cancers. As millions of Americans are currently taking statins to lower cholesterol and prevent heart disease, we wanted to test if simvastatin could inhibit prostate carcinogenesis in the TRAMP (TRansgenic Adenocarcinoma of the Mouse Prostate) model. We hypothesized that simvastatin would inhibit the most aggressive form of cancer in the model, poorly differentiated carcinoma (PDC), similar to what has been seen in epidemiological studies. Mice were fed a Western Diet to mimic the high-fat diet common among men in the United States (n=25 per group). Two additional groups were fed the Western diet supplemented with either 0.025% or 0.050% w/w simvastatin. The control mice on the Western diet had an increase of PDC when compared to a low-fat AIN93 casein based diet (48% vs. 32%). While the 0.025% simvastatin Western diet reduced PDC incidence from 48% to 38%, the 0.050% simvastatin Western diet drastically reduced PDC incidence from 48% to 16% when compared to Western controls ($p=0.0153$ by Chi square analysis). Our working **hypothesis** is that statins are lowering the concentration of oxysterols, like 27-hydroxycholesterol, and are thus removing a competitor for binding to the Estrogen Receptors. By doing so, statins are allowing for the beneficial effects from estradiol binding of Estrogen Receptor β over ERα to lower PDC incidence. In conclusion, our results show that simvastatin can reduce the most aggressive stage of prostate cancer in the TRAMP model and supports the epidemiological observations that simvastatin may reduce the risk of developing advanced prostate cancer. Sources of Research Support: NIH R01-AT002978 and NIH 1P50AT006273. Nothing to Disclose: SKD, GAJ, JLB, CDH, JDB, KLF, CEW, CLB-W, DBL

Pub #	P2-26
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	GTx-758 Reduces Serum Testosterone without Increasing Fat Mass in Mature Male Cynomolgus Monkeys
Author String	A Jones, R Samadfam, JT Dalton, KA Veverka GTx, Inc, Memphis, TN; Charles River Laboratories, Montreal, Canada
Body	Gonadotropin releasing hormone (GnRH) agonists are associated with increases in fat mass resulting in a change of body composition that may contribute to metabolic syndrome and other adverse outcomes in men with prostate cancer. GTx-758 (Capesaris[trade]) is as an oral, selective estrogen receptor alpha (ERα) agonist that induces castrate levels of serum testosterone through feedback inhibition of the hypothalamic-pituitary-gonadal axis. We compared the effects of GTx-758 and leuprolide acetate, a GnRH agonist, on serum hormones and body composition in aged male non-human primates. Male cynomolgus monkeys (n = 3-7/group, [ge] 4 years of age), received daily gavage of vehicle, GTx-758 (1, 10 and 100 mg/kg) or leuprolide acetate (0.1 mg/day, subcutaneous injection) for a period of 39 weeks, with a 4-week treatment-free recovery period (n = 2/group). Serum testosterone levels were measured by enzyme immunoassay and body composition was evaluated by peripheral quantitative computed tomography (pQCT). Serum testosterone levels were equally reduced by treatment with GTx-758 (100 mg/kg) and leuprolide acetate, with castrate levels ([le] 50 ng/dL) achieved by day 28 and maintained through 39 weeks. However, leuprolide acetate, but not GTx-758, induced a surge in serum testosterone after 3 days of treatment. Serum testosterone levels returned to normal once treatment was discontinued (recovery period). GTx-758 (10 and 100 mg/kg) and leuprolide acetate significantly reduced mean body weight by 12, 22 and 11%, respectively, after 39 weeks of treatment. Both treatments decreased lean mass. GTx-758 significantly reduced fat mass, whereas a 20% increase in fat mass was observed with leuprolide acetate. GTx-758 and leuprolide acetate reduced serum testosterone to castrate levels and decreased body weight and lean body mass in aged monkeys. However, contrary to the effects observed with leuprolide acetate, GTx-758 reduced fat mass in aged monkeys and therefore represents an oral therapeutic alternative for advanced prostate cancer that may not be associated with the body composition change that is characteristic of GnRH agonists. Disclosures: AJ: Researcher, GTx, Inc. JTD: Vice President, GTx, Inc. KAV: Principal Investigator, GTx, Inc Nothing to Disclose: RS

Pub #	P2-27
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Vitamin D Action in Prostate Cancer Cells Expressing or Lacking the TMPRSS2:ERG Fusion
Author String	J-S Kim, MN Washington, NL Weigel Baylor College of Medicine, Houston, TX
Body	We and others have reported that 1,25-dihydroxyvitamin D_3 (1,25D) decreases growth of many prostate cancer (PCa) cell lines; growth of LNCaP PCa cells in vitro and LNCaP xenografts in vivo are inhibited by treatment with EB1089, a less calcemic vitamin D receptor (VDR) agonist. 1,25D reduces expression of c-myc, and in LNCaP derived C4-2 cells, depleting c-myc is sufficient to mimic 1,25D mediated growth inhibition and cell cycle arrest. However, none of the commonly studied PCa lines contain the genomic rearrangement that fuses the promoter of the androgen regulated TMPRSS2 gene to the coding region of an ETS factor, most commonly ERG (T/E). These fusions, expressed in the majority of PCa increase motility and invasiveness and promote growth in vitro and in vivo. The growth stimulatory activity has been attributed to c-myc, an ERG target. We found that 1,25D induces TMPRSS2 and the T/E in VCaP PCa cells. Despite this, 1,25D inhibited growth of VCaP cells and reduced c-myc levels in vitro. The goals of the current study are to assess the net effect of VDR activity in VCaP xenografts and to evaluate the contribution of down-regulation of c-myc to the overall actions of VDR. A pilot xenograft study showed that EB1089 did not inhibit growth of VCaP tumors. Moreover, EB1089 significantly induced T/E expression and its target gene, c-myc, in tumors. However, the mice were fed a normal chow diet, which results in much higher levels of serum 25-hydroxyvitamin D (25D) than is typical for humans, potentially minimizing effects of EB1089. A larger xenograft study using a diet that yields low normal levels of serum 25D is ongoing. To determine whether c-myc down-regulation is necessary for 1,25D mediated growth inhibition, we have generated a doxycycline inducible c-myc expressing C4-2 cell line. As expected, the inducibly expressed c-myc maintains cell growth and c-myc target gene expression (E2F1 and Cdc25a) when an siRNA targeted to the 3' UTR of c-myc is used to deplete endogenous c-myc. However, 1,25D inhibited growth of cells overexpressing c-myc suggesting additional 1,25D mediated growth inhibitory pathways. To distinguish between c-myc dependent and c-myc independent pathways, the activity of a myc responsive reporter was measured. 1,25D reduced c-myc transcriptional activity in the c-myc over-expressing cells. Thus 1,25D reduces c-myc activity by inhibiting the transcriptional activity of c-myc as well as by down-regulating c-myc RNA and protein. Nothing to Disclose: J-SK, MNW, NLW

Pub #	P2-28
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Enhanced Efficacy of New Synthetic Hsp90 Inhibitors in Human Prostate Cancer Tissues
Author String	LM Butler, MM Centenera, J Gillis, A Hanson, P Sutherland, WD Tilley University of Adelaide and Hanson Institute, Adelaide, Australia; Royal Adelaide Hospital, Adelaide, Australia
Body	The molecular chaperone heat shock protein 90 (Hsp90) is an important target for cancer therapy as it is required for the correct maturation and function of its various client proteins, many of which are known oncogenes. In prostate cancer, targeting Hsp90 is particularly attractive as the androgen receptor (AR), the key mediator of prostate cancer cell growth and survival, is also an Hsp90 client protein. Despite promising results in pre-clinical studies, the first-in-class Hsp90 inhibitor 17-allylamino-demethoxygeldanamycin (17-AAG) has shown little efficacy in Phase I and II clinical trials for advanced prostate cancer due to limitations in formulation, poor pharmacokinetics and hepatoxicity. In this study, we used cell based assays and an explant culture model of human prostate tumors to examine the efficacy of two new synthetic inhibitors of Hsp90, namely (i) NVP-AUY922 that has emerged as the most potent Hsp90 inhibitor developed to date, and (ii) the orally available HSP990. We demonstrate that both agents are significantly more potent than 17-AAG in killing prostate cancer cells. In the AR positive LNCaP cell line, a 40nM dose of NVP-AUY922 or HSP990 induced cell death in 70% and 30% of cells, respectively, while no increase in cell death was observed for 40nM 17-AAG. The AR negative cell line PC3 was more sensitive to both agents, with a 40nM dose of NVP-AUY922 or HSP990 causing 80-90% cell death. Both NVP-AUY922 and HSP990 significantly reduce steady state protein levels of the Hsp90 client proteins HER2, c-RAF-1 and AR, in addition to the AR-regulated protein PSA, and both inhibitors altered cell cycle distribution. In addition to our cell line studies, we have developed a unique model of human prostate cancer where specimens collected from men undergoing radical prostatectomy are cultured as explants. Using this model, we report for the first time how human prostate tumour tissue responds to NVP-AUY922 and HSP990, and demonstrate modulation of the established clinical biomarkers of Hsp90 inhibition, namely HSP70, c-RAF-1 and CDK4, in addition to AR. In summary, we provide the first extensive evaluation of the synthetic Hsp90 inhibitors NVP-AUY922 and HSP990 in prostate cancer cells and in human prostate cancer tissue. Our findings support the clinical development of these agents for the treatment of men with prostate cancer. Nothing to Disclose: LMB, MMC, JG, AH, PS, WDT

Pub #	P2-29
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Inhibition of PI3K Reduces Tumor Growth in a Mouse Model of Type 2 Diabetes
Author String	EJ Gallagher, Y Fierz, A Vijayakumar, N Haddad, S Yakar, D LeRoith Mount Sinai Medical Center, New York, NY
Body	Type 2 diabetes (T2DM) has been associated with increased breast cancer incidence and mortality. The increased risk of breast cancer risk has been correlated with increased insulin levels in humans and increased insulin receptor (IR) expression has been reported in breast cancer cell lines. Insulin signaling through the IR leads to activation of the phosphatidylinositol 3-kinase (PI3K) signaling pathway and accelerated mammary tumor development in animal models of T2DM (1). These models have shown increased activation of IR and PI3K signaling, in the presence of hyperinsulinemia. PI3K signaling is also regulated by the tumor suppressor gene PTEN (phosphatase and tensin homolog deleted on chromosome 10). PTEN is the second most common tumor suppressor gene to be mutated in breast cancer; mutations of PTEN can lead to increased activation of the PI3K pathway. Therefore, pharmacological inhibitors of PI3K have been developed that could potentially be used to treat breast cancer with increased IR and PI3K signaling. We aimed to examine the effects of inhibiting PI3K activity in a mouse model of T2DM, firstly to see if blocking PI3K would reduce mammary tumor growth in the setting of hyperinsulinemia and increased IR signaling; and secondly to explore the metabolic effects of blocking PI3K in a mouse model of T2DM. We used the female MKR mouse model of T2DM. The female MKR mouse is non obese but has severe insulin resistance and hyperinsulinemia and has been previously demonstrated to develop increased mammary tumor growth, compared to wild type (WT) mice (1,2). We induced tumors in MKR and WT mice by implantation of PyVmT or Neu/ErbB2 expressing mammary tumor cells. We then treated the mice with either the PI3K inhibitor BKM120 (50mg/kg/d) or the PI3K/mTOR inhibitor BEZ235 (40mg/kg/d) by oral gavage for 2 weeks. Decreased mammary tumor growth was seen in MKR mice treated with BKM120 and BEZ235. BKM120 and BEZ235 were seen to reduce signaling downstream of PI3K in MKR and WT mice. Blocking PI3K led to significant hyperglycemia in MKR and WT mice. Therefore, in a mouse model of T2DM, with hyperinsulinemia and more aggressive tumor growth, inhibiting PI3K using either the PI3K inhibitor BKM120 or the PI3K/mTOR inhibitor BEZ235 can reduce tumor progression. However, hyperglycemia developed in MKR and WT mice, with both BKM120 and BEZ235 due to blocking the PI3K signaling pathway. (1) Novosyadlyy R et al., Cancer Res 2010; 70: 741-751. (2) Fernandez AM et al., Genes Dev. 2001; 15: 1926-1934 Sources of Research Support: Grants from the Swiss National Science Foundation [PBBSB-120851 and PBBSB3-120851], the Novartis Foundation, the Roche Research Foundation and the Oncosuisse Foundation awarded to YF. Nothing to Disclose: EJG, YF, AV, NH, SY, DL

Pub #	P2-30
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Estrogen Receptor β as Drug Target for the Treatment of Castration-Resistant Prostate Cancer
Author String	P Balanathan, D Pook, U Chandrasiri, S McPherson, G Risbridger Monash University, Melbourne, Australia
Body	Prostate cancer (PC) is the most common cancer, and the second leading cause of death from cancer, in males in most Western countries. Advanced PC is initially sensitive to androgen deprivation therapy, but usually progresses to the castration-resistant state. Although several new therapies have recently become available for the treatment of castrate-resistant prostate cancer (CRPC), the disease remains universally incurable and demands novel therapeutic approaches. Selective estrogen receptor (ER) modulators are a class of drugs with mixed estrogen agonistic/antagonistic activity that holds promise in fulfilling this need. Although the expression pattern and specific action of ERβ during PC progression has been unclear, we previously reported ERβ1 expression in human PC cell lines as well as in Gleason Grade 7 human specimens (1). We also showed that the ERβ specific agonist, 8β-VE2, causes apoptosis in androgen-independent cell lines (PC3/DU145) and xenografted PC tissues that is mediated via caspase-8. These data provided the first evidence that ERβ-induced action in PC tissues targets cells in an androgen-independent manner and therefore we hypothesize that ERβ has therapeutic potential as a drug target. This study is the first to report ERβ1 expression in CRPC. The anti-ERβ antibody (Leica) was used in immunohistochemistry to characterize the expression of ERβ in CRPC TURP specimens (n=8). Nuclear ERβ staining was detected in 7/8 specimens and there was no difference in the immunostaining in benign and cancer regions within the tissue. We further tested the therapeutic potential of ERβ activation by combining *in vitro* assays and *in vivo* imaging approaches; we analyzed the effects of 8β-VE2 on PC proliferation, apoptosis, expression of tumourigenic factors and tumour growth in cultured cells and in xenografts using DsRed-labelled PC3 cells. Our data show that 8β-VE2 not only significantly reduces VEGF-C expression, but also induces a broader anti-tumour phenotype by decreasing the proliferation rate, and increasing apoptosis, *in vitro*. The clinical significance of 8β-VE2 was evident by an increase in PC3-DsRed tumour doubling time (~2 fold) following 8β-VE2 treatment and concurrent with a significant increase in apoptosis and reduction in proliferation. Our data highlight a pivotal role for ERβ as potential drug target for the treatment of CRPC and establish the rationale for the use of ERβ specific modulators as new options for the treatment of CRPC. (1) McPherson et al., PNAS 2010; 107:3123 Nothing to Disclose: PB, DP, UC, SM, GR

Pub #	P2-31
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Functional and Structural Effects of ERβ Agonist: Targeting Tumor Repopulating Cells in the Prostate
Author String	S Hussain, SJ Mcpherson, C Lo, SL Hedwards, M Frydenberg, GP Risbridger Department of Anatomy & Developmental Biology, Monash University, Clayton, Australia; Princess Alexandra Hospital, Brisbane, Australia; Monash University, Clayton, Australia; Monash Medical Centre, Clayton, Australia; Monash University, Clayton, Australia
Body	Prostate cancer is now the most common malignancy occurring in aging men and is commonly treated by the removal of androgens. Androgen ablation in modern society is most commonly achieved by either blocking the production of testosterone, or its more potent form, dihydrotestosterone. Whilst this causes initial tumor regression, the tumor eventually adapts, and returns in an androgen-independent form for which there is currently no cure. Our laboratory has previously used transient treatments of the estrogen receptor β (ERβ) specific agonist; 8β-VE2 to target P63+ castration resistant prostatic cells (1) which are implicated in prostatic regeneration and in cancer initiation (2). Using mouse models and a cyclic model of treatment, we now show that in addition to increasing apoptosis in castrate resistant murine prostatic cells, transient 8β-VE2 exposure also alters the composition of prostatic secretions following recovery, unlike in castrated mice treated with testosterone to restore androgen levels. Furthermore, agonist treated prostates showed areas of cystic atrophy, suggesting the targeting of a group of cells important in prostatic regeneration and homeostasis. Using stereology and fluroscent markers, we were able to show that these functional and structural changes may have been due to the loss of a subset of basal and transient amplifying cells. In order to further elucidate the functional implications of transient ERβ agonist treatment in a disease setting, we treated the castrate-resistant human prostatic epithelial cancer line; PC3, and evaluated their motility and migratory function. Here we showed that whilst ERβ agonist was able to significantly decrease the motility of PC3 cells, transient therapy appeared not to inhibit the migratory capabilities of the cells. These results are currently being further validated using other normal & cancer primary cell lines. Our results indicate that not only does the drug target castrate resistant cells implicated in disease initiation, but it also has sustained functional and structural alterations in both murine and human prostate cells following transient exposure. In conclusion, these data demonstrate the potential of 8β-VE2 to be used as a novel therapy and potentially a preventative agent for prostatic disease. (1) Hussain, S., et al., Estrogen receptor beta activated apoptosis in benign hyperplasia and cancer of the prostate is androgen-independent and TNF-A Mediated. Proc Natl Acad Sci U S A, 2010. 107(7): p. 3123-8. (2)Goldstein, A.S., et al., Identification of a cell of origin for human prostate cancer. Science, 2010. 329 (5991): p. 568-71. Nothing to Disclose: SH, SJM, CL, SLH, MF, GPR

Pub #	P2-32
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	A Role for Estrogen Receptor Beta in the Inhibition of Prostate Cancer Cell Growth
Author String	A Ibragimov, L Hinds, RJ Handa University of Arizona, Phoenix, AZ
Body	Prostate cancer (PC) and benign prostatic hypertrophy (BPH) are highly prevalent neoplasms. Studies have demonstrated the androgen-dependent nature of benign and pathologic growth of prostate cells. The presence of both androgen receptors (AR) and estrogen receptor beta (ERb) have been well-characterized in these cells Although ARs have strong proliferative activity in prostate, recent studies have implicated an anti-proliferative role for ERb. This study investigated the effects of ERb stimulation on normal prostate growth in a mouse model and on PC cell growth to better elucidate a mechanism for the proposed anti-proliferative actions of ERb. We also determined the interplay between concurrent androgen and ERb stimulation on PC cell proliferation in vitro. Our hypothesis is that ERb activation will decrease cell growth and increase cell death in PC cells. Three different ERb-activating compounds were each examined for ability to inhibit prostate weight in adult male mice and to prevent human PC cell (LnCAP and PC3 lines) growth. The dihydrotestosterone (DHT) metabolite 5 alpha androstane-3 beta 17b diol (3bdiol), the selective ERb agonist diarylpropionitrile (DPN), and the isoflavone metabolite, equol, a daidzein-derived compound with ERb-binding properties were tested. DPN (2mg/Kg) treatment of adult male C57BL/6 mice for 21 days caused a significant decrease in dorsolateral (but not anterior or ventral) lobe weight as compared to control (P=.0002). Given this finding, we examine the effect of ERb agonists on the growth of LnCAP and PC3 cells in vivo. As expected in both cell lines, DHT increased prostate cell proliferation as indicated by a relative decrease in number of dead cells as compared to control, suggesting an anti-apoptotic effect. Furthermore, DPN treatment of LNCaP cells decreased cell proliferation, an effect that was overcome by concurrent treatment with DHT. Interestingly, equol also showed anti-proliferative effect in cells when alone as well as in the presence of DHT. 3bdiol did not alter cell growth. These data suggest an anti-proliferative role of some ERb agonists, notably DPN and equol. Furthermore, in vitro data strongly suggest an antagonistic action of Equol on DHT activity not seen by DPN or 3bdiol. The anti-androgen effects of Equol are of paramount importance in regulating/counteracting hormone-induced PC cell proliferation and may have future clinical implication for this disease condition. Nothing to Disclose: AI, LH, RJH

Pub #	P2-33
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	ERbeta Overexpression Is Anti-Proliferative in the Prostate Cancer DU145 Cell Line
Author String	R Aesoey, P Balanathan, EF Need, G Buchanan, GP Risbridger Monash University, Melbourne, Australia; The University of Adelaide, Adelaide, Australia
Body	Prostate cancer (PCa) develops as a consequence of abnormal androgenic stimulation and androgen-deprivation remains the predominant effective therapy for advanced PCa, although it invariably fails with the emergence of fatal castrate-resistant disease. Recent studies have shown that estrogens also affect PCa development. While estrogen receptor alpha (ERalpha) activity promotes malignant prostate cell growth, estrogen receptor beta (ERbeta) inhibits growth of PCa cell lines and tissue. The aim of our study was to analyse the effects of ERbeta overexpression in the ERalpha-negative castrate-resistant prostate cancer cell line DU145. The cells were transduced with an empty Lentiviral vector or a Lentiviral vector for Flag-ERbeta expression, and colonies stably expressing ERbeta were selected. Cells transduction with Flag-ERbeta showed a 200 fold increase in ERbeta mRNA level and a 15 fold increase in ERbeta protein level in comparison to parental or empty-vector transduced DU145 cells. Transactivation activity in the ERbeta transduced cells demonstrated a 4 fold increase in basal activity compared to cells transduced with empty vector. Overexpression of ERbeta led to a reduction in proliferation in the presence of either E2 or 8beta-VE2 (an ERbeta selective agonist) compared to vehicle treated cells, while this effect was not observed in parental or empty-vector transduced DU145 cells. Preliminary data also suggests that ERbeta up-regulation affects DU145 cell death/apoptosis, migration and invasion potential. Moreover, treatment of parental DU145 cells with 8beta-VE2 decreased expression of the tumourigenic factors VEGF-A and VEGF-C. Collectively, these results suggests that ERbeta may play an antiproliferative role in prostate cancer cells in response to estrogens and may therefore explain in part the effectiveness of estrogens as prostate cancer therapeutics. Nothing to Disclose: RA, PB, EFN, GB, GPR

Pub #	P2-34
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Modulation of Pro-Inflammatory Phenotype in Prostate Cancer: Role of Hypoxia
Author String	L Ravenna, L Principessa, L Salvatori, G Coroniti, C Moretti, MA Russo, E Petrangeli National Research Council (CNR), Rome, Italy; Sapienza University of Rome, Rome, Italy; University of Rome Tor Vergata, Rome, Italy
Body	Previous studies have underlined the role of tumor cells in the endogenous synthesis of pro-inflammatory molecules (1). The malignant progression of prostate cancer appears to be associated with the activation of a phenotype typical of the innate immune system. The expression of alarmin receptors such as the receptor for advanced glycation end products (RAGE) and the purinoreceptor (P2X7R), the inducible pro-inflammatory enzymes and the acute-phase protein pentraxin-3 (PTX3) were up-regulated in prostate cancer samples in the absence of a detectable leukocyte infiltrate, with respect to controlateral non-tumor tissues. Besides, the expression levels of pro-inflammatory genes positively correlated with the estrogen receptor alpha isoform, but not with beta isoform, showing that only the former is involved in the abnormal prostate cancer activation of pro-inflammatory response. The presence of hypoxic and necrotic areas in prostate cancer tissues has been described. Therefore, we investigated the role of hypoxia in the modulation of the pro-inflammatory phenotype in cancer prostate LNCaP, PC3 and DU145 cells. We performed real-time and western blot analysis to evaluate gene expression. After oxygen withdrawal, we compared the activation kinetics of HIF-1A and of NF-kB (p65 e p50) and we evaluated the expression levels of a number of genes (VEGF, RAGE, P2X7R, COX2, HMOX1, CXCR4) involved in inflammation and metastasis Nuclear translocation of HIF-1A followed comparable kinetics in all prostate cells: early start, top after four hours and decline by 24. Expression of HIF1A mRNA was stable for 4 hours, then abruptly decreased and stabilized under the base level. Hypoxia dependent increase in NF-kB nuclear translocation was observed in PC3 and DU145 cell lines but not in the more differentiated LnCaP. When expressed, a clear up-regulation of almost all the studied pro-inflammatory and metastasizing genes was observed, peaking after 24 h (DU145, LNCaP) or between 48 and 72h (PC3). Our data show that prostate tumor cells express molecules of the inflammatory response and that hypoxic microenvironment strongly modulates this phenotype. By silencing HIF isoforms and p65 we intend to clarify the individual contribution of each transcription factor to the pro-inflammatory phenotype in prostate and identify potential therapeutic targets. L. Ravenna et al., Prostate 2009, 69 (11):1245-1255 Sources of Research Support: MIUR, Italy. Nothing to Disclose: LR, LP, LS, GC, CM, MAR, EP

Pub #	P2-35
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Suppression of Truncated Androgen Receptor AR-V7 Transcriptional Activity
Author String	SN Mediwala, H Sun, A Szafran, S Hartig, G Sonpavde, MA Mancini, M Marcelli Baylor College of Medicine, Houston, TX; Baylor College of Medicine, Houston, TX; Michael E DeBakey VA Medical Center, Houston, TX
Body	Background: The androgen receptor (AR) is a member of the nuclear receptor super-family. AR is involved ir prostate cancer (PC) growth, and androgen-ablation therapy is the primary therapy if surgical resection fails o is not an option. Androgen ablation therapy, however, invariably fails, resulting in castration resistant prostate cancer (CRPC). Once PC has transitioned to CRPC it may progress to a fatal outcome, and results in the death of approximately 29,000 Americans a year. Although CRPC patients are androgen-depleted by chemical or surgical castration, androgen receptor (AR) signaling remains active and appears necessary to drive tumor growth. The recent description of truncated AR isoforms (T-ARs) in CRPC cells lines may explain the persistence of AR signaling in CRPC despite androgen ablation. T-ARs are truncated so that they lack a ligand binding domain, but maintain functional activity. These truncated AR isoforms are constitutively active, and thus allow for the growth of prostate cancer cells even in the clinical setting of androgen ablation. Based on this hypothesis, suppression of activity of the truncated androgen receptor AR-V7 could prove to be a critical aspect of future prostate cancer therapies. Hypotheses: We hypothesize that wild type AR suppresses AR-V7 transcriptional activity in a bicalutamide independent fashion. Further, we hypothesize that PI3 and MAP kinase pathway inhibitors and the experimental AR antagonist MDV3100 are capable of modulating AR-V7 transcriptional activity. Methods: The AR negative PC3 prostate cancer cell line was transiently transfected with AR-V7, or AR-V7 and wild type AR, and a probasin driven luciferase reporter. Relative luciferase activity was measured in the presence and absence of androgen, bicalutamide, PI3K and MAPK inhibitors, and MDV3100. Results: Wild type AR suppresses the androgen independent transcriptional activity of AR-V7. Bicalutamide and MDV3100 do not inhibit AR-V7 induced transcriptional activity. Several PI3 and MAP kinase inhibitors are shown to inhibit AR-V7 by relative luciferase activity and high content analysis. Conclusions: Dissecting the interaction of AR-V7 with wild type AR and further investigation of kinase pathway inhibitors may reveal avenues to ablate AR signaling in castration resistant prostate cancer. Sources of Research Support: South Central VA Healthcare Network Pilot Project Grant (SNM); VA MERIT review program (M. Marcelli); Department of Defense Prostate Cancer Research Program (M. Marcelli, M. Mancini); John S. Dunn Gulf Coast Consortia for Chemical Genomics (M. Mancini). Nothing to Disclose: SNM, HS, AS, SH, GS, MAM, MM

Pub #	P2-36
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Evaluating the Consequences of Expressing Constitutively Active Androgen Receptor Splice Variants in Androgen-Dependent LNCaP Prostate Cancer Cells
Author String	WC Krause, M Nakka, A Shafi, H Zhao, W Li, NL Weigel Baylor College of Medicine, Houston, TX; Baylor College of Medicine, Houston, TX
Body	Prostate cancer (PCa) is an androgen-dependent disease. There is compelling evidence that tumors that become resistant to androgen ablation therapy remain androgen receptor (AR)-dependent. Among the potential contributors to this resistance are the recently discovered constitutively active AR splice variants. These variants typically contain the amino-terminal transactivation domain and the DNA binding domain but lack the carboxyl-terminal hormone binding domain of AR. Instead, they have small amounts of unique sequence derived either from an intron or from out of phase translation of an exon. Antibodies have been raised to the unique sequence of one of these (V7 also termed AR3) and its expression in castration-resistant tumors has been measured. Initial studies depleting expression of this variant in 22RV1 cells suggest that the activity of the variant may differ from full-length AR and that the variant might not induce PSA (prostate-specific antigen), an AR target gene. This raises the question of how the activity of the splice variants differs from AR and whether differences in activity are due to the unique sequence or to the lack of the hormone binding domain. To address these issues, we have used lentiviruses encoding AR-V7 (amino acids 1-627 of AR with the 16 amino acids unique to the splice variant) or AR-NTD (amino acids 1-660 of AR) to prepare derivatives of androgen-dependent LNCaP cells that inducibly express the variants in response to doxycycline. We established conditions to express the variants at levels comparable to endogenous, full-lengt AR. Both variants constitutively activate an AR-dependent luciferase reporter as expected. At variant levels comparable to the level of full-length AR, both variants stimulated cell growth in hormone-depleted medium. Moreover, both induced the AR target genes PSA and TMPRSS2 showing that either can at least partially compensate for the lack of androgen in these models. Whether the endogenous, full-length AR, which ordinarily is inactive under these conditions, plays a role in this activity remains to be determined. Higher levels of variant show a reduced ability to stimulate transcription of these targets. Although 22RV1 cells express higher levels of variants than AR, variants are expressed at lower levels than AR in most tumors. Our RNA Seq studies (in progress) will comprehensively address the differences between the activities of AR and the variants. Nothing to Disclose: WCK, MN, AS, HZ, WL, NLW

Pub #	P2-37
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Small Molecule Inhibitors of the Androgen Receptor in Prostate Cancer Cells Identified by High-Throughout Screening
Author String	MT Cherian, EM Wilson, DJ Shapiro University of Illinois, Urbana, IL; University of Illinois, Urbana, IL; University of North Carolina, Chapel Hill, NC
Body	Androgen receptor (AR) bound to androgens plays a key role in primary and castration-recurrent prostate cancer. Although endocrine therapy based on blocking androgen production or the use of competitor ligands i initially effective, recurrent prostate cancers, which sometimes overexpress AR, are usually resistant to currently available antagonists. We performed a cell-based screen of 150,000 small molecules to identify novel antagonists of AR activity. To simulate an environment of high AR, as seen in some advanced prostate cancers, we used a stably transfected cell line that expresses high levels of wild-type AR, and an androgen responsive prostate specific antigen (PSA)-luciferase reporter gene. Hits from the primary screen were also evaluated in a stably transfected cell line expressing AR in a range of levels seen in AR positive prostate cancer cell lines. Candidate small molecules were evaluated for their ability to inhibit androgen-dependent growth of AR positive LNCaP, human prostate cancer cells, with little or no effect on the growth of AR negative PC3, prostate cancer cells, or on estrogen receptor mediated gene expression. Perhaps because the primary screen used cells expressing high levels of AR, none of the small molecule inhibitors evaluated in detail down-regulated AR protein levels. Here we describe one of several small molecule inhibitors we identified. IN19 inhibited expression of an androgen-responsive luciferase reporter gene with an IC_{50} of ~75 nM in cells expressing moderate levels of AR and ~5 [micro]M in cells expressing high levels of AR. IN19 is largely AR-specific and inhibited androgen-dependent growth of LNCaP and LAPC4 cells, without inhibiting the growth of PC3 cells. In LNCaP cells, IN19 inhibited AR-mediated induction of the endogenous PSA and TMPRSS2 genes with an IC_{50} of ~500 nM. IN19 also shows robust inhibition of all AR-regulated genes tested so far in LNCaP and LAPC4 cells. These studies show that a high throughput screening strategy, using cells expressing elevated levels of AR sometimes seen in recurrent prostate cancer, can identify small molecule inhibitors that inhibit AR-mediated gene expression and growth of castration-recurrent prostate cancer cells. Sources of Research Support: DOD Prostate Cancer Research Program grant W81XWH-09-1-0309; National Cancer Institute Center grant P01-CA77739. Nothing to Disclose: MTC, EMW, DJS

Pub #	P2-38
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	The Role of PKA in DHT-Induced Transactivation of the EGFR in Prostate Cancer Cells
Author String	H Prizant, SR Hammes University of Rochester School of Medicine and Dentistry, Rochester, NY
Body	Recent data in prostate cancer cells demonstrate that androgens via interaction with the classical androgen receptor (AR) can lead to matrix metalloproteinase (MMP)-mediated release of membrane-bound EGF receptor (EGFR) ligands, which in turn activate the EGFR and subsequent Akt and MAPK signaling. These signals can then mediate important cellular functions such as proliferation and migration. The purpose of this work is to study the underlying mechanisms that regulate androgen-induced transactivation of the EGFR in prostate cancer cells. Studies in the *Xenopus laevis* oocyte have suggested that androgens modulate kinase signaling by altering G-protein actions at the cell surface, perhaps via interactions with the molecule proline, glutamate and leucine rich protein 1 (PELP-1). Given these observations, along with the knowledge that G-protein coupled receptors (GPCR) can transactivate the EGFR, we postulated that G-proteins may similarly regulate androgen-induced ERK activation in LnCap cells. Inhibition of Gβγ as well as Gαi had no effect on ERK activation in response to dihydrotestosterone (DHT); however, blockade of PKA by H89 resulted in the drastic reduction of DHT-induced ERK activation, suggesting that Gαs and cAMP are regulating androgen-induced EGFR transactivation. Importantly, while PELP-1 knockdown by siRNA markedly reduced the expression of PSA mRNA, no effect was detected on androgen-mediated ERK activation. Thus, in prostate cancer cells, PELP-1 appears to be primarily a regulator of transcription rather than kinase signaling. Notably, we and others have shown in several cell lines that Gαs-coupled seven-transmembrane domain receptors can transactivate the EGFR via PKA signaling. Furthermore, we demonstrate that activation of PKA by forskolin activates ERK, suggesting through transactivation of the EGFR. Together, these observations suggest that PKA serves as a common activator of EGFR signaling, and that, at or near the membrane, classical ARs and GPCRs transactivate the EGFR in a similar PKA-mediated fashion. These extranuclear signals can then modulate transcription factors, including the AR, to regulate proliferation and other cellular processes. Sources of Research Support: NIH Grant DK59913 awarded to SRH. Nothing to Disclose: HP, SRH

Pub #	P2-39
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Crystal Structure of Human Type 5 17β-Hydroxysteroid Dehydrogenase (AKR1C3) in Complex with 3-(4-(Trifluoromethyl)Phenylamino)benzoic Acid
Author String	M Chen, AO Adeniji, BM Twenter, Y Jin, JD Winkler, DW Christianson, TM Penning University of Pennsylvania, Philadelphia, PA; University of Pennsylvania, Philadelphia, PA
Body	Human type 5 17β-hydroxysteroid dehydrogenase (AKR1C3) has been implicated in the proliferation of a wide variety of cancers, including prostate cancer, breast cancer, endometrial cancer, and leukemia. Our recent focus is on castration resistant prostate cancer (CRPC), where the successful clinical trials with abiraterone actetate, a CYP17A1 inhibitor, suggest that intratumoral androgen biosynthesis is the culprit for tumor progression. Over-expressed AKR1C3 in CRPC enhances androgen receptor signaling by transforming the weak androgen [Delta]^4-androstene-3,17-dione to the potent androgen testosterone and by converting 5α-androstane-3,17-dione to 5α-dihydrotestosterone. Since AKR1C3 acts further downstream from CYP17A, it i an attractive target for CRPC because AKR1C3 inhibitors may not affect adrenal steroidogenesis. An ideal AKR1C3 inhibitor should be selective towards the enzyme but impose minimal effects on the other AKR1C isoforms involved in androgen metabolism in the prostate. Based on the crystal structure of AKR1C3[bull] $NADP^+$[bull]flufenamic acid (PDB# 1S2C), we have synthesized and screened a family of *N*-phenylanthranilate AKR1C3 inhibitors. One of our lead compounds, 3-(4-(trifluoromethyl)phenylamino) benzoic acid (TFPB), has nanomolar affinity for AKR1C3 and exhibits greater than 250-fold selectivity for AKR1C3 over the other AKR1C isoforms, whereas flufenamic acid shows only a seven-fold selectivity between AKR1C3 and AKR1C2. Here we report the X-ray crystal structure of AKR1C3 in complex with $NADP^+$ and TFPB (at 2.5 [Aring] resolution) obtained by co-crystallization and determined by molecular replacement. TFPB is anchored to the oxyanion site through the carboxylate group and its trifluoromethyl substituted *N*-phenyl ring extends into the same binding subpocket as flufenamic acid. However, due to the *meta*-substitution in the benzoic acid ring, the *N*-phenyl ring is shifted and penetrates more deeply into the subpocket. The penetration is likely to prevent binding of TFPB to the other AKR1C isoforms and is the basis of the observed selectivity of this agent on AKR1C3 over the other AKR1C isoforms. Sources of Research Support: NIH Grants R01-DK40715, R01-CA-90744, Prostate Foundation Challenge Grant, and P30-ES013508 awarded to TMP; and GM-056838 awarded to DWC. Nothing to Disclose: MC, AOA, BMT, YJ, JDW, DWC, TMP

Pub #	P2-40
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	The Association between Metformin and Cancer Incidence and Outcomes: A Systematic Review
Author String	IC Lega, LL Lipscombe, P Rochon University of Toronto, Toronto, Canada

Body

Background: Patients with diabetes have an increased risk of cancer and poorer cancer related outcomes. Cancer risk in diabetic patients is strongly mediated by hyperinsulinemia which appears to affect carcinogenesis both directly through insulin-mediated mechanisms, and indirectly through insulin-like growth factors and other hormone levels. Emerging evidence suggests that insulin-modifying therapies, such as metformin, may affect cancer incidence and outcomes. Our objective was to systematically review and summarize the most recent literature which reports on a relationship between metformin and cancer incidence or cancer outcomes in diabetic patients.

Methods: Using PubMed, EMBASE, Cochrane library and conference abstract databases, we searched for articles that reported on metformin and cancer incidence or cancer outcomes (mortality, prognosis, recurrence).

Results: We included 12 observational studies in our review based on relevance and quality assessment (1-12). Of these, 9 studies reported on cancer incidence, two on cancer mortality, and one on cancer outcome. In terms of cancer sites, 4 reported on all cancer sites, three on prostate, two on breast, two on hepatocellular and one on pancreatic cancers. A total of 7131 cancer events were reported and 529 cancer deaths. Metformin was associated with a decrease incidence in all-site cancer, as well as hepatic, pancreatic and breast cancers. Overall cancer mortality was reduced among metformin users (7,8). For breast cancer incidence, a dose-response relationship was reported with greatest decrease in incidence of breast cancer seen with greater than 5 years of metformin use (5). The association between metformin and prostate cancer incidence was not consistent among studies (10,11). Furthermore, one study reported on cancer outcomes after radical prostatectomy for prostate cancer and found there to be no benefit to metformin use on rates of biochemical recurrence (9).

Conclusion:

Our review corroborates existing evidence that metformin may decrease both cancer risk and cancer mortality in patients with diabetes. Little evidence exists on the impact of metformin on cancer outcomes. This emerging observational evidence thus supports a potential role for metformin for prevention of cancer and perhaps even treatment, and underscores the need for clinical trials to confirm this benefit.

(1) Libby et al., Diabetes Care 2009; 32:1620-5
(2) Currie et al., Diabetologia 2009; 52:1766-77
(3) Li et al., Gastroenterology 2009; 137(2):482-8
(4) Wright JL, et al., Cancer Causes Control 2009; 20:1617-22
(5) Bodmer et al., Diabetes Care Diabetes Care 2010; 33:1304-8
(6) Hassan et al., Cancer 2010; 116:1938-46
(7) Bowker et al., Diabetologia 2010; 53(8):1631-7
(8) Landman et al., Diabetes Care 2010; 33(2):322-26
(9) Patel et al., Urology. 2010 Nov;76(5):1240-4
(10) Donadon V et al., Liver Inte 2010; 30(5):750-8
(11) Azoulay L et al., Cancer Epidemiol Biomarkers Prev; epub 2010 Dec 10
(12) Bosco JL et al., Cancer Epidemiol Biomarkers Prev; 2011 Jan 20(1):101-11

Nothing to Disclose: ICL, LLL, PR

Pub #	P2-41
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Prostate Health & Safety Parameters: Hypogonadal Patients on TRT Are Not at Higher Risk Than Eugonadal Subjects of Age-Matched Control Group -- Prospective Comparative 6-Year FU Analysis
Author String	AA Yassin, AD-J Yassin, A Haider, F Saad Institute of Urology & Andrology, Norderstedt-Hamburg, Germany; Asklepios Klinik Barmbek, Hamburg, Germany; Private Urologic Practice, Bremerhaven, Germany; Bayer Schering Pharma AG, Berlin, Germany
Body	Background: Prostate Safety, LUTS, BPH and the risk of prostate cancer are still the major concern when treating testosterone-deficient men with testosterone. Objective: To evaluate prostate safety parameters in subjects under TRT in comparison with age-matched control group prospectively in follow up of many years. Material & Methods: 154 testosterone deficient patients (baseline average age 58 ± 1.7 years and mean follow-up of 42 months, range: 38-61 months), receiving longacting injectable testosterone undecanoate 1000mg) were compared to a control cohort of 160 eugonadal men (average age 59 ± 2.8 years) with similar characteristics visiting the clinic for preventive medical check up. They underwent monitoring at baseline and 6-monthly including co-morbidities, concomitant medication, International Prostate Symptom Score (IPSS), prostate-specific antigen (PSA), digital rectal examination (DRE), total prostate volume and transitional zone measured by transrectal ultrasound (TRUS). Residual postvoiding urine volume and measuring bladder wall thickness. TRUS-guided biopsies were performed when indicated by PSA velocity > 0.75 [mu]g/L, or elevation over 4.0 [mu]g/L. Results: At baseline, hypogonadal patients showed lower PSA values and lower prostate volumes (0.68 ± 0.4 [mu]g/L and 25.6 ± 1.4 ml, respectively). Subjects in the control group had PSA levels of 2.42 ± 1.2 [mu]g /L and prostate volume 38.4 ± 2.42 ml at baseline. IPPS, residual postvoiding urine volume and bladder wall thickness were slightly improving. Prostate transitional zone and total volume increased as higher as in contro group. No acute urinary retention and/or surgery had been noticed in TRT group, but in controls. Conclusions: 1) Subjects with T-deficiency have lower prostate volumes and PSA levels than eugonadal ones. 2) Testosterone therapy does not worsen LUTS/BPH symptoms 3) Within 6 years follow up, the group on testosterone treatment had no adverse events such as AUR or prostate surgery as met in controls. 4) Hypogonadism offers no protection against the development of symptomatic BPH. Low to normal levels o total testosterone or free testosterone were associated with an increased risk of cancer. Nothing to Disclose: AAY, AD-JY, AH, FS

Pub #	P2-42
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Testosterone and Prolactin Increase Carboxypeptidase-D and Nitric Oxide Levels To Promote Survival of Prostate Cancer Cells
Author String	TJ Morehouse, LN Thomas, CKL Too Dalhousie University, Halifax, Canada; Dalhousie University, Halifax, Canada

Body

We have previously reported that prolactin (PRL) induces plasma-membrane carboxypeptidase-D (CPD) that releases C-terminal arginine from extracellular substrates (1). Arginine is converted by intracellular nitric oxide synthase to nitric oxide (NO). NO is a pleiotropic regulator of many physiological processes, and it also plays a role in tumour growth and progression (2). In prostate cancer (PCa) cells, PRL and androgen receptor signalling pathways interact synergistically to stimulate gene transcription (3). Therefore, we sought to determine the effects of PRL and testosterone on CPD expression in PCa cells, and the role(s) of CPD on NO production and PCa cell survival. The present study first showed that 10 ng/ml PRL or 10 nM testosterone upregulated CPD mRNA and protein levels in a time-dependent manner in human LNCaP PCa cells. However, in the prostate, testosterone is converted to the more potent dihydrotestosterone (DHT) by the enzyme 5α-reductase (5αR), of which there are two well-characterized isozymes, 5αR1 and 5αR2. Secondly, we investigated whether CPD is differentially regulated by testosterone as compared to DHT. Therefore, LNGK9 cells (LNCaP subclone) were transfected with the vector pTRE (control) or with pTRE-5αR1 to overexpress isozyme 5αR1. In pTRE transfectants, 1 nM testosterone for 6 h upregulated CPD mRNA levels by about 2-fold over hormone-free cells, whereas a lower dose of 0.1 nM testosterone was sufficient to produce a similar upregulation in pTRE-5αR1 transfectants. Thirdly, intracellular NO levels were measured in LNCaP cells, using fluorescent DAF2-DA assay. NO levels were low in LNCaP cells cultured in arginine-free medium but increased upon addition of CPD substrate, furylacryloyl-Ala-Arg (FAR). In the presence of FAR, NO production was enhanced by PRL and/or testosterone but decreased by CPD inhibitor, 2-mercaptomethyl-3-guanidinoethylthiopropionic acid. Lastly, small interfering RNAs targeting CPD (siCPDs) were used to knockdown CPD gene expression. LNCaP cells transfected with siCPDs showed a decrease in NO production. This was accompanied by a decrease in cell viability and an increase in apoptosis, as determined using MTS and annexin-V assays, respectively. In summary, PRL and testosterone upregulate CPD and NO levels in PCa cells; DHT is even more potent than testosterone in bringing about such changes. CPD plays a role in NO production to promote PCa cell survival.

(1) Abdelmagid SA and Too CKL, Endocrinology 2008; 149:4821
(2) Fukumura D et al., Nat Rev Cancer 2006; 6:521
(3) Tan et al., Cancer Res 2008; 68:236

Sources of Research Support: Canadian Institutes of Health Research (CIHR-RPP); Nova Scotia Health Research Foundation; Dalhousie Cancer Research Program.

Nothing to Disclose: TJM, LNT, CKLT

Pub #	P2-43
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Metabolic Classification between Benign Prostatic Hyperplasia and Prostate Cancer Evaluated by GC-MS Based Steroid Signatures
Author String	J-Y Moon, MH Choi, H-J Jung, MH Moon, BC Chung KIST, Seoul, Korea; Yonsei University, Seoul, Korea
Body	Abnormalities in steroid hormones synthesized from cholesterol in the adrenal cortex, ovaries, and testes are responsible for development and prevention of many diseases including cancer. Due to their biochemical roles in endocrine system, the quantitative evaluation of steroid hormones is needed to elucidate altered expression of steroids. The present method was used for GC-MS profiling of 84 urinary steroids as their trimethylsilyl derivatives, containing 25 androgens, 17 estrogens, 23 corticoids, 14 progestins, and 5 sterols, obtained from 59 patients with benign prostatic hyperplasia (BPH) and 19 prostate cancer (PCa) to evaluate changes in metabolic patterns versus 41 healthy male subjects. After obtaining quantitative data for each steroid, data were z-score transformed for visualization in the heat-map generated by hierarchical-clustering analysis to allow steroid signatures. Urinary concentrations of 11b-hydroandrosterone, epiandrosterone, 17a-OH-pregnenolone, cortisol, corticosterone, 11-deoxycortisol, 11-deoxycorticosterone, cortisone, 11-dehydrocorticosterone, tetrahydrocortisol, tetrahydrocorticosterone, a-cortolone, b-cortolone, a-cortol, 20a-dihydrocortisone were significantly higher ($P < 0.001$) in patients with PCa than BPH patients. In multi-substrate enzyme assays, 5a-reductase activity (DHT/T ; $P <$ 1.4 [times] 10^{-5}, allo-DHB/B ; $P <$ 9.8 [times] 10^{-8}) known as a marker of BPH were present in much higher levels in both PC and healthy cases, while oxidoreductase activity (11b-HSD, 17b-HSD, 20a-HSD; $P < 0.001$) were significantly higher in patients with PCa than BPH patients.Multi-targeted profiling analysis of steroid hormones generates quantitative results and the metabolic signature of steroids manipulated by multivariate data analysis may be a useful tool for clinical diagnosis as well as mining biomarker in prostate diseases. Nothing to Disclose: J-YM, MHC, H-JJ, MHM, BCC

Pub #	P2-44
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Testosterone Treatment in Elderly Hypogonadal Patients Does Not Increase Prostate Cancer Risk: Prospective Comparative 6-Year Follow-up Analysis with Age-Matched Controls
Author String	AA Yassin, AD-J Yassin, A Haider, F Saad Institute of Urology & Andrology, Norderstedt-Hamburg, Germany; Asklepios Klinik Barmbek, Hamburg, Germany; Private Urologic Practice, Bremerhaven, Germany; Bayer Schering Pharma AG, Berlin, Germany
Body	Objective: Evaluation of prostate safety parameter including prevalence of prostate cancer in elderly hypogonadal subjects under testosterone treatment in comparison with age- and characteristic matched contro groups.Methods: 154 testosterone deficient patients (average age 58 ± 1.7 years and mean follow-up of 42 months, range: 38-61 months), receiving inj. long-acting TU 1000 mg, compared to a control cohort of 160 eugonadal men (average age 59 ± 2.8 years) with similar characteristics visiting clinic for preventive medical check up. They underwent monitoring at baseline and 6-monthly including co-morbidities, concomitant medication, International Prostate Symptom Score (IPSS), prostate-specific antigen (PSA), digital rectal examination (DRE), total prostate volume and transitional zone measured by transrectal ultrasound (TRUS). TRUS-guided biopsies were performed when indicated by PSA velocity > 0.75 [mu]g/L, or elevation over 4.0 [mu]g/L. Results: At baseline, hypogonadal patients showed lower PSA values and lower prostate volumes(0.68 ± 0.4 [mu]g/L and 25.6 ± 1.4 ml, respectively).Subjects in the control group had PSA levels of 2.42 ± 1.2 [mu]g /L, and prostate volume 38.4 ± 2.42 ml at baseline. Hypogonadal patients whose PSA velocity in the observation period was > 0,75 [mu]g /L, underwent TRUS-guided prostate biopsies (10 cores 2.2 cm each or saturating biopsies 24-32 cores 2.2 cm each in those men for whom a repeat biopsy was indicated). We found CaP in 5/22 biopsies, three of them unilateral with up to 10% tumor cells in a core. Gleason scores were 3+2 or 3+3. Two patients had a high grade prostate intra-epithelial neoplasia (PIN). In the 160 control subjects, 16/39 subjects who underwent biopsies showed CaP, 4 of them bilateral, with significantly higher Gleason score of 3+3 till 4+5 and up to 80% tumor cells in a core. No subject of both groups showed any abnormality in rectal palpation. Conclusions: Subjects with T-deficiency have lower prostate volumes and PSA levels than eugonadal ones. Testosterone therapy does not increase CaP incidence. The group on testosterone treatment had smaller tumors and less malignancy (better differentiation).Hypogonadism offers no protection against the development of biopsy-detectable prostate cancer. Lower levels of total testosterone or free testosterone were associated with an increased risk of cancer. Hypogonadal patients untreated with TRT could be at higher risk to have bigger and more aggressive prostate cancers. Nothing to Disclose: AAY, AD-JY, AH, FS

Pub #	P2-45
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Prostate Cancer (1:30 PM-3:30 PM)
Title	Natural History of Hypogonadism and Effects on Prostate Health and Function: The Registry of Hypogonadism in Men (RHYME)
Author String	RC Rosen, AB Araujo, FGW Wu, HM Behre, AS Dobs, CG Roehrborn, F Schroder, GR Cunningham, M Maggi, EJ Meuleman, TH Jones, FS Siami, JVV Brewer New England Research Institutes, Watertown, MA; School of Biomedicine, The University of Manchester Manchester Royal Infirmary, Manchester, UK; Martin-Luther University, Halle-Wittenberg, Halle (Saale), Germany; Johns Hopkins University School of Medicine, Baltimore, MD; UT Southwestern Medical Center at Dallas, Dallas, TX; Erasmus Medical Center, Rotterdam, Netherlands; Baylor College of Medicine, St Luke's Episcopal Hospital, Houston, TX; AOU Careggi, Florence, Italy; VU Medical Centre, Amsterdam, Netherlands; Barnsley Hospital NHS FT, Barnsley, UK
Body	Male hypogonadism is a clinical disorder consisting of reduced circulating testosterone levels in addition to characteristic symptoms. Despite the prevalence and potential impact of the problem, little is known about the natural history of hypogonadism in men particularly regarding prostate and sexual health outcomes. Current treatment approaches include a variety of hormonal therapies, although little information is available about the extent of their use, and long-term efficacy and safety. A multi-national patient registry of treated and untreated men with hypogonadism (RHYME) has been initiated based on current guidelines from the Agency for Healthcare Research and Quality (AHRQ). 1000 hypogonadal men are being recruited in the registry by experienced clinicians (urology, endocrinology, and primary care) in six countries (Germany, Italy, Netherlands, Spain, Sweden, and United Kingdom). Patients in the registry include men with: (1) late-onset hypogonadism; (2) hypogonadism secondary to medical illness; and (3) classical hypogonadism (e.g., Klinefelter's syndrome, Kallmann's syndrome). The primary endpoint for the study is the rate of positive prostate biopsy, with other measures of prostate health (e.g., BPH), quality of life and sexual function being studied as secondary endpoints. Patients are excluded for a history of prostate cancer (PCa) or prior testosterone therapy. All patients provide informed consent to participate in the registry and four (4) patient visits are scheduled to occur at baseline, 3-6 months, 12, and 24 months (Schedule of Visits below). Data collection includes a complete medical history, physical examination, blood sampling, and patient questionnaire, all in accordance of standard of care guidelines for hypogonadism. In addition, any serious adverse events that are potentially related to treatment for hypogonadism are systematically monitored. Blood samples are analyzed in a central laboratory for serum testosterone (by mass spectrometry), prostate-specific antigen (PSA), luteinizing hormone (LH), and sex hormone-binding globulin. Prostate, cardiovascular and other clinical endpoints are centrally adjudicated. All data are entered into a centralized database. RHYME will provide much-needed data on the natural history of hypogonadism and the efficacy and safety of testosterone therapy. Sources of Research Support: A Bayer Schering Pharma AG group company. Nothing to Disclose: RCR, ABA, FGWW, HMB, ASD, CGR, FS, GRC, MM, EJM, THJ, FSS, JVVB

Pub #	P2-46
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	Nodal Signaling Stimulates Cyclin G2 Transcription by Up-Regulating FoxO3A Expression and Promoting Synergistic Interaction between FoxO3A and SMADS
Author String	G Fu, C Peng York University, Canada
Body	Nodal, a member of the transforming growth factor-β (TGF-β) superfamily, has been recently shown to suppress cell proliferation and to stimulate the expression of cyclin G2 (CCNG2) in human epithelial ovarian cancer cells (1). However, the precise mechanisms underlying these events are not fully understood. In this study, we investigated the transcriptional regulation of CCNG2 by the Nodal signaling pathway. In an ovarian cancer cell line, OV2008, overexpression of Nodal or its receptors, activin receptor like kinase 7 (ALK7) or ALK4, resulted in an increase in CCNG2 promoter activity. Several putative FoxO3a binding sites are present in the human CCNG2 promoter and overexpression of FoxO3a enhanced the CCNG2 promoter activity. The functional FoxO3a binding element (FBE) was mapped to a proximal region located between -398 to -380bp (FBE1) through deletion and mutation analyses, as well as ChIP assay. Interestingly, mutation of the FBE1 not only abolished the effect of FoxO3a, but also blocked Nodal-induced CCNG2 transcription. Nodal stimulated FoxO3a mRNA and protein expression through the canonical Smad pathway and suppressed FoxO3a inactivation by inhibiting Akt activity. Silencing of FoxO3a using siRNA significantly reduced the effect of Nodal on the CCNG2 promoter activity. On the other hand, overexpression of Smad2 and 3 enhanced the FoxO3a induced CCNG2 promoter activity and knockdown of Smad4 blocked the activity of FoxO3a. Furthermore, immunoprecipitation assays revealed that FoxO3a formed complexes with Smad proteins and that Nodal facilitated the binding of FoxO3a to CCNG2 promoter. Finally, silencing of FoxO3a reversed the inhibitory effect of Nodal on cell proliferation. Taken together, these findings demonstrated that Nodal signaling promotes CCNG2 transcription by upregulating FoxO3a expression, inhibiting FoxO3a phosphorylation, and enhancing its interaction with Smads. These results also suggest that FoxO3a is an important mediator of Nodal signaling in ovarian cancer cells. Xu G, Bernaudo S, Fu G, Lee DY, Yang BB, Peng C (2008). Cyclin G2 is degraded through the ubiquitin-proteasome pathway and mediates the antiproliferative effect of activin receptor-like kinase 7. Mol Biol Cell 19: 4968-79. Sources of Research Support: CIHR grant MOP-89931 and OWHC/CIHR mid-career award to CP. Nothing to Disclose: GF, CP

Pub #	P2-47
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	Differential Regulation of the Human Adiponectin Promoter by a Non-Synonymous Single Nucleotide Polymorphism in Human ID3
Author String	JL Kirby, MJ Lipinski, JL Kaplan, AM Taylor, PT Hallowell, B Schirmer, CA McNamara University of Virginia, Charlottesville, VA; University of Virginia, Charlottesville, VA; University of Virginia, Charlottesville, VA; University of Virginia, Charlottesville, VA

Body

Adiponectin has emerged as an important adipokine involved in the regulation of metabolism. Hypoadiponectinemia is seen in obese, insulin-resistant patients and correlates with the development of type 2 diabetes. Previous studies have revealed a role for the inhibitor of differentiation-3 (Id3) in the regulation of the murine adiponectin gene both in vivo and in vitro (1). However, less is known about the regulation of the human adiponectin gene. Recently, a functionally significant single nucleotide polymorphism (SNP) in the human ID3 gene has been identified (2) raising the possibility that this SNP may be involved in the regulation of adiponectin expression in humans. Quantification of adiponectin mRNA in human subcutaneous adipose tissue revealed significantly increased levels in subjects heterozygous for the SNP compared to subjects homozygous for the ancestral allele. Promoter-reporter studies conducted in a pre-adipocyte cell line with 1.1 kb of the human adiponectin promoter revealed a 23-fold activation by SREBP-1c, which was reduced 68% by the ancestral form of human Id3, but not by the variant form (encoded by the SNP). Mammalian two-hybrid studies confirmed that there is not a direct interaction between SREBP-1c and either form of human Id3, suggesting that the differential effect is mediated in an indirect manner. Previous data showed that the E-protein, E47, synergizes with SREBP-1c to promote murine adiponectin promoter activation and the interaction of Id3 and E47 results in the inhibition of SREBP-1c-mediated promoter activation (1). The two forms of human Id3 have a differential interaction with E12, another E-protein, in co-immunoprecipitation experiments (2). To begin to understand how the two forms of Id3 may differentially regulate the human adiponectin promoter, co-immunoprecipitation experiments were carried out with various members of the E-protein family. These experiments revealed that E12 is unique among the E-proteins as the only member that demonstrated a differential interaction with the ancestral and variant Id3 proteins. However, both E12 and E47 resulted in repression of SREBP-1c-mediated adiponectin promoter activation. These data provide evidence that SNP-dependent attenuation of Id3:bHLH interactions is restricted to specific interaction partners and reveal a role for the ID3 SNP in the regulation of the human adiponectin promoter.

(1) Doran AC et al., Circ Res 2008; 103:624
(2) Doran AC et al., Circ Res 2010; 106:1303

Sources of Research Support: NIH PPG P01 HL55798, Project 3 (C.A.M.) and NIH Training Grant T32 HL007355-29 (J.L.K. and M.J.L.).

Nothing to Disclose: JLK, MJL, JLK, AMT, PTH, BS, CAM

Pub #	P2-48
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	FTO Promoter CpG Methylation in Sardinian Obese Children and Adolescents
Author String	A Loche, P Zavattari, A Lomniczi, SR Ojeda, A Ibba, S Loche Oregon National Primate Research Center - Oregon Health and Science University, Beaverton, OR; Ospedale Regionale per le Microcitemie, Cagliari, Italy
Body	Background: Numerous genetic variants at the level of FTO first intron have been shown to be associated witl obesity. However, the function of the gene as well as the role of these variants in determining an increased risk of developing obesity are not fully understood. Aim of this study was to evaluate the degree of CpG methylation in the FTO promoter region in genetically predisposed obese individuals and controls. Patients and methods: We have confirmed the association of the SNP rs9939609 with obesity (p-value 5.1 x 10-10) in a selected and genetically isolated population of Sardinian obese children and adolescents (N=912, BMI-SDS 2.65±0.03, M±SE) and controls (N=543, BMI <24 Kg/m2). We then performed a pyro-sequencing analysis of the FTO promoter (NCBI GRCh37.p2 Ref primary assembly: NC_000016.9, from position 53737715 to 53737895 at Chr 16, total 15 CpG islands) to estimate the percentage of methylation of each CpG island in this area in four subgroups of our subjects selected for their genotype at the rs9939609 (A/T) SNP. Particularly, two groups of obese subjects carrying respectively the risk allele (A/A, n=8) and the [ldquo]protective[rdquo] allele in homozygosis (T/T, n=8), and two groups of genetically-matched controls (n=8 per subgroup). Results: Within the analyzed region we report a decrease in methylation of three CpG islands ($p<0.05$) between A/A and T/T obese subjects. Furthermore, we have observed changes in methylation levels ($p<0.05$) at six CpG islands between control A/A and T/T subjects. Interestingly, we found that the CpG island in the closest vicinity of the transcriptional starting site (53737879) was significantly less methylated (~50% decrease, $p<0.05$) in severe obese individuals carrying the risk allele (A/A) compared to the other three subgroups, indicating a possible functional role for methylation at the level of this CpG site. We found no significant changes in global methylation within all subgroups analyzed. Conclusions: We have shown that genetically different subgroups have different methylation levels at certain CpG islands within the FTO promoter region. The lack of change in global methylation in our subgroups suggests that should a methylation-specific mechanism influencing gene expression exist, it must be specific to single CpG islands. Further investigation is needed in order to verify possible mechanisms of methylation-dependent protein-DNA interaction at the level of differentially methylated CpG islands. Nothing to Disclose: AL, PZ, AL, SRO, AI, SL

Pub # P2-49

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)

Title Genetic Determinants of Serum Testosterone Concentrations in Men

Author String C Ohlsson, R Haring
University of Greifswald, Greifswald, Germany; Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Body **Background:** Testosterone concentrations in men are associated with cardiovascular morbidity, osteoporosis, and mortality and affected by age, smoking, and obesity. Because of its high heritability, we investigated the genetic determinants of testosterone concentrations in men.
Methods: We performed a genome-wide association study of testosterone concentration among 14,429 Caucasian men from 10 cohorts. Seven cohorts were designated discovery cohorts (n = 8,938), one *in silico* replication cohort (n = 871), and two *de novo* replication cohorts (n = 4,620). Inverse variance weighted fixed effect model meta-analysis of study-specific results was performed. Serum testosterone < 300 ng/dl was deemed low.
Results: Two independent variants at the sex hormone-binding globulin *(SHBG)* locus (17p13-p12) reached genome-wide significance in the discovery cohorts and were confirmed in the replication cohorts (combined p-value rs12150660, $p=1.2x10^{-41}$; rs6258, $p=2.3x10^{-22}$). Subjects with [ge]3 risk alleles of these variants had 6.5-fold higher risk of having low serum testosterone than subjects with no risk allele. The rs5934505 polymorphism near *FAM9B* on the X chromosome was associated with total testosterone ($p=5.6x10^{-16}$) and free testosterone ($p=6.7x10^{-15}$) concentrations. The rs6258 polymorphism in exon 4 of *SHBG* affected SHBG's affinity for testosterone binding and the free testosterone fraction ($p < 0.01$).
Conclusion: Genetic variants in the *SHBG* locus and on the X chromosome are associated with a substantial variation in testosterone concentrations and increased risk of low testosterone. rs6258 is the first reported *SHBG* polymorphism, which affects testosterone binding to SHBG and the free testosterone fraction and coul influence the calculation of free testosterone using law-of-mass-action equation.

Please find here the correct order & affiliations of all co-authors (all without any disclosures):
Claes Ohlsson1* (claes.ohlsson@medic.gu.se)
Henri Wallaschofski2* (henri.wallaschofski@uni-greifswald.de)
Kathryn L. Lunetta3,4* (klunetta@bu.edu)
Lisette Stolk5,6* (l.stolk@erasmusmc.nl)
John R.B. Perry7* (john.perry@pms.ac.uk)
Annemarie Koster8* (kostera@mail.nih.gov)
Ann-Kristin Petersen9* (ann-kristin.petersen@helmholtz-muenchen.de)
Joel Eriksson1* (joel.erik.eriksson@gmail.com)
Terho Lehtim[auml]ki10* (terho.lehtimaki@uta.fi)
Ilpo T. Huhtaniemi11* (ilpo.huhtaniemi@imperial.ac.uk)
Geoffrey L. Hammond12* (ghammond@cw.bc.ca)
Marcello Maggio13 (marcellomaggio2001@yahoo.it)
Andrea D. Coviello14 (coviello@bu.edu)
EMAS Study Group15
Luigi Ferrucci16 (FerrucciLu@grc.nia.nih.gov)
Margit Heier17 (heier@helmholtz-muenchen.de)
Albert Hofman6,18 (a.hofman@erasmusmc.nl)
Kate L. Holliday19 (kate.holliday@manchester.ac.uk)
John-Olov Jansson20 (joj@neuro.gu.se)
Mika K[auml]h[ouml]nen21 (mika.kahonen@uta.fi)
David Karasik22 (karasik@hsl.harvard.edu)
Magnus K. Karlsson23 (Magnus.Karlsson@med.lu.se)
Douglas P. Kiel22 (kiel@hsl.harvard.edu)
Yongmei Liu24 (yoliu@wfubmc.edu)
[Ouml]sten Ljunggren25 (osten.ljunggren@medsci.uu.se)
Mattias Lorentzon1 (Mattias.Lorentzon@medic.gu.se)
Leo-Pekka Lyytik[auml]inen10 (Leo-Pekka.Lyytikainen@uta.fi)
Thomas Meitinger26,27 (Meitinger@helmholtz-muenchen.de)
Dan Mellstr[ouml]m1 (dan.mellstrom@vgregion.se)
David Melzer28 (david.melzer@pms.ac.uk)
Iva Miljkovic29 (miljkovici@edc.pitt.edu)
Matthias Nauck2 (matthias.nauck@uni-greifswald.de)
Maria Nilsson1 (maria.e.jansson@vgregion.se)
Brenda Penninx30 (B.Penninx@vumc.nl)
Stephen R. Pye19 (Stephen.Pye@manchester.ac.uk)
Vasan Ramachandran3,31 (vasan@bu.edu)

Martin Reincke32 (Martin.Reincke@med.uni-muenchen.de)
Abdelouahid Tajar19 (Abdelouahid.Tajar@manchester.ac.uk)
Alexander Teumer33 (ateumer@uni-greifswald.de)
André G. Uitterlinden5,6,18 (a.g.uitterlinden@erasmusmc.nl)
Jagadish Ulloor31 (julloor@bu.edu)
Jorma Viikari34 (jorma.viikari@utu.fi)
Uwe V[ouml]lker33 (voelker@uni-greifswald.de)
Henry V[ouml]lzke35 (voelzke@uni-greifswald.de)
H. Erich Wichmann9,36,37 (wichmann@helmholtz-muenchen.de)
Tsung-Sheng Wu12 (zenith6912g@yahoo.com.tw)
Wei Vivian Zhuang4 (statinfo@bu.edu)
Elad Ziv38,39 (elad.ziv@ucsf.edu)
Frederick C.W. Wu40** (frederick.wu@manchester.ac.uk)
Olli Raitakari41** (olli.raitakari@utu.fi)
Anna Eriksson1** (anna.eriksson@pharm.gu.se)
Martin Bidlingmaier32** (Martin.Bidlingmaier@med.uni-muenchen.de)
Tamara B. Harris8** (harrist@gw.nia.nih.gov)
Anna Murray7** (anna.murray@pms.ac.uk)
Frank de Jong5** (f.h.dejong@erasmusmc.nl)
Joanne M. Murabito3,31** (murabito@bu.edu)
Shalender Bhasin3,31** (bhasin@bu.edu)
Liesbeth Vandenput1** (liesbeth.vandenput@medic.gu.se)
Robin Haring2** (robin.haring@uni-greifswald.de)
1Center for Bone and Arthritis Research, Department of Internal Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
2Institute of Clinical Chemistry and Laboratory Medicine, University of Greifswald, Germany
3The National Heart Lung and Blood Institute's Framingham Heart Study, Framingham, MA, USA
4Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA
5Dept of Internal Medicine, Erasmus MC Rotterdam, the Netherlands
6Netherlands Consortium of Healthy Ageing, Rotterdam, the Netherlands
7Genetics of Complex Traits, Peninsula Medical School, University of Exeter, UK
8Laboratory for Epidemiology, Demography and Biometry, National Institute on Aging, Bethesda, Maryland
9Institute of Epidemiology, Helmholtz Zentrum Munich, Neuherberg, Germany
10Department of Clinical Chemistry, University of Tampere and Tampere University Hospital, Tampere, Finland
11 Department of Surgery and Cancer, Hammersmith Campus, Imperial College London, London, UK
12 Child and Family Research Institute, and Department of Obstetrics and Gynaecology, University of British Columbia, Canada
13 Department of Internal Medicine and Biomedical Sciences, Section of Geriatrics, University of Parma, Italy
14Sections of General Internal Medicine, Preventive Medicine, Cardiology and Endocrinology, Diabetes and Nutrition, Department of Medicine, Boston University School of Medicine, Boston, MA, USA
15 the European Male Ageing Study
16Longitudinal Studies Section, Clinical Research Branch, National Institute on Aging, Baltimore, Maryland, USA
17Institute of Epidemiology II, Helmholtz Zentrum Munich, Neuherberg, Germany
18Department of Epidemiology, Erasmus MC Rotterdam, the Netherlands
19Arthritis Research UK Epidemiology Unit, University of Manchester, Manchester Academic Health Science Centre, Manchester, UK
20Department of Physiology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
21Department of Clinical Physiology, University of Tampere and Tampere University Hospital, Tampere, Finland
22Hebrew SeniorLife Institute for Aging Research and Harvard Medical School, Boston, MA, USA
23Clinical and Molecular Osteoporosis Research Unit, Department of Clinical Sciences and Department of Orthopaedics, Lund University, Skane University Hospital, Malm[ouml], Sweden
24Department of Epidemiology & Prevention, Wake Forest University Health Sciences
25Department of Medical Sciences, University of Uppsala, Uppsala, Sweden
26Institute of Human Genetics, Technische Universit[auml]t München, München, Germany
27Institute of Human Genetics, Helmholtz Zentrum Munich, German Research Center for Environmental Health, Neuherberg, Germany
28Peninsula Medical School, University of Exeter, Exeter, UK
29University of Pittsburgh, Department of Epidemiology, USA
30Department of Psychiatry and EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, the Netherlands
31Sections of General Internal Medicine, Preventive Medicine, Cardiology and Endocrinology, Diabetes and Nutrition, Department of Medicine, Boston University School of Medicine, Boston, MA, USA

32Medizinische Klinik Innenstadt, Ludwig-Maximilians-University, Munich, Germany
33Interfaculty Institute for Genetics and Functional Genomics, University of Greifswald, Germany
34Department of Medicine, University of Turku and Turku University Hospital, Finland
35Institute for Community Medicine, University of Greifswald, Germany
36Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians- University, Munich, Germany
37Klinikum Gro[szlig]hadern, Munich, Germany
38Division of General Internal Medicine, Department of Medicine, University of California San Francisco, San Francisco, California, USA
39Department of Epidemiology and Biostatistics, Institute for Human Genetics, University of California San Francisco, San Francisco, California, USA
40Andrology Research Unit, Developmental & Regenerative Biomedicine Research Group, The University o Manchester, Manchester Academic Health Science Centre, Manchester Royal Infirmary, Manchester, UK
41Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku and the Department of Clinical Physiology, Turku University Hospital, Finland
*these authors jointly contributed to this work
**these senior authors jointly oversaw this work

Nothing to Disclose: CO, RH

Pub #	P2-50
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	New Polymorphisms in Human Angiotensinogen Gene Promoter and Their Role in Regulation of Blood Pressure
Author String	S Jain, VG Pandey, B Mopidevi, A Kumar New York Medical College, Valhalla, NY
Body	Human angiotensinogen gene (hAGT) has an A/G polymorphism at -6 and -6A allele is associated with increased blood pressure. However, transgenic mice containing 1.2 Kb of the promoter with -6A of the hAGT gene show neither increased plasma AGT level nor increased blood pressure compared to -6G. We have found that hAGT gene has three additional SNPs (A/G at -1670, C/G at -1562 and T/G at -1561). Variants -1670A, -1562C, and -1561T almost always occur with -6A (-6A haplotype); and variants -1670G, -1562G, and -1561G almost always occur with -6G (-6G haplotype). We show that these polymorphisms affect the binding of HNF-1α and GR to the promoter and reporter construct containing 1.8 Kb hAGT gene promoter with -6A haplotype has four fold increased glucocorticoid induced promoter activity as compared to -6G haplotype. In order to understand the physiological significance of these haplotypes in an *in vivo* situation, we have generated double transgenic mice containing either -6A or -6G haplotype of the hAGT gene and human renin gene. Our ChIP assay shows that HNF1α and GR have stronger affinity to the chromatin obtained from the liver and kidney of transgenic mice containing -6A haplotype. Our studies also show that transgenic mice containing -6A haplotype have increased expression of hAGT in liver, kidney and plasma and also increased blood pressure as compared to -6G haplotype. Our studies explain the molecular mechanism involved in association of -6A allele of the hAGT gene with hypertension. Sources of Research Support: NIH grants HL081752 and HL66296 to AK. Nothing to Disclose: SJ, VGP, BM, AK

Pub #	P2-51
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	Association between Polymorphisms in the Genes Coding for Oxidant and Antioxidant Enzymes and Overt Nephropathy in Type 1 Diabetes
Author String	MB Monteiro, SM Vieira, T Marques, M Nery, M Queiroz, SA Dib, MF Vendramini, MJ Azevedo, D Giannella-Neto, LH Canani, ML Correa-Giannella University of São Paulo, São Paulo, Brazil; Instituto de Assistencia Medica ao Servidor, São Paulo, Brazil; Hospital das Clinicas, São Paulo, Brazil; Federal University of São Paulo, São Paulo, Brazil; Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil; University of São Paulo, São Paulo, Brazil
Body	Oxidative stress is recognized as a major pathogenic factor in chronic complications. NOX4/NADPH oxidase is highly expressed in kidney and its activation requires association with subunit p22phox and generates ROS. In the opposite way, glutathione and thioredoxin (TXN) are important endogenous antioxidants. The aim of this study was to test whether SNPs in genes coding NOX4, p22phox (CYBA), glutathione peroxidase-4 (GPX4) and TXN are associated with overt diabetic nephropathy (DN) in patients with type 1 diabetes. The SNPs *NOX4* -1435C[rarr]A, *TXN* -224T[rarr]A and *CYBA* -675T[rarr]A are located in putative sites for transcription factors binding in the promoter region and the SNP *GPX4* 718C[rarr]T is located in the 3'UTR. 366 type 1 diabetic patients were divided into 2 groups: without overt DN (normo or microalbuminuria; n=259) or with overt DN (persistent macroalbuminuria, proteinuria, ESRD or renal replacement therapy; n=107). Also, 118 non-diabetic subjects were included as controls. SNPs were genotyped by PCR using fluorescent-labelled probes (TaqMan, Applied Biosystems). The allelic distribution of all SNPs was consisten with Hardy-Weinberg equilibrium and no differences were found in their frequency between diabetic and non diabetic subjects. The 2 groups were comparable regarding age (33.3 vs 35 years old), disease duration (21 vs 21.4 years) and HbA1C (8.6 vs 8.3%), but frequency of hypertension and triglyceridemia were higher in the group with overt DN (62.9 vs 34.4% and 97 vs 78 mg/dL, respectively). After logistic regression for all confusion variables, the genotypes CT+TT of the SNP *GPX4* 718C[rarr]T conferred protection against overt DN in male patients (OR=0.27; CI95%=0.1-0.69; p=0.0064) and the genotype AA of the SNP *TXN* -224 T [rarr]A conferred risk to overt DN in the overall population (OR=2.7, CI 95%=1.21-5.98, p=0.0136). SNP *GPX4* 718C[rarr]T was previously characterized as functional and SNP *TXN* -224T[rarr]A was selected for its potential to be functional; Genomatix software predicted the generation of a binding site for transcription factor AP-4 when the ancestral allele is replaced by the variant allele. The evaluated SNPs, which may influence the antioxidant status, are associated with overt DN in this series of patients with type 1 diabetes. Sources of Research Support: Fapesp. Nothing to Disclose: MBM, SMV, TM, MN, MQ, SAD, MFV, MJA, DG-N, LHC, MLC-G

Pub #	P2-52
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	Analysis of Aberrantly Spliced *PRKAR1A* Transcripts and the Resulting Proteins in Carney Complex
Author String	YS Jang, HS Kim, JH Kim, JM Lee, SD Moon The Catholic University of Korea, Seoul, Republic of Korea; The Catholic University of Korea, Seoul, Republic of Korea
Body	Carney complex (CNC) is an autosomal dominant disorder featuring cardiac or cutaneous myxoma, multiple endocrine and non-endocrine tumors, psammomatous melanotic schwannoma and spotty pigmentations in mucosa and skin. The endocrine manifestations are growth hormone secreting pituitary adenoma, thyroid follicular benign and malignant neoplasms, testicular large-cell calcifying sertoli-cell tumors (LCCSCT), and primary pigmented nodular adrenocortical disease (PPNAD). The majority (62 %) carries a variety of mutations of the gene encoding the protein kinase A type 1-α regulatory subunit (*PRKAR1A*). *PRKAR1A* is located on chromosome 17q22-24 and germline heterozygous inactivating mutation in CNC has been reportec since 2000. Until now, 117 *PRKAR1A* mutations have been identified all over the world. We experienced a typical case of CNC and found a novel mutation of *PRKAR1A* at splice-junctional region (IVS4-2A>G). We identified altered transcripts resulting from alternatively splicing of the *PRKAR1A* gene in a CNC. We also investigated the stability of the altered *PRKAR1A* transcripts and translation products produced in the CNC. We quantified the differentially expressed *PRKAR1A* mRNAs using real time RT-PCR and examined the expression of *PRKAR1A* proteins in the CNC. The relative quantification ratios of the wild type *PRKAR1A* mRNA, expected 41-bp deleted *PRKAR1A* mRNA, and 62-bp deleted *PRKAR1A* mRNA in the CNC were 0.46, 0.00 and 0.08, respectively. But endogenous *PRKAR1A* protein expression was not detectable in the adrenal tumor of the CNC. The altered *PRKAR1A* mRNAs resulting from the splice site mutation in the CNC were stable, but their *PRKAR1A* translation products from the CNC were probably degraded rapidly. Additional studies that aim to fully characterize the consequences of altered *PRKAR1A* mRNAs in CNC are required to explore these possibilities. Nothing to Disclose: YSJ, HSK, JHK, JML, SDM

Pub #	P2-53
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	Transcriptional and Post-Transcriptional Regulation of the Nescient Helix-Loop-Helix 2 (Nhlh2) Gene
Author String	N Al_Rayyan, DJ Good Virginia Polytechnic Institute and State University, Blacksburg, VA
Body	Nescient helix-loop-helix 2 (Nhlh2) is one of the basic helix-loop-helix transcription factor members. It plays a role in exercise energy expenditure and body weight control. Hypothalamic Nhlh2 gene expression is decreased by food deprivation and increased by feeding or leptin injection following deprivation. This suggests that Nhlh2 gene expression responds positively to increased energy availability and negatively to reduced energy availability. Evidence suggests that both transcriptional and post-transcriptional regulation mechanisms exist for the Nhlh2 gene. Electrophoresis mobility shift assay (EMSA) demonstrated that NF [kappa]B can bind to one of the NF[kappa]B binding site on the Nhlh2 promoter at -144, but not to the other site at -77 on the Nhlh2 promoter. Promoter elements conferring transcriptional regulation of Nhlh2 has been narrowed down to a 163 bp proximal promoter fragment, containing three Stat3 sites, two NF[kappa]B sites, and an Ap-1 binding site. Mutagenesis of the NF[kappa]B site at -144 resulted in increased expression of the Nhlh2 promoter reporter in the presence of leptin in the hypothalamic cell line N29/2. Conversely, mutation o Stat3 site at -91 on the Nhlh2 promoter resulted in a significantly decreased expression of Nhlh2. These results suggest that NF[kappa]B may serve as a negative transcription factor and Stat3 as a positive transcription factor for leptin-induced Nhlh2 expression. In analyzing possible post-transcriptional regulation of Nhlh2, we found that the 3' tail of both the human and mouse Nhlh2 genes have numerous miRNA binding sites and mRNA stability motifs. A single nucleotide polymorphism (SNP) in human Nhlh2 (Nhlh2A1568G) has the potential to affect a putative microRNA binding site which is highly conserved between mice and humans. Transfection of N29/2 cells with a construct which includes the mouse Nhlh2 coding region and 3' UTR, and either two polymorphisms showed that constructs containing the Nhlh2A1568G SNP resulted in decreased overall Nhlh2 expression. Actinomycin D analysis showed this occurred by decreasing mRNA stability relative to the wild-type control construct. These data indicate that the Nhlh2 is regulated at the post-transcriptional level through its 3' UTR, and potentially through miRNA binding motifs. Together this study indicates that both transcriptional and post-transcriptional mechanism contribute to modulate the expression of Nhlh2 under differential energy availability conditions. Nothing to Disclose: NA_R, DJG

Pub #	P2-54
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	Association of the Non-Synonymous CD226 (DNAM1) Gly307Ser Variant with Autoimmune Polyendocrinopathy and Isolated Addison Disease
Author String	EH Gan, AL Mitchell, K MacArthur, SHS Pearce Newcastle University, Newcastle, UK
Body	Genome-wide-association studies have discovered various susceptibility alleles that are shared among different autoimmune conditions, implicating several biochemical pathways in the pathogenesis of autoimmunity. A non-synonymous polymorphism in exon 7 of the gene encoding the lymphocyte cell-surface CD226 (DNAM1) receptor, Gly307Ser (rs763361), has recently been identified as conferring risk for type 1 diabetes, multiple sclerosis, systemic lupus erythematosus and systemic sclerosis. Associations with autoimmune thyroid disease and rheumatoid arthritis have also been suggested but are less robust. We performed a case-control study to determine if the CD226 307Ser variant is also associated with autoimmune Addison's disease (AAD). We genotyped rs763361 in a UK cohort of 300 AAD subjects (167 with associated autoimmune conditions-autoimmune polyendocrinopathy syndrome type-2 [APS2]) and 260 healthy controls, using a Taqman genotyping assay. All datasets were in Hardy-Weinberg equilibrium. The susceptibility 'T' allele at rs763361 was found in 51.2% of AAD patients compared to 47.5% of controls (p-value 0.23, OR 1.16; 95% CI 0.92-1.47). However, comparing the APS2 subgroup to healthy controls, the T allele was found in 54.8% vs. 47.5% in controls (OR 1.34; CI 1.02-1.76, p[SP1] -value 0.04). In contrast, the T allele frequency was 46.9% in isolated Addison's disease (p-value 0.88 vs. healthy controls). Thus, there was a suggestion of allelic heterogeneity between isolated AAD and APS2 with regards rs763361 genotypes, with higher frequency of the T allele observed in APS2 group (54.8% vs. 46.9%; OR 1.37; 95% CI 0.99-1.90; p- value: 0.07). It seems likely that the 307Ser variant of the CD226 receptor is associated with APS2 because of its underlying association with type 1 diabetes and autoimmune thyroid disease. The strength of association in patients with isolated AAD appears to be weak or non-existent compared to that in APS2. To confirm our findings that CD226 307Ser contributes to the susceptibility to APS2 and possibly to that of isolated AAD, we need to study additional patient cohorts. Nothing to Disclose: EHG, ALM, KM, SHSP

Pub #	P2-55
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	Loss-of-Function Mutation in an Inhibitory UORF in CDKN1B 5[prime]UTR Associated with MEN4 Phenotype
Author String	G Occhi, D Regazzo, G Trivellin, F Ceccato, F Boaretto, S Ferasin, P De Lazzari, G Opocher, NS Pellegata, I Mantero, C Scaroni University of Padova, Padova, Italy; Veneto Institute of Oncology, Padova, Italy; Helmholtz Zentrum, Munich, Germany
Body	Mutations in CDKN1B gene, which encodes the cyclin dependent kinase inhibitor p27KIP1, predispose to multiple endocrine neoplasia (MEN) syndrome both in human (MEN4) and rats (MENX). MEN4 affected patients develop MEN1-related lesions such as parathyroid and pituitary tumors. So far only 6 germline mutations have been reported, thus a genotype-phenotype association is difficult to determine and a wider spectrum of affected organs need to be defined. It is hence of great interest to further examine the possible contribution of CDKN1B mutations in patients with MEN. Mutation screening of the coding and untranslated regions (UTRs) of CDKN1B was performed by direct sequencing of genomic DNA of 24 patients with MEN1-like phenotype. A c.-456_-453del was detected in the 5'UTR of an acromegalic patient with a pancreatic lesion. Investigation was extended to 14 MEN1-like patients free from mutations in CDKN1B coding regions and splice sites. In a patient with pheochromocytoma and silent corticotrophinoma a 5'UTR DNA change (c.-469C>T) was detected. Neither variant was found in 300 control subjects. LOH studies for the c.-456_-453del in the pancreatic lesion revealed the retention of the wild-type allele. To clarify the possible role of the 5'UTR DNA changes further functional studies have been performed. The 5'UTR of the CDKN1B gene is highly structured containing regulatory elements such as an IRES, an uORF and a G/C-rich hairpin domain. The uORF, which codes for a 29 aa peptide, is highly conserved among vertebrates both in position, length and sequence, suggesting a functional role. The c.-456_-453del shifts the uORF frame, leading to a delay of its termination codon and to the lengthening of the uORF encoded peptide (158 aa), while the c.-469C>T represent a silent substitution in the uORF. Using an in-vitro approach based on dual luciferase reporter constructs we found a reduced translation efficiency for the c.-456_-453del containing construct compared to the wild type (3 times), while as expected, no differences were found when the c.-469C>T was evaluated. The reduced distance between the delayed STOP codon and the main ATG may prevent the reinitiation of CDKN1B translation explaining the impaired expression of p27KIP1 observed by IHC in the pancreatic lesion of mutation carrier. Although further studies are needed to confirm the role of the c.-456_-453 del this represent the first report in which mutations in the 5'UTR of the CDKN1B gene was associated to MEN4. Nothing to Disclose: GO, DR, GT, FC, FB, SF, PDL, GO, NSP, FM, CS

Pub #	P2-56
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	Hypothyroidism in a Girl with Thyroid Dysgenesis Due to Mutations in the *NKX2.5* Gene and the *PAX8* Promoter
Author String	P Hermanns, H Grasberger, S Refetoff, J Pohlenz Johannes Gutenberg-University, Mainz, Germany; University of Michigan, Ann Arbor, MI; University of Chicago, Chicago, IL
Body	A girl was found to be hypothyroid on neonatal screening; her ultrasound of the neck suggested athyreosis. A brother, parents, and grandparents had normal thyroid function tests and normally located thyroid glands. No mutations in known candidate genes involved in thyroid development (*TSHR, NKX2.1* and *TTF2*) were detected. However, further investigation revealed a new heterozygous *NK2 homeobox 5* (*NKX2.5*) mutation, which was inherited from the father, and a new heterozygous mutation in the *paired box 8* (*PAX8)* promoter region transmitted from the mother. Both mutations were neither found in hundred normal alleles nor reportec previously. *In vitro* studies were performed to unravel the mechanisms by which these two mutations cause congenital hypothyroidism. Immunofluorescence microscopy exhibited a correct nuclear localisation of the wild-type (WT) and the mutant NKX2.5 proteins. Electromobility shift assays (EMSA) demonstrated that the mutant NKX2.5 binds to the NKE_2 and other target promoters (DIO2, TPO, TG) equally well as the WT protein. However, in transient transfection studies the mutant NKX2.5 protein showed a 30-40% reduced transactivation of the *thyroglobulin*- and the *thyroid peroxidase* promoters. Moreover, in presence and also in absence of NKX2.1, a dominant negative effect of the mutant NKX2.5 was observed. EMSA studies of WT and mutant *PAX8* promoter sequences incubated with nuclear extracts isolated from PCCL3 and FRTL5 cells showed a severely reduced protein binding capacity of the mutated allele. Reduced protein binding to the mutated promoter element was accompanied by a significantly reduced transcriptional activity in a *luciferase* reporter system. In summary, we identified new partial loss-of-function mutations in both *NKX2.5* and *PAX8* genes in a girl with congenital hypothyroidism due to thyroid dysgenesis. This is the first case of thyroid dysgenesis where heterozygous mutations in two different genes involved in thyroid development have been identified and characterized. Nothing to Disclose: PH, HG, SR, JP

Pub #	P2-57
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	Mechanism of Repression of the Fetal Adrenal Specific Enhancer (FAdE) of NrR5a1 by Dax1 *In Vivo*
Author String	M Zubair, D Simon, I Finco, K Krill, E Starnes, M Wood, J Heaton, G Hammer University of Michigan, Ann Arbor, MI
Body	The nuclear receptor Steroidogenic Factor-1 (Ad4BP/SF-1/NrR5a1) is essential for adrenal and gonadal development. During the course of embryogenesis, the outer definitive/adult adrenal cortex gradually displaces the early fetal adrenal cortex that regresses after birth. We previously identified a fetal adrenal enhancer (FAdE) in the *NrR5a1* gene locus that directs expression of *Nr5a1* solely in cells of the fetal adrenal cortex as opposed the adult cortex. As this enhancer is autoregulated by Nr5a1, we questioned whether Dax1 might participate in the transition of a fetal to adult cortex by suppressing FAdE-mediated Nr5a1 expression in fetal adrenal cells. Because Dax1 can repress Nr5a1-mediated expression of a tranfected FAdE luciferase constuct in cell culture and is detected only in the outer region of the developing adrenal cortex, we chose to examine FAdE activity in the fetal adrenal cortex in the absence of Dax1 *in vivo*. Dax1 knockout (KO) mice were crossed with mice expressing lacZ driven by the FAdE enhancer. At developmental stages of E11.5-E17.5, the expression of LacZ and the organization of the fetal and definitive cortex of Dax1 KO mice were indistinguishable from wild type littermates. However after birth at P16, while wild-type mice display lacZ positive cells only in a thin rim of remaining cells of the inner adrenal, mice lacking Dax1exhibit an increase in LacZ-positive cells that are arranged in a broader and more diffuse pattern. Importantly, the increasing lacZ-positive cells also stain with the x-zone marker 20alpha HSD, confirming that the x-zone is derived from fetal adrenal zone. Interestingly, the absence of lacZ in the outermost cortex is consistent with a role for additional regulatory factors involved both in FAdE repression and in the emergence of the adult/definitive cortex. Current efforts examine the molecular mechanisms of FAdE repression and the activation of *Nr5a1* expression in the definitive/adult adrenal cortex. Disclosures: GH: Ad Hoc Consultant & Study Investigator, OSI; Ad Hoc Consultant, Orphagen; HRA Pharma; Study Investigator, Corcept. Nothing to Disclose: MZ, DS, IF, KK, ES, MW, JH

Pub #	P2-58
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	Characterization of miRNAs That Are Differentially Regulated in Epithelial Cells from Human Fetal Lung Explants during Differentiation in Culture
Author String	H Benlhabib, CR Mendelson UT Southwestern Medical Center, Dallas, TX
Body	Growth and differentiation of the fetal lung and development of the capacity to synthesize pulmonary surfactant are highly orchestrated processes that are essential for transition of the fetus from an aqueous to an air-breathing environment. In recent years, considerable progress has been made in our understanding of the transcriptional mechanisms for differentiation of the developing lung and expression of genes encoding the surfactant proteins. To further define mechanisms for type II cell differentiation and developmental induction of surfactant synthesis, we are investigating the potential role of miRNAs; evolutionarily-conserved, potent regulators of gene expression. miRNAs are ~22 nucleotide, single-stranded RNAs that inhibit gene expressior by binding to 3'-untranslated regions of target mRNAs through base-pairing, resulting in inhibition of mRNA translation and/or enhancement of mRNA degradation. Previously, we observed that midgestation human feta lung explants differentiate spontaneously in organ culture in serum-free medium. The rate of type II cell differentiation and expression of the gene encoding the major surfactant protein, SP-A, are markedly induced by cAMP. The goal of the present study was to identify and characterize miRNAs that are differentially regulated in mid-gestation human fetal lung explants upon type II cell differentiation in culture in the presenc of Bt_2cAMP. To this end, we performed miRNA microarray analysis of RNA isolated from epithelial cells from human fetal lung explants before and after 48 and 96 h of culture in the presence of Bt_2cAMP. Eight miRNAs were found to be significantly (>2-fold) upregulated and sixteen were significantly downregulated after 48 and 96 h of cAMP-treatment. Two significantly downregulated miRNAs, hsa-miR-199a-5p and hsa-miR-199a-3p, are synthesized as part of a 6-kb anti-sense transcript (Dnm3os). Known targets of hsa-miR-199a-5p include COX-2, NF-[Kappa]B (p50) and IKK[Beta], factors found to positively regulate *SP-A* expression in human fetal lung. We also identified and validated several upregulated miRNAs, including hsa-miR-29a, hsa-miR-200c, hsa-miR-21 and hsa-let-7i. Interestingly, expression of these miRNAs are known to be inversely correlated with cell proliferation. Because of their important functions in other systems, further studies will be carried out to determine the roles of these differentially expressed miRNAs in type II cell differentiation and regulation of *SP-A* gene expression. Sources of Research Support: (NIH R37HL050022). Nothing to Disclose: HB, CRM

Pub #	P2-59
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	The Role of Hedgehog Signaling and Primary Cilia in Adrenal Development and Function
Author String	KF Cogger, L Guasti, PJ King William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK
Body	The adrenogonadal primordium develops from a thickening of the coelomic epithelium covering the urogenital ridge. After segregation of the primordium into the bipotential gonad and the adrenocortical primordium, the cortex is encapsulated by mesenchymal cells and infiltrated by migrating sympathoadrenal cells which form the medulla. Our laboratory and others have demonstrated that the cell fate regulator Sonic hedgehog (Shh) is not required for the formation of the adrenocortical primordium but is required for its subsequent growth and development. Its expression is restricted to relatively undifferentiated cortical subcapsular cells and it signals to cells within the mesenchymal capsule. Lineage tracing studies in mice demonstrate that these signal receiving cells enter the cortex and adopt a steroidogenic phenotype, becoming zona glomerulosa and zona fasciculata cells, at least in part, via a Shh-expressing intermediate cell population Genetic ablation of Shh results in small adrenals with thin capsules. Primary cilia play an essential role in Shh signalling. These are sensory organelles found on almost all vertebrate cells which play key roles in development, cell signalling and cancer. Shh pathway components are localised in cilia and mutations affecting cilia formation and function exhibit phenotypes similar to Shh mutations. Our immunofluorescence studies on H295R cells, a human adrenocortical carcinoma cell line, have demonstrated that they form primary cilia. We have shown that knockdown of IFT88, a major component of the intraflagellar transport system involved in cilium assembly and maintenance, reduces the expression of Hh pathway components, and affects steroidogenesis. Although there has been no report of ciliopathies associated with adrenal insufficiency to date, our studies have documented adrenal phenotypes in BBS 4 and 6 null mice consistent with impaired Shh signalling. Taken together, these data indicate a requirement for primary cilia in adrenal development, further implicating Shh signalling in these processes, and suggest that primary cilia also play a key role in determining the phenotype of adrenocortical cells during differentiation. Sources of Research Support: Medical Research Council. Nothing to Disclose: KFC, LG, PJK

Pub #	P2-60
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	Delineating the Expression and Localization Pattern of FoxO6 in Mouse Skeletal Muscle
Author String	C-W Su, S-T Chen, S-L Chen National Central University, Jhongli City, Taiwan; Chang Gung Memorial Hospital, Taoyuan, Taiwan
Body	Subfamily O of the forkhead-box transcription factors includes FoxO1, FoxO3, FoxO4, and FoxO6. Among these FoxOs, the expression and function of the recently discovered FoxO6 in most physiological processes has not been studied before. We detected FoxO6 mRNA in mouse tissues by Q-PCR and observed that the highest expression of FoxO6 was found in brain, especially in pituitary. Considerable amount of FoxO6 was also expressed in heart, liver, kidney, and muscle. We also found that FoxO6 expression decreased with increasing age. The FoxO6-speific domain (amino acid 229~492) was used as antigen to generate FoxO6-specific polyclonal antibody in rabbits. After titration, we confirmed that this antibody recognized mouse FoxO6 (about 78 kD in size) specifically without cross-reacting with other FoxOs. Besides, this antibody also recognized human FoxO6 protein that yet to be cloned in several human cells lines, including HEK293 and RD cells, and its size is much smaller than that of mouse FoxO6. Using immunofluororescence, we demonstrated that FoxO6 accumulated mostly in nucleus of proliferating myoblasts, and this nuclear localization was further increased when myoblasts became confluent. After terminal differentiation, homogeneous distribution of FoxO6 was detected in the cytoplasm and nucleus of myotubes. Since the localization pattern of FoxO6 is similar to other FoxOs, it suggests that the role played by FoxO6 in myogenesis is probably the same as other FoxOs do. We are currently using gain and loose of function assays to address this issue. Nothing to Disclose: C-WS, S-TC, S-LC

Pub #	P2-61
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	Regulation of α-Inhibin Expression in Placental Tissue from Pregnancies Affected by Fetal Down Syndrome
Author String	J Kipp, G Lambert-Messerlian, G Rodriquez, E Eklund, F Gundogan DePaul University, Chicago, IL; Alpert Medical School of Brown University and Women and Infants Hospital, Providence, RI
Body	Maternal serum inhibin A is a clinically accepted marker in assessing risk of fetal Down syndrome during pregnancy. Second trimester levels of inhibin A are, on average, about two times higher in affected than in control pregnancies, although the pathological basis of this alteration is not understood. The genes for inhibin A are not located on chromosome 21, eliminating a simple dosage effect. Nevertheless, previous studies have shown up-regulated inhibin A subunit mRNA levels and protein content in placental tissue from pregnancies affected with Down syndrome. We sought to determine the regulatory factors responsible for up-regulated inhibin A expression in Down syndrome placenta as a means to better understand the basis of increased maternal serum levels in this condition. Residual placental tissue was collected after routine examination at Women and Infants Hospital. Samples were obtained in the second trimester after spontaneous loss or termination, and in the third trimester after delivery. Chorionic villous tissue was sampled by Perinatal Pathology staff and stored in RNA later at -80 C before processing. Total RNA was extracted using Trizol, digested with DNase, and analyzed for quality. Following reverse transcription, the mRNA levels of select genes were determined by real time PCR. Ribosomal protein L19 (Rpl19) mRNA was used as an internal control. Ten samples from Down syndrome pregnancy and eight control samples were collected at gestational weeks 14 - 39. There was a trend for higher inhibin alpha and beta-A subunit mRNA levels in Down syndrome than in control placental tissues, as expected. Among all regulatory factors studied, levels of WT1 mRNA, a repressor of the alpha inhibin subunit promoter, were reduced in Down syndrome placentas suggesting its importance in the regulation of inhibin A. There were also trends for increased levels of GATA-6 and GATA-2 mRNA, alpha inhibin promoter activators, in Down syndrome. Paradoxically, LRH-1 mRNA levels were decreased in Down syndrome placentas relative to controls. These data contribute to understanding the mechanisms regulating placental inhibin production and may be helpful in ultimately understanding the biological basis of increased maternal serum inhibin A levels in Down syndrome pregnancy. Sources of Research Support: Grant from Beckman Coulter Inc. (GM) and University Research Council and Faculty Summer Research grants from DePaul University (JK). Disclosures: GL-M: Principal Investigator, Beckman Coulter Inc. Nothing to Disclose: JK, GR, EE, FG

Pub #	P2-62
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	*Desert Hedgehog* Is Not Testis-Specific during Early Development in a Marsupial Mammal
Author String	WA O'Hara, WJ Azar, MB Renfree, AJ Pask The University of Connecticut, Storrs, CT; University of Melbourne, Melbourne, Australia
Body	*Desert hedgehog* (*Dhh*), a secreted intercellular signal transducer of the hedgehog gene family, is critical for normal testis development in humans and mice. In mice, *Dhh* is expressed in the testes from 11.5dpc through to adult stages and is essential for Leydig cell proliferation, maintenance of the male germ line and spermatogenesis. In contrast, female mice lacking the *Dhh* gene develop normally. Hedgehog signals through a multi-component receptor complex comprised of PTCH receptors (PTCH1 or PTCH2) and Smoothened (SMO) protein. While *Ptch1* is widely expressed throughout mouse embryo, *Ptch2* is found at high levels in the skin and testis. The majority of genes involved in testis differentiation are highly conserved among the vertebrates. While no *DHH* orthologues have been identified in birds, they are present in fish, reptile and amphibian genomes. To determine the conserved function of DHH in mammalian gonad development we have examined the conservation of DHH signalling in a marsupial. Marsupials occupy a unique lineage of the mammalian tree, and have been evolving independently of eutherian mammals for over 148 million years. *DHH* was expressed in both the ovary and testis from the bipotential gonad through to adult stages. In males DHH was localized to the pre-Sertoli cells at early developmental stages and localized to Sertoli and germ cells in the adult testis, consistent with protein distribution in the mouse. DHH was found throughout the developing ovary at early stages. Once ovarian differentiation began, DHH was localized to the follicles at all stages of development, the steroidogenic corpus luteum and weakly to the interstitium. PTCH1 showed diffuse staining throughout development in both males and females and was slightly elevated within the cords in testes and follicles in ovaries compared to the interstitial tissue. In the testis PTCH2 was weak in the bipotential gonad, but increased within the cords and Leydig cells as the gonad matured. In the adult testes, PTCH2 was localized to Leydig and Sertolli cells, and weakly throughout the interstitium. In the ovary, PTCH2 mirrored PTCH1 expression at all stages but appeared to be more abundant. Taken together, our findings indicate that DHH signaling plays an essential and conserved role in the formation of the mammalian testis and ovary. Furthermore, it suggests a persistent role for hedgehog signaling in early marsupial ovarian development that is not seen in the mouse. Nothing to Disclose: WAO, WJA, MBR, AJP

Pub #	P2-63
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	Nandrolone Induces Numb Expression through Inhibition of Mdm2 in C2C12 Myoblast Cells
Author String	X-H Liu, J Pan, Y Wu, W Qin, W Bauman, C Cardozo James J Peter VA Medical Center, Bronx, NY; Mount Sinai School of Medicine, New York, NY
Body	Nandrolone, an anabolic steroid, slows denervation atrophy of rat muscle. The molecular mechanisms responsible for this effect, however, are not well understood. We have previously observed that nandrolone prevented denervation-induced nuclear accumulation of Notch intracellular domain (NICD), and this effect was accompanied by an elevated numb expression in rat muscle. Numb acts as an inhibitor of Notch signaling and has been identified as a protein involved in the determination of cell fate. Numb has also been shown to promote differentiation of satellite cells to the myogenic lineage. Moreover, expression of numb has been reported to be regulated by Mdm2, an E3 ubiquitin ligase. With these considerations in mind, the effects of nandrolone on numb and Mdm2 expression in C_2C_{12} myoblastic cells were investigated. When C_2C_{12} cells were cultured in a differentiation-favorable medium (MEM containing 2% horse serum), numb protein was mainly located in the nuclear fraction. Rt-PCR and Western blotting revealed that nandrolone increased both numb mRNA and protein levels in a time-dependent manner. Peak levels of numb mRNA and protein were detected at 48h and 72h, respectively. In addition, nandrolone reduced the expression of Mdm2 mRNA and protein. To determine whether the lower levels of Mdm2 induced by nandrolone were responsible for increased numb expression, Mdm2 siRNA was employed to inhibit Mdm2 expression in C_2C_{12} cells. Compared to the cells transfected with scrambled siRNA (negative control), basal numb protein expression was upregulated and the nandrolone-induced increase in numb protein was diminished. The half-lives of Mdm2 and Numb protein were examined in the presence or absence of nandrolone. Nandrolone prolonged numb half-life from 10h to 18h but shortened Mdm2 half-life from 19h to 11h. Our data suggest that nandrolone-induced numb expression may, at least in part, be via suppression of Mdm2 expression and that nandrolone may be able to differentially modulate intracellular protein degradation systems to control the levels of numb and Mdm2 proteins. Sources of Research Support: Veterans Health Administration, Rehabilitation Research and Development Service (grants B4162C and B3347K). Nothing to Disclose: X-HL, JP, YW, WQ, WB, CC

Pub #	P2-64
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	The Repression of MRF Transactivation by Bhlhe40 Is Mediated by Its C-Terminal α-Helixes
Author String	SP Hsiao, G-G Liou, SL Chen National Central University, Jhongli, Taiwan; National Health Research Institutes, Miaoli, Taiwan
Body	Myogenic regulatory factors (MRFs), including MyoD, Myf-5, Myogenin and MRF4, work together with myocyte enhancer factor 2 (MEF2) proteins to promote myogenic differentiation. Recently we found that the expression level of Bhlhe40, a ubiquitously expressed transcriptional repressor, was up regulated during myogenic differentiation and it could bind to PGC-1α, M-cadherin, and Myogenin promoters in vitro and in vivo. Furthermore, we found that this binding repressed MRF transactivational activity and which could be relieved by P/CAF. In this study, we found that Bhlhe40 repressed MyoD transactivated M-cadherin promoter activity and this repression could be relieved by disrupting the Bhlhe40 targeted E3-box (-32~-27). Duplicating the E3-box by replacing the MRF-specific E4-box (-2~+4) with E3-box in the M-cadherin promoter dramatically increased Bhlhe40 binding and repressive activity but repressed MyoD transactivation. Surprisingly, Bhlhe40 repressive activity remained after E3-box was substituted by E4-box, even though its direct binding had been abolished. Interestingly, the Bhlhe40 repressive activity vanished when any one of its 3 C-terminal α helixes, but not the DNA binding domain, was deleted. These observations suggest that Bhlhe40 repressive activity is mediated by its C-terminal α helixes to recruit unknown bHLH factors to repress the MRF transactivity. In the future we like to identify these unknown factors and further clarify the interactions between these factors (MRFs, Bhlhe40, and P/CAF) on the promoters and enhancers of myogenic genes. Besides, we also like to know if other transcriptional cofactors, both coactivators and corepressors, are involved in the modulation of MRF transactivational activity by Bhlhe40. Nothing to Disclose: SPH, G-GL, SLC

Pub #	P2-65
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	Regulatory Mechanisms Controlling Differential Expression of the Inhibin α-Subunit Gene in Rat and Human Luteinized Granulosa Cells
Author String	KM Meldi, GA Gaconnet, SM Kilen, KE Mayo Northwestern University, Evanston, IL; Northwestern University, Evanston, IL
Body	The ovarian hormone inhibin is a key regulator of the female reproductive cycle. In granulosa cells, inhibin is positively regulated by FSH and then transiently repressed by LH. Following luteinization, inhibin repression is maintained in the rat, while inhibin A is strongly expressed in the human ovary. Therefore, despite having highly conserved promoter sequences, there is a marked differential regulation of inhibin between species at luteinization. In this study, we sought to investigate mechanisms responsible for the regulation of the inhibin α subunit observed in rat and human luteinized granulosa cells. We demonstrate that inhibin α mRNA is dramatically reduced and unresponsive to hCG in rat granulosa cells luteinized in culture as compared to primary human granulosa-lutein cells. Because our earlier studies in the rat had demonstrated a key role for the inducible cyclic AMP early repressor (ICER) in negatively regulating inhibin expression in response to LH, we asked whether an absence of ICER function might explain the continued expression of inhibin α mRNA in the human. We found that ICER mRNA is robustly induced by hCG in the human cells, and ICER overexpression can repress human inhibin α promoter activity. These results indicate that ICER-mediated repression is not likely a primary determinant of differential inhibin regulation between species. An alternative hypothesis is that DNA methylation might be responsible for the observed silencing of the rat inhibin gene. Bisulfite sequencing was performed on the inhibin α proximal promoter from rat preovulatory follicles, rat corpora lutea, and human granulosa-lutein cells. There was a 3-fold increase in the number of clones containing methylated CpG sites as well as the complexity of the methylation pattern in the corpora lutea as compared to the follicles, suggesting that DNA methylation might contribute to inhibin α repression in the rat. The CpG dinucleotide of the cyclic AMP response element (CRE) was a common target of methylation, and methylation of this site reduces CREB binding *in vitro*. Initial results indicate the inhibin α promoter is not methylated in the human cells where the gene remains highly expressed, suggesting that promoter methylation may contribute to inhibin gene regulation as well as the differential expression between species. Current studies are examining recruitment of methyl-CpG binding proteins and histone modifications on the rat and human promoters. Sources of Research Support: NIH Specialized Cooperative Centers Program in Reproductive Research U54 HD041857 awarded to KEM; NICHD Reproductive Biology Training Grant T32 HD07068 awarded to KMM. Nothing to Disclose: KMM, GAG, SMK, KEM

Pub #	P2-66
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	*SHBG* $(TAAAA)_n$ Is Associated with Serum SHBG in a PCOS Case-Control Cohort
Author String	CM Ackerman, OA Garcia, RS Legro, A Dunaif, M Urbanek Northwestern University, Feinberg School of Medicine, Chicago, IL; Penn State University College of Medicine, Hershey, PA
Body	Sex hormone-binding globulin (SHBG) is glycoprotein synthesized by the liver that binds sex steroid hormones and regulates their bioavailability. Several polymorphisms in *SHBG* have been associated with alterations in circulating SHBG levels. Low SHBG levels are an early indicator of insulin resistance and predict the development of type 2 diabetes in men and women. Since hyperandrogenemia is a common feature of polycystic ovary syndrome (PCOS), the *SHBG* gene is proposed as being a PCOS candidate gene. A pentanucleotide repeat $(TAAAA)_n$ in the promoter of the *SHBG* gene may affect transcription levels of the gene with the shorter repeat lengths being associated with more efficient transcription. We, therefore, investigated the possible association of the *SHBG* microsatellite polymorphism $(TAAAA)_n$ with PCOS and its metabolic phenotypes. 605 women with PCOS and 657 control women of European ancestry were included in the study. PCOS was diagnosed according to the NIH criteria. Control women consisted of 566 population-based controls and 91 phenotyped controls. Phenotyped controls were women with normal androgen levels and regular menses. Genotyping of the $(TAAAA)_n$ polymorphism in the *SHBG* gene was performed. Serum total testosterone (cases n=602; controls n=91), unbound testosterone (cases n=591; controls n=91), SHBG (cases n=432; controls n=30), fasting insulin (cases n=539; controls n=80), and fasting glucose (cases n=584; controls n=90) concentrations were determined. Linear regression analyses, adjusted for BMI and age, were used to test for associations between *SHBG* $(TAAAA)_n$ repeat length and PCOS and all metabolic traits. Neither *SHBG* average repeat length nor any of the individual alleles were significantly associated with PCOS. *SHBG* $(TAAAA)_n$ average repeat length was negatively associated with serum SHBG levels, with a 2.3% decrease in SHBG for every additional repeat present (P=0.04) in women with PCOS. *SHBG* $(TAAAA)_n$ average repeat length was not associated with any other metabolic traits tested. The *SHBG* microsatellite polymorphism $(TAAAA)_n$ is associated with circulating SHBG levels, but not with PCOS *per se*. This polymorphism may be an important determinant of circulating SHBG levels and, consequently, the amount of testosterone that is biologically available to target tissues in PCOS. Nothing to Disclose: CMA, OAG, RSL, AD, MU

Pub #	P2-67
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	Assessment of Selenoprotein Expression in Human Placentas and Correlation with Selenium, Mercury and Fish Consumption
Author String	CL Gilman, LA Seale, A Seyedali, MJ Berry John A Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI
Body	Selenium (Se) is an essential element known for its antioxidant properties. Se exerts its biological function through selenoproteins by means of the 21st amino acid, selenocysteine. There are 25 selenoprotein genes in the human genome, the biological functions of which many are unknown. In mouse knockout studies, genetic deletion of glutathione peroxidase 4 and thioredoxin reductase 1 and 2 results in embryonic lethality. The work presented here is part of a larger study in Hawaii examining the correlations between maternal seafood consumption and the levels of mercury (Hg), Se and omega-3 fatty acids (FA) in placenta and maternal and cord blood, as well as neurodevelopmental outcomes. Recent studies have indicated the protective effect of Se against Hg toxicity as Se is able to bind and sequester Hg with high specificity. Methyl mercury (MeHg) is especially harmful to health and can have devastating effects on the neurodevelopment of a growing fetus as MeHg readily crosses the placenta and the blood-brain barrier. Ocean fish contains high levels of omega-3 FA, which are beneficial to neurodevelopment, and high Se:Hg ratios. In this study, selenoprotein expression in different compartments of human placenta was investigated. Lobes near the umbilical cord and periphery of the placentas were cut, and from each lobe additional cuts were made from maternal, middle, and fetal compartments. Quantitative real time polymerase chain reaction (qPCR) was utilized to measure the gene expression of glutathione peroxidase 1 and 4 (GPx1 and GPx4), deiodinase type II and III (DIO2 and DIO3), thioredoxin reductase 1 and 2 (TrxR1 and TrxR2), and selenoprotein P (SelP). qPCR results indicate overall high expression of GPx4 and SelP at both regions of the placenta. GPx1 expression is higher, though not significant, in the fetal compartment than in the other two compartments in both regions. In the fetal compartment, DIO2 and TrxR1 are highest near the cord and DIO3 at the periphery. Western blotting techniques will be utilized to measure protein expression of these selenoproteins. Fish consumption has not yet been unblinded in this study. Understanding the correlations between maternal seafood consumption during pregnancy, Se, Hg, omega-3 FA and neurodevelopmental outcomes will shed light on the potential protective effects of Se in ocean fish against Hg, as well as the benefit from omega-3 FA. Sources of Research Support: HCF Grant 433291 awarded to MJB; NIH Grant U54RR026136. Nothing to Disclose: CLG, LAS, AS, MJB

Pub #	P2-68
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	Gene Expression Changes in Mouse Uterus during the Estrus Cycle
Author String	KA Stanley, A Suvorov, J Connerney, DJ Waxman Boston University, Boston, MA
Body	Previous studies have reported how changes in hormone levels alter the expression of select genes in mouse uterus and ovary during the estrus cycle (1-3). However, a global analysis of gene expression changes in mouse uterus in the proestrus and estrus stages of the reproductive cycle has not been reported. We conducted a genome-wide transcriptional profiling study of uterine horn tissue isolated from CD-1 mice in the proestrus and estrus stages of the reproductive cycle. Mice were bred in house, and the estrus cycle of female offspring from multiple litters was monitored beginning at 6 wk of age by taking vaginal smears daily between 0800 and 0900 hr. Following the monitoring of 2 estrus cycles, to confirm that mice went through complete cycles, mice in proestrus and estrus were euthanized between 1300 and 1600 hr. Uterine horns were collected from 1 individuals in proestrus and 8 individuals in estrus and total RNA was extracted and analyzed on Agilent two color microarrays. Thresholds for statistical significance of differential expression of each gene of >2-fold-change in expression and $p < 0.005$ were applied to the microarray data. Over 2900 genes were differentially expressed in estrus relative to proestrus, including 1339 up regulated and 1626 down regulated genes. Genes up regulated in estrus (Wnt1 (10-fold), Serpinb7 (31-fold), and Lgr5 (20-fold) and down regulated in estrus (Prl6a1 (24.5-fold), Uts2 (13.6-fold), and Spink3 (13.2-fold)) were analyzed by qPCR to verify microarray data. DAVID analysis was performed to determine enriched functional groups. Genes that were up regulated in estrus relative to proestrus were significantly enriched (>2.8-fold) in several functional groups including extracellular matrix, cell cycle process, and the Wnt superfamily, among others. Genes that were down regulated in estrus relative to proestrus were significantly enriched (>5-fold) in several functional groups including extracellular matrix, protease inhibitor, and serine protease. Top differentially expressed genes from our study may be used as markers of estrus and proestrus. (1) Miller, C., A. Pavlova, and D.A. Sassoon, Differential expression patterns of Wnt genes in the murine female reproductive tract during development and the estrous cycle. Mech Dev, 1998. 76(1-2): p. 91-9. (2) Salgado, R.M., et al., Hormone-regulated expression and distribution of versican in mouse uterine tissues. Reprod Biol Endocrinol, 2009. 7: p. 60. (3) Tan, Y.F., et al., Global gene profiling analysis of mouse uterus during the oestrous cycle. Reproduction, 2003. 126(2): p. 171-82. Sources of Research Support: In part by Superfund Basic Research Program at Boston University, NIH grant 5 P42 ES07381 (to DJW). Nothing to Disclose: KAS, AS, JC, DJW

Pub #	P2-69
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	Genome-Wide Profiling of DNA Methylation in Human Uterine Leiomyomas
Author String	A Navarro, P Yin, D Monsivais, J-J Wei, SE Bulun Northwestern University, Chicago, IL
Body	Uterine leiomyomas, or fibroids, are the most common benign tumors of the female genital tract and become symptomatic in 30% of women and up to 70% of African American women of reproductive age. The clinical symptoms are pelvic pressure and pain, reduced fertility, frequent pregnancy loss, and abnormal uterine bleeding which can lead to anemia. There is increasing evidence suggesting that epigenetic dysregulation is involved in leiomyomas, and to our knowledge, a genome-wide profile of DNA methylation in these benign tumors has not yet been performed. We conducted a genome-wide DNA methylation profiling in bisulphite converted DNA from 18 leiomyoma cases along with normal matched myometrium using the Illumina Infinium Beadchips, which looks at 27,578 CpG dinucleotides located predominantly in CpG islands within proximal promoter regions between 1.5 kb upstream and 1 kb downstream of the transcription start sites of 14 475 genes. To complement the genome-wide DNA methylation studies, we also performed genome-wide gen expression profiles using the same 18 leiomyoma and matched normal myometrium samples, which were hybridized to the HumanHt-12v3 expression beadchips, targeting more than 25,000 genes. We primarily focused on the inverse association between the differential DNA methylation levels correlating with their respective gene expression profiles. We found that approximately 34 genes are hypermethylated and 10 genes are hypomethylated in leiomyomas compared to normal myometrium. We randomly selected 3 of these hypermethylated genes and validated their methylations status by bisulfite sequencing and their mRNA level by qRT-PCR. Our results clearly demonstrate that there is differential DNA methylation patterns in human uterine leiomyoma tissues compared to matched normal myometrium, and identified a subset of genes whose differential DNA methylation correlates to differential gene expression. Our findings will identify how epigenetics, in particular DNA methylation contributes to the etiology and pathogenesis of leiomyomas, whic is important for the development of novel strategies for treatment and prevention of leiomyomas. Nothing to Disclose: AN, PY, DM, J-JW, SEB

Pub #	P2-70
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Human Polymorphisms, Development & Reproduction (1:30 PM-3:30 PM)
Title	CRH Gene Induction Involves Rapid and Specific CpG Demethylation
Author String	P Xin, C Abou-Seif, M Allars, Y Chen, D Mossman, R Scott, R Nicholson Hunter Medical Research Institute, Newcastle, Australia; Hunter Medical Research Institute, Newcastle, Australia
Body	Background: Corticotropin Releasing Hormone (CRH), is expressed in many regions of the central nervous system, in some peripheral tissues, and it plays an important role in determining gestational length. In placenta, a cAMP regulatory site (CRE) is crucial for CRH gene regulation. The promoter of CRH gene has 9 CpG sites, which should be the targets of epigenetic regulation by DNA methylation. The BeWo cell line, derived from human gestational choriocarcinoma, has been widely used as an in vitro model for the placenta, and cytotrophoblast differentiation. BeWo cells only produce CRH after exposure to cAMP. Preliminary experiments showed that 5-aza-cytidine, a DNA methyl-transferase (DNMT) inhibitor, stimulates CRH expression 2.5-fold in cAMP treated BeWo cells, indicating the CRH promoter as a target of DNMTs. Objective: To evaluate in BeWo cells the methylation differences of the 9 CpG sites in CRH gene promoter and correlate them to cAMP mediated gene induction. Methods: BeWo cells treated or not with cAMP. RNA was isolated and CRH expression was quantified by PCR. Genomic DNA was extracted and sodium bisulfite conversion was used to modify the genomic DNA. PCR was used to amplify the CRH promoter region with primers that did not contain CpG sites. The PCR products were cloned and sequenced. The CpG methylation status of each sample was obtained by comparing the sequencing results with the original sequence. Results: In non-stimulated cells (control) 7 CpG sites were methylated in every clone, CpG -4 was methylated in 50% and CpG -6 was methylated in 75% of the clones. This methylation pattern was also observed after 6 hours of cAMP treatment. However, after 12 hours of cAMP treatment there was complete removal of methylation at CpG-2 (within the CRE) in all of the clones, partial methylation at CpG-1 and 3 (60%), CpG-4 and 5 (40%) and there was 100% methylation at CpG sites 6 through 9. This specific pattern of demethylation around the CRE remained after 24 hours of exposure to cAMP. CRH mRNA began to increase from 12-16 hours following cAMP treatment, correlating perfectly with the timing of CpG methylation removal. These results indicate a direct link exists between expression of the CRH gene and a rapid and specific CpG de-methylation in and around the CRE. Nothing to Disclose: PX, CA-S, MA, YC, DM, RS, RN

Pub #	P2-71
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	Laminarin from *Laminaria digitata* Regulates EGFR Signaling Pathway in Human Colon Cancer Cells
Author String	H-K Park, I-H Kim, J Ryu, Y-H Choi, T-J Nam Pukyong National University, Busan, Korea
Body	The laminarin is found in marine brown algae with potential biological activities. Algae in recent years are highlighted as an anticancer medicine and laminarin is a tropical plant traditionally used Chinese medicine. Laminarin is used as a carbohydrate food reserve in phytoplankton. But laminarin has not been investigated for biological activities. In this study, we examined how laminarin from *Laminaria digitata* regulates HT-29 cell and the influence of laminarin from *Laminaria digitata* on the ErbB signaling pathway. Using the MTS assay, we obtained laminarin from *Laminaria digitata* induced cell death in a dose-dependent manner. The Western blotting revealed that laminarin inhibited HRG-stimulated phosphorylation of ErbB2, ErbB3. Decreased proliferation was dependent on ErbB, which was localized to activated P-JNK and P-Akt. Therefore, these results have important implications for understanding the roles of EGFR in colon cancer cell tumorigenesis. Therefore, laminarin could be a potential source of bio-functional food to have anticancer effect in human colon cancer. Nothing to Disclose: H-KP, I-HK, JR, Y-HC, T-JN

Pub #	P2-72
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	hPTTG Promotes Mitogenic Mechanisms in Thyroid Cells through Autocrine Pathways of Interaction with Growth Factors
Author String	G Lewy, G Ryan, S Stewart, M Read, V Smith, J Fong, A Warfield, M Eggo, R Seed, N Sharma, P Kwan, S Melmed, J Franklyn, C McCabe, K Boelaert University of Birmingham, Birmingham, UK; University of California, Los Angeles, CA
Body	The human Pituitary Tumor Transforming Gene (hPTTG) is overexpressed in thyroid cancers; it induces genetic instability and propagates growth through the induction of growth factors. We investigated the pathways of interaction between hPTTG and epidermal growth factor (EGF), transforming growth factor-α (TGF-α), insulin-like growth factor-1 (IGF-1) and basic fibroblast growth factor (FGF-2) in vitro and in vivo. EGF(5nM), TGF-α(5nM) and IGF-1(10 ng/l) induced hPTTG protein in synchronised K1(2-fold, 3.6-fold and 2.3-fold respectively,p=<0.01) and TPC-1 papillary thyroid carcinoma cells(2-fold, 4-fold and 2.6-fold, p=<0.01), as well as in human primary thyrocytes(3-fold, 2.4-fold and 2-fold,p=<0.01). The effects of EGF and TGF-α on hPTTG expression were abrogated by treatment with MAPK inhibitor PD98059(30[mu]M), bu not with PKC inhibitor BIS-I(50nM), whereas IGF-1-mediated hPTTG induction was reduced following addition of PI3-kinase inhibitors Wortmannin (20[mu]M) and LY294002(50[mu]M). FGF-2(5nM) significantly upregulated hPTTG in TPC-1 cells(7-fold,p=<0.01) but had no effect in K1 cells nor primary thyrocytes. Following transient transfection of primary thyrocytes with hPTTG, we detected increased EGF (1.7-fold,n=4,p=0.004), IGF-1(1.6-fold,n=5,p=0.002) and TGF-α(1.6-fold,n=3,p=0.024) mRNA expression, suggesting induction of growth factors by hPTTG. In vivo evaluation of our transgenic mouse model with thyroid-targeted hPTTG overexpression confirmed increased mEGF(2.7-fold,n=3,p=0.012) and mIGF-1 (2.0-fold,n=3,p=0.02) mRNA when comparing 6-week-old hPTTG+/+ mice to age-matched WT mice. Further, mEGF mRNA expression was downregulated in the thyroids of PTTG-/- knockout mice (0.4-fold,n=4,p=0.001). Unexpectedly, 6-week-old hPTTG mouse thyroids exhibited reduced weights (0.86-fold,p=0.005) and lower cyclin D1 (0.59-fold,p=0.015) and PCNA protein expression(0.61-fold,p=0.07) wher compared with age and gender matched WT mice, in keeping with the reported inhibition of cellular proliferation by high hPTTG expression. Conclusion: hPTTG is involved in autocrine signalling mechanisms with TGF-α, EGF, IGF-1 and FGF-2 in the thyroid, where aberrant control of these pathways may enhance tumour development. Interestingly, hPTTG overexpression simultaneously represses proliferative pathways through inhibition of mitosis. Further elucidation of these complex interactions may provide novel therapeutic targets for the prevention of thyroid tumour growth and progression. Sources of Research Support: Medical Research Council. Nothing to Disclose: GL, GR, SS, MR, VS, JF, AW, ME, RS, NS, PK, SM, JF, CM, KB

Pub #	P2-73
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	IGF-I Proliferative Effects Are Inhibited by Targeting PKC in Human Endocrine Tumor Cells
Author String	D Mole, T Gagliano, E Gentilin, M Bondanelli, F Tagliati, E degli Uberti, MC Zatelli Section of Endocrinology, University of Ferrara, Ferrara, Italy
Body	Insulin-like Growth Factor I (IGF-I) is a well known stimulator of cell proliferation also in the settings of neuroendocrine tumors. Previous evidence has shown that IGF-I stimulates cell proliferation and resistance to pro-apoptotic stimuli both in human pancreatic endocrine tumor (PET) and in medullary thyroid carcinoma (MTC) cell lines and primary cultures. IGF-I signals through many pathways, including protein kinase C (PKC). We here investigate whether PKC may mediate IGF-I proliferative stimuli in two different in vitro models, represented by PET primary cultures and the BON1 cell line and by MTC primary cultures and the TT cell line, by using a novel PKC inhibitor, Enzastaurin. We found that Enzastaurin inhibits IGF-I stimulated cell proliferation at 5 and 10 [micro]M (concentrations reached also at plasma level in human clinical trials) by inducing caspase-mediated apoptosis both in PET-derived and in MTC-derived cells. We found that Enzastaurin also reduces IGF-I stimulated phosphorylation of glycogen synthetase kinase 3 beta (GSK3-β), a downstream target of PKC pathway and a pharmacodynamic marker for Enzastaurin in BON1 and TT cells. These data indicate that in endocrine tumor cell lines Enzastaurin blocks IGF-I induced proliferative stimuli inducing apoptosis, with a mechanism likely involving GSK3β signaling, indicating that PKC plays a crucial role in the control of human neuroendocrine tumor proliferation and survival and that PKC inhibitors may represent a new pharmacological target in neuroendocrine tumors. Disclosures: EdU: Principal Investigator, Pfizer, Inc. Nothing to Disclose: DM, TG, EG, MB, FT, MCZ

Pub #	P2-74
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	Signaling Synergy of Growth Hormone and Insulin-Like Growth Factor-1 and Its Relationship to Glucose in Pancreatic Islet Beta-Cells
Author String	Z Wei, J Lu, J Balducci, Y Huang St Joseph's Hospital and Medical Center, Phoenix, AZ
Body	Type 2 diabetes is characterized by progressive pancreatic beta-cell dysfunction and reduced beta-cell mass. The beta-cell mass that is determined by a dynamic balance of beta-cell growth and death can change in response to metabolic status and insulin demand. Certain nutrients such as glucose, amino acids, and free fatty acids, and hormones/growth factors such as growth hormone (GH) and insulin-like growth factor-1 (IGF-1), play essential roles in regulation of beta-cell proliferation, survival, differentiation, and insulin secretion. However, the GH and IGF-1-mediated signaling pathways and potential crosstalk, especially their relationships to glucose concentrations, remain poorly understood. In this study, we used the rat pancreatic beta-cell line (INS-1), which responses to glucose within the physiologically relevant concentration range (3-15 mM), as a model to explore the GH-IGF-1 signaling crosstalk. Acute stimulation of INS-1 cells with bovine GH, but not IGF-1, caused robust activation of both JAK2 (Janus kinase 2) and STAT5 (signal transducer and activator of transcription 5) in a glucose-dependent manner ($P<0.01$). Although glucose itself activated ERK1/2 (extracellular signal-regulated kinase-1 and 2), IGF-1 (but not GH) further increased the ERK activity in addition to the glucose effect ($P<0.05$). Interestingly, in the absence of exogenous glucose, costimulation with GH and IGF-1 augmented the GH-induced JAK2 activation (pJAK2) and STAT5 phosphorylation (pSTAT5) by 52.4% ($P=0.0001$) and 45.8% ($P=0.0006$), respectively. In contrast, the levels of ERK activation (pERK1/2) were not significantly different when GH and IGF-1 cotreatment was compared with single hormone treatments with or without glucose. Furthermore, we observed synergistic activation of Akt (protein kinase B) by GH and IGF-1 costimulation under glucose-deprivation condition ($P=0.001$ vs. GH alone and $P=0.002$ vs. IGF-1 alone). Taken together, these results suggest previously unappreciated GH and IGF-1 signaling synergy under glucose fasting in the glucose-sensitive INS-1 cells. We are currently investigating the underlying mechanisms and biology consequences of such crosstalk and its precise relationship to glucose, which may have important implications in pathogenesis of type 2 diabetes. Sources of Research Support: American Heart Association (AHA) Beginning Grant-in-Aid Award, Science Foundation Arizona (SFAz) Competitive Advantage Award, and St. Joseph's Foundation (SJF) Startup Fund (to Y.H.). Nothing to Disclose: ZW, JL, JB, YH

Pub #	P2-75
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	Efficacy of an AKT Inhibitor, MK-2206, on Leiomyoma Cell Survival and Proliferation
Author String	E Sefton, Z Lu, J Kim Northwestern University, Chicago, IL
Body	Leiomyomas (uterine fibroids) cause significant morbidity of 25% of women in their 30's. Leiomyomas are the top reason for hysterectomy in pre-menopausal women in the U.S. largely because therapies are limited and unsafe for long term use. Activation of the PI3K/AKT pathway has been associated with tumor growth and AKT has been shown to be highly phosphorylated in leiomyomas. The goal of our research was to pre-clinically test an AKT inhibitor, MK-2206 (MK), for possible treatment of leiomyoma. Treatment of primary leiomyoma cells, primary myometrial cells, and immortalized leiomyoma (DD-HLM), and immortalized myometrial (myo-hTERT) cells with MK-2206 (100nM) for 24 hours decreased levels of phospho(Ser473) -AKT. Furthermore, phosphorylation of PRAS40, a component of the mTORC1 complex, and a direct target of AKT, decreased with MK treatment. Localization of FOXO1 was monitored upon MK treatment since FOXO1 can be phosphorylated by AKT and translocated to the cytoplasm. Accordingly, 24 hour MK treatment increased levels of FOXO1 in the nucleus of primary leiomyoma and myometrial cells. Importantly levels of phospho-SGK1, another ACG family kinase not regulated by AKT, remained constant with MK treatment indicating that MK is specific for AKT. Since activation of the AKT pathway promotes cell proliferation and survival, we tested the effect of MK on leiomyoma cell viability. A dose response (from 50nM to 20 uM) over 48 hrs revealed that MK decreases cell viability of primary leiomyoma and myometrial cells. In summary, these data indicate that MK inhibits AKT in leiomyoma and myometrial cells possibly leading to reduced cell viability. We are currently testing the effects of MK on additional biological outcomes such as cell cycle, proliferation, and apoptosis. We also plan to test MK in-vivo using human leiomyoma tissue in a mouse xenograft model. Further investigation on the efficacy of MK in leiomyomas in vitro and in-vivo will provide the evidence to determine the importance of AKT in leiomyoma survival and growth and may provide a novel therapeutic route. Sources of Research Support: NIH P01HD057877. Nothing to Disclose: ES, ZL, JK

Pub #	P2-76
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	Cell-Specific Effects of IGF-I Receptor Deficiency on Akt Activation and Protection Against Oxidative Stress-Induced Apoptosis
Author String	S Thakur, ML Adamo University of Texas Health Science Center at San Antonio, San Antonio, TX
Body	The antiapoptotic effect of the activated IGF-1R by its ligand IGF-1 is well established pathway for cell survival in a wide array of cell types, mainly mediated through IRS-1/PI3kinase/Akt as downstream regulators. Recent reports suggest existence to alternative pathways for averting apoptosis in addition to primary pathways. Moreover, studies in our lab have demonstrated that *in vivo*, haploinsufficiency of the IGF-1R paradoxically confers resistance to oxidative stress. The current work was undertaken in order to determine the mechanism by which oxidative stress induced apoptosis is altered in IGF-1R deficiency. An siRNA-mediated approach was used to knockdown IGF-1R in C2C12 myoblasts. Treating the IGF-1R deficient myoblasts with H_2O_2 resulted in greater phosphorylation of Akt as compared to cells having normal expression of IGF-1R. Similar results were obtained with UV treatment, another inducer of stress. This enhanced activation of Akt was associated with reduced level of cleaved caspase-3 and PARP. To confirm these results to be cell specific (C2C12), same experiments were repeated in other cell lines, NIH-3T3 fibroblasts and MC3T3 osteoblasts. The loss of IGF-1R by siRNA directed knockdown in these cell lines was associated with reduced level of phosphorylated Akt on treatment with H_2O_2 /UV as compared to control cells. These preliminary results support the notion that reduced levels of the IGF-1R can lead to resistance to oxidative-stress induced apoptosis by enhancing Akt signaling specifically in C2C12 myoblasts. These results suggest a novel mechanism of protection against oxidative stress induced apoptosis and cell death in IGF-1R deficient C2C12 myoblasts. Ongoing work in our laboratory involves further evaluating these findings as well as their mechanism. Sources of Research Support: R01 AG026012 awarded to MLA. Nothing to Disclose: ST, MLA

Pub #	P2-77
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	Minocycline Attenuates *In Utero* Cocaine Exposure[en dash]Induced Fetal Cardiac Myocyte Apoptosis through Inhibition of Oxidative Stress, JNK and p38 MAPK-Mediated Mitochondria-Dependent Death Pathway
Author String	M Mangubut, AP Sinha Hikim, R Shen, SK Mahata, I Sinha-Hikim Charles R Drew University, Los Angeles, CA; University of California, La Jolla, CA
Body	**Background and Objective:** Abuse of cocaine during pregnancy exposes ~ 100,000 infants per year to cocaine in the United States. Cocaine readily crosses the placenta into the fetal circulation affecting the placenta and fetus with numerous adverse outcomes, including fetal cardiac myocyte death. This study investigates the molecular mechanisms by which minocycline, a second generation tetracycline, prevents cardiac myocyte death induced by in utero cocaine exposure. **Experimental Design:** Timed mated pregnant Sprague-Dawley (SD) rats received one of the following treatments twice daily from embryonic (E) day 15 to 21 (E15-E21): i) intraperitoneal (IP) injections of saline (control); ii) IP injections of cocaine (15 mg/kg BW); and iii) IP injections of cocaine + oral administration of 25 mg/kg BW of minocycline. Pups were killed on postnatal day 15 (P15). Additional pregnant dams received twice daily IP injections of cocaine (from E15-E21) + oral administration of a higher (37.5 mg/kg BW) dose of minocycline. Minocycline treatment continued from E15 until the pups were sacrificed on P15. **Results:** In utero cocaine exposure resulted in an increase in oxidative stress and fetal cardiac myocyte apoptosis through activation c-Jun-NH_2-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK)-mediated mitochondria-dependent apoptotic pathway. Continued minocycline treatment from day E15 through P15 significantly prevented oxidative stress, kinase activation, perturbation of BAX/BCL-2 ratio cytochrome c release, caspase activation, and attenuated fetal cardiac myocyte apoptosis after prenatal cocaine exposure. **Conclusions:** These results show *in vivo* cardioprotective effects of minocycline in preventing fetal cardiac myocyte death after prenatal cocaine exposure. Minocycline with its proven clinical safety and its ability to cross placental barrier and enter into the fetal circulation may become an effective therapy for preventing cardiac consequences of *in utero* cocaine exposure. Sources of Research Support: NIH R24DA017298-01, MIDARP. Nothing to Disclose: MM, APSH, RS, SKM, IS-H

Pub #	P2-78
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	Inhibition of Inducible Nitric Oxide Synthase Suppresses Monosodium Urate Crystal-Induced Gouty Inflammation in Mice
Author String	S-Y Park, T-J Ju, K-C Won, H-J Kim, E-G Hong College of Medicine, Yeungnam University, Daegu, Korea; College of Medicine, Yeungnam University, Daegu, Korea; EulJi University College of Medicine, Seoul, Korea; Hallym University College of Medicine, Seoul, Korea
Body	In the present study, we examined the effect of inhibition of inducible nitric oxide synthase (iNOS) on gouty inflammation in mice. Gouty inflmmation was induced by injection of monosodium urate crystal (MSU) into the both sole of C57BL/6J mice feet (4 mg/50 ml). The iNOS-specific inhibitors L-N(6)-(1-iminoethyl)lysine (L-NIL, 10 mg/kg/day) was administered intraperitoneally before injection of MSU. The thickness of foot wa measured with digital caliper as indicator of inflammatory edema. MSU injection significantly increased the thickness of feet and the gene expression of tumor necrosis factor-a (TNF-a) and interleukin 1-b (IL-b) indicating that MSU induced inflammatory edema. L-NIL treatment partially reduced feet thickness and the gene expression of TNF-a and IL-b suggesting MSU-induced inflammatory edema was suppressed by the inhibition of iNOS. MSU increased the gene expression and protein level of iNOS, the NO metabolites nitrate and nitrite, and nitrotyrosin level in mice feet and the L-NIL treatment attenuated them. The phosphorylation of ERK and p38 were increased by MSU and L-NIL treatment reduced the phosphorylation of both ERK and p-38. These results suggest that inhibition of iNOS attenuated MSU-induced inflammation and ERK and p38 may be involved. Nothing to Disclose: S-YP, T-JJ, K-CW, H-JK, E-GH

Pub #	P2-79
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	Green Tea (-)-Epigallocatechin Gallate Inhibits IGF-II Stimulation of 3T3-L1 Preadipocyte Mitogenesis Via the 67-Kilodalton Laminin Receptor, but Not AMP-Activated Protein Kinase, Pathways
Author String	H-C Ku, Y-W Tsuei, C-C Kao, C-H Huang, H-S Liu, L-J Shih, C-M Lin, C-L Lin, H-H Chang, C-W Liu, P-F Hung, Y-H Kao National Central University, Jhong-li, Taiwan; Armed Forces Tao-Yuan General Hospital, Taoyuan, Taiwan; Cathay General Hospital, Taipei, Taiwan
Body	Insulin-like growth factors (IGFs) and (-)-epigallocatechin gallate (EGCG) have been reported to regulate fat cell mitogenesis and adipogenesis. This study investigated the pathways involved in EGCG modulation of IGF-II-stimulated mitogenesis in 3T3-L1 preadipocytes. EGCG inhibited IGF-II stimulation of preadipocyte proliferation in a dose- and time-dependent manner. EGCG suppressed IGF-II-stimulated phosphorylation of the p66Shc and MAPK pathway proteins, RAF1, MEK1/2, and ERK1/2, but not JNK, AKT, p52Shc, or p46Shc. Furthermore, EGCG did not alter levels of total IGF-II receptor in the presence and absence of IGF-II, but inhibited the IGF-II-stimulated association of IGF-II receptor with G_{ai-2} protein. These data suggest that EGCG mediates anti-IGF-II signals and selectively affects particular types of Shc and MAPK family members. Generally, EGCG was more effective than epicatechin, epicatechin gallate, and epigallocatechin in modulating IGF-II-stimulated mitogenic signaling. We have previously identified the EGCG receptor (also known as the 67-kilodalton laminin receptor; 67LR) in fat cells and reported herein that pretreatment of preadipocytes with 67LR antiserum prevented the effects of EGCG on IGF-II-stimulated phosphorylation of ERK1/2 and preadipocyte proliferation (cell number and bromodeoxyuridine incorporation). Although EGCG alone stimulated phosphorylation of preadipocyte AMPK, the enzyme inhibitor compound C did not block the anti-IGF-II effects of EGCG. These data suggest that EGCG mediates anti-IGF-II signals in preadipocyte mitogenesis via the 67LR, but not AMPK, pathways. Nothing to Disclose: H-CK, Y-WT, C-CK, C-HH, H-SL, L-JS, C-ML, C-LL, H-HC, C-WL, P-FH, Y-HK

Pub #	P2-80
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	Regulation of 11β-Hydroxysteroid Dehydrogenase Type 1 by NF[kappa]B in Stromal Cells: Is Tissue-Specific Enzyme Inhibition Possible?
Author String	M Ahasan, C Jones, R Hardy, Z Hassan-Smith, G Lavery, EH Rabbitt, CD Buckley, K Raza, PM Stewart, MS Cooper University of Birmingham, Birmingham, UK; University of Birmingham, Birmingham, UK
Body	The 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) enzyme is a tissue specific regulator of glucocorticoid action that is linked to the development of osteoporosis, inflammatory arthritis and myopathy. Systemic inhibition of 11β-HSD1 enzyme activity has been proposed as a therapeutic option in these conditions. However, 11β-HSD1 activity has also been reported to be important in regulation of the immune response and the HPA axis. We have previously demonstrated that proinflammatory cytokines, glucocorticoids and their combination dramatically increase 11β-HSD1 expression and activity in osteoblasts, synovial fibroblasts and myoblasts. The mechanisms underlying this increase in activity are unknown. We have now examined the mechanisms underlying the regulation of 11β-HSD1 activity in stromal cells. Regulation of activity was not due to any previously described mechanism. The increase in activity induced by TNFα/IL-1β was independent of new protein synthesis. 5' RACE analysis and luciferase gene reporter studies demonstrated that this effect was mediated via the classical proximal HSD11B1 promoter. Chemical inhibitor studies demonstrated that this increase in activity, along with basal enzyme activity, was mediated via the NF-[kappa]B pathway. Furthermore 11β-HSD1 expression in mouse embryonic fibroblasts (MEFs) from mice with targeted disruption of canonical NF-[kappa]B activation (RelA-/-) failed to respond to TNFα in contrast to wildtype or MEFs with alternate NF-[kappa]B inactivation (RelB-/-. Unexpectedly, the induction of 11β-HSD1 by TNFα/IL-1β treatment (but not basal 11β-HSD1 activity) was increased in the presence of inhibitors of the p38MAPK pathway. This suggested that TNFα/IL-1β induced activation of the p38MAPK pathway resulted in an inhibition of 11β-HSD1 expression. Glucocorticoids had a minor inhibitory effect on NF-[kappa]B nuclear translocation and induction of 11β-HSD1 but appeared to have their stimulatory effect on 11β-HSD1 expression through inhibition of TNFα/IL-1β induced p38MAPK activity. The mechanism by which expression of 11β-HSD1 is regulated in stromal cells appears distinct from that reported in non-stromal cells such as hepatocytes. The finding that stromal cell expression of 11β-HSD1 activity is regulated by NF-[kappa]B opens up new opportunities to inhibit 11β-HSD1 activity in a tissue restricted manner. Nothing to Disclose: MA, CJ, RH, ZH-S, GL, EHR, CDB, KR, PMS, MSC

Pub #	P2-81
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	Increased Fibroblast Growth Factor 21 Expression Causes Growth Inhibition Directly at the Growth Plate
Author String	A Levenson, S Wu, A Kharitonenkov, F De Luca St Christopher's Hospital for Children, Philadelphia, PA; Drexel University College of Medicine, Philadelphia, PA; Lilly Research Laboratories, Indianapolis, IN
Body	Background: Fibroblast Growth Factor 21 (FGF21), which is induced in the liver during fasting, functions as a potent regulator of glucose and lipid metabolism. Previous evidence indicates that transgenic mice overexpressing FGF21 exhibit reduced growth and impaired Growth Hormone (GH) action in the liver. In light of the inhibitory effects on growth plate chondrogenesis mediated by other FGFs, we hypothesized that FGF21 may cause growth inhibition by acting directly at the long bones' growth plate. Results: In chondrocytes isolated from fetal (dpc 20) mouse metatarsal growth plates, we first demonstrated mRNA and protein expression (by real-time PCR and Western, respectively) of FGF21, b-klotho and Fibroblast Growth Factor Receptor 1 (FGFR1) (b-klotho and FGFR1 are the cell-surface co-receptor and one of the receptors mediating FGF21 signaling, respectively). We then cultured mouse growth plate chondrocyte in the absence or in the presence of graded concentrations of rhFGF21 (1-5-10 ng/ml), with or without rhGH (10 ng/ml). The highest FGF21 concentration (10 ng/ml) inhibited chondrocyte thymidine incorporation ($p<0.01$ vs. control) and collagen X mRNA expression (by real-time PCR, $p<0.05$ vs. control). 10 ng/ml FGF21 also induced Erk1/Erk2 phosphorylation (by Western), FGFR1 and b-klotho mRNA expression ($p<0.05$ vs. control) in chondrocytes. In addition, the stimulatory effects of GH on chondrocyte thymidine incorporation and collagen X mRNA expression ($p<0.01$ vs. control) were significantly reversed by co-culturing chondrocytes with GH and FGF21 (thymidine incorporation: GH + 1 ng/ml FGF21 vs. GH, $p<0.05$; GH + 5 ng/ml FGF21 or GH + 10 ng/ml FGF21 vs. GH, $p<0.01$. Collagen X mRNA expression: GH + 5 ng/ml FGF21 or GH + 10 ng/ml FGF21 vs. GH, $p<0.01$). The GH-mediated induction of Stat5 phosphorylation in chondrocytes was reversed by the addition of FGF21 in the culture medium in a concentration-dependent manner. Conclusion: High concentrations of FGF21 directly inhibit mouse growth plate chondrocyte proliferation and differentiation by activating the FGF21 signaling pathway. In addition, FGF21 antagonizes the direct GH stimulatory effects on chondrocyte proliferation and differentiation. Thus, our findings suggest that increased expression of FGF21 during fasting/starvation may lead to the attenuation of growth by a dual mechanism: by antagonizing GH action both in the liver and in the growth plate, and by directly inhibiting growth plate chondrogenesis. Nothing to Disclose: AL, SW, AK, FDL

Pub #	P2-82
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	The Inflammatory and Angiogenic Properties of Chemerin: Implications for the Metabolic Syndrome
Author String	J Kaur, J Chen, R Adya, H Lehnert, HS Randeva University of Warwick (UoW), West Midlands, UK; Medical School, Lübeck, Germany
Body	Chemerin, a novel adipokine, circulates at high concentrations in inflammatory and obese states, and acts *via* its distinct G protein-coupled receptor, denoted as CMKLR1. Circulating chemerin levels are associated with various facets of the metabolic syndrome including impaired glucose tolerance, insulin resistance, central obesity, dyslipidaemia and hypertension. These dysfunctional metabolic states are associated with an increased incidence of cardiovascular diseases (CVDs), dysregulated angiogenesis and inflammation. However, little is known of the mechanistic links between chemerin and dysregulated angiogenesis, in particular inflammation. We therefore sought to investigate the inflammatory and angiogenic properties of chemerin in human endothelial cells (HEC). We were able to demonstrate the presence of CMKLR1 in HEC at both gene and protein level. Under pro-inflammatory conditions, after stimulation with TNF-a, IL-6 and IL-1β, we observed CMKLR1 expression to increase significantly. More importantly, chemerin exhibited pro-inflammatory behaviour by promoting endothelial cell migration, proliferation and capillary tube formation; critically controlled processes of angiogenesis in normal and pathophysiological conditions. We were able to further extend our novel observations by showing that chemerin enhanced NF-[kappa]B activity in a concentration-dependent manner, a key transcription factor which plays a major role in the process of inflammation; NF-[kappa]B activity increased synergistically when chemerin was co-incubated with IL-1β in HEC. Furthermore, chemerin significantly increased protein expression levels and secretion of cell adhesion molecules, including Intracellular Cell Adhesion Molecule (ICAM)-1, Vascular Endothelial Cells (VCAM)-1 and E-selectins, all critical for inflammatory and angiogenic processes. Investigating the molecular signalling pathways involved in these processes, we were able to show that chemerin activated the PI3K/Akt pathway in a concentration-dependent manner. Chemerin also activated key MAPKs pathways, including ERK1/2, ERK 5, p38 and JNKs, the latter being the key regulator of inflammation and insulin resistance. In summary, our novel findings provide a mechanistic link between chemerin and inflammation/angiogenesis processes crucial in human obesity and related complications. Nothing to Disclose: JK, JC, RA, HL, HSR

Pub #	P2-83
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	Grass Carp SOCS and CISH: Molecular Cloning, Functional Characterization, and Regulation of Transcript Expression in Grass Carp Hepatocytes
Author String	X Jiang, W Wong, A Wong The University of Hong Kong, Hong Kong, China
Body	Suppressors of cytokine signaling (SOCS) are intracellular feedback inhibitors for signal transduction couplec to cytokine receptors. To date, seven members of the SOCS family have been identified, including SOCS1 to SOCS7 and their functional homologue cytokine-inducible SH2 domain-containing protein (CISH). Although the functional role of individual members of the SOCS/CISH family has been studied extensively in mammals, not much is known regarding the structure, function and regulation of SOCS and CISH in lower vertebrates, including fish models. Recently, using grass carp as an animal model, we have shown that SOCS1 can down-regulate growth hormone (GH) receptor signaling via the JAK2/STAT5 pathway. To further examined the role of other members of the SOCS/CISH family in GH signaling, the structural identities of grass carp SOCS1, SOCS2 and CISH were established by 5'/3' RACE and their protein sequences deduced from cDNAs obtained were found to be highly homologous to their zebrafish counterparts, especially in SOCS box and SH2 domain. Using Southern blot, SOCS1, SOCS2 and CISH were confirmed to be single copy genes in the carp genome. Unlike SOCS2, which was detected a tissue-specific manner, SOCS1 and CISH were shown to be widely expressed in various tissues. Similar to SOCS3, mRNA expression of SOCS1, SOCS2 and CISH were consistently located in the carp liver. Functional study of these newly cloned SOCS/CISH cDNAs in CHO cells also confirmed that STAT5-responsive promoter activation induced by GH treatment or STAT5b expression could be suppressed by over-expression of grass carp SOCS1, SOCS2 and CISH, respectively. In primary cultures of carp hepatocytes, TNFα treatment was effective in increasing SOCS1, SOCS3 and CISH, but not SOC2 mRNA levels. Transcript expression of SOCS1, SOCS2, SOCS3 and CISH, however, could all be elevated in parallel experiments with GH stimulation. Our results, taken together, provide evidence that the members of SOCS/CISH family, including SOCS1, SOC2 and CISH, are expressed in the carp species. At the hepatic level of carp model, TNFα and GH induction can up-regulate gene expression of these SOCS/CISH family members, which may interfere GH signaling by inhibiting JAK2/STAT5 pathway. Sources of Research Support: Grants from Research Grant Council, Hong Kong. Nothing to Disclose: XJ, WW, AW

Pub #	P2-84
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	Multi-Targeted Activity of the HSP90 Inhibitor Ganetespib (STA-9090) in Prostate Cancer Cells
Author String	S He, Y Wada, DA Proia Synta Pharmaceuticals, Lexington, MA
Body	Heat Shock Protein 90 (Hsp90) is emerging as an important target in cancer therapy because its inactivation results in the simultaneous blockade of multiple oncogenic signaling pathways and sensitizes cancer cells to other chemotherapeutic agents. Ganetespib (formerly STA-9090), a second generation Hsp90 inhibitor, is in numerous Phase II trials across a broad range of indications, including stage IIIB and IV non-small cell lung cancer where it has demonstrated encouraging clinical activity and is well tolerated. In light of this, we sought to determine the potency of ganetespib in prostate cancer (PCa) cells given the importance of several Hsp90 client proteins in mediating PCa progression. We examined the effectiveness of ganetespib or the first generation Hsp90 inhibitor 17-AAG in both hormone-dependent (LNCaP) and hormone-independent (PC-3, DU-145) PCa cell lines. Ganetespib displayed low nanomolar activity regardless of the cell's AR status, with IC50's 3-7 fold less than 17-AAG. In the treated cultures, ganetespib increased the population of apoptotic (Annexin V positive) cells, whose appearance paralleled the dose-dependent degradation of the anti-apoptotic protein Mcl-1. In all of the cell lines, the master cell cycle regulator Cdk1 and the DNA damage checkpoint protein Chk1 were completely destabilized by ganetespib exposure. This led to the cells arresting in G2/M. Interestingly, expression of a distinct isoform of Chk2 was enhanced in response to the down-regulation of Chk1, suggesting a potential feedback loop. We also evaluated the stability of several client proteins (AR, IGF-1R, EGFR, RAF1 and JAK2) and their effectors responsible for PCa progression in response to ganetespib and observed significant degradation/inactivation, albeit with variable kinetics. In conclusion, ganetespib is a highly potent Hsp90 inhibitor that displays preclinical activity in a panel of prostate cancer cel lines due to its ability to target the key signaling components required for PCa cell growth, survival and cell division. Thus, further investigation of ganetespib as a new treatment for patients with prostate cancer is warranted. Nothing to Disclose: SH, YW, DAP

Pub #	P2-85
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	Cofilin Phosphorylation Regulates Prostate Cancer Cell Adhesion, Migration and Invasion Independently of TGF-β Signaling
Author String	J Collazo, N Kyprianou University of Kentucky, Lexington, KY
Body	The actin depolimerizing factor (ADF) cofilin, a small (15-18KD) actin binding protein have been shown to be an intracellular effector of transforming growth factor beta (TGF-β) signaling. Cofilin and its signaling pathway effectors have been identified as one of the proteins involved in the early steps in cell motility by promoting actin depolimerization. Cofilin is regulated by phosphorylation of a Serine residue at position 3 which inhibits actin binding and depolimerization. This study investigated the impact of a mutation in cofilin phoshorylation site (Serine 3 residue; S3ACFL) in the human androgen-independent prostate cancer cells, PC-3 that overexpress TGF-β. We found significant differences in migration, invasion and attachment potential between wild type and S3ACFL expressing PC-3 cells. After 24 hours of seeding, a wounding assay demonstrates an increase in migration potential for S3ACFL compared to wild type. There was s significant decrease in the invasion ability using the matrigel invasion chamber for S3ACFL compare to wild type. Cell attachment assays for each of the cell lines showed a lower percent of S3ACFL cell attachment on fibronectin and collagen compare to wild type. These results demonstrate that cofilin is functionally involved in prostate cancer cell motilility and invasion and in prostate cancer cell migration independently of TGF-β. The identification of a role for cofilin as a determinant of prostate tumor cell aggressive behavior provides new insights into the molecular pathways contributing to the initiation and promotion of prostate tumor progression to metastasis in the context of actin reorganization and dynamic of the tumor microenvironment. Sources of Research Support: NIH/NCI Grant #R01 CA10757. Nothing to Disclose: JC, NK

Pub #	P2-86
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	The Breast Cancer-Predisposing Fibroblast Growth Factor Receptor 2 Governs Epithelial-Mesenchymal Interactions
Author String	C Wei, C Cassol, W Liu, SL Asa, S Ezzat Ontario Cancer Institute, Toronto, Canada; Ontario Cancer Institute, Toronto, Canada
Body	**Background & Rationale:** Genome-wide association studies have recently identified FGFR2 as one of few genes linked with increased breast cancer risk. We reported that FGFR2 levels are down-regulated in micro-dissected primary human breast cancer cells when compared with normal epithelium. However, there is limited knowledge of the role of FGFR2 in breast cancer initiation and/or progression. **Methods & Results:** Breast cancer MDA-MB-231 and the near normal breast epithelial MCF-10A cells were used for retroviral transduction of FGFR2-IIIb and its alternatively spliced FGFR2-IIIc variant. Unexpectedly we found that both FGFR2 isoforms blocked cell proliferation, reduced cell invasion in matrigel, and inhibited anchorage-independent growth in soft agar. Expression of markers of epithelial to mesenchymal transition (EMT) including E-cadherin and Snail1 supported this growth arrest in response to FGFR2. Similarly, FGF stimulation revealed decreased phosphorylation of classical targets including ERK1/2 and STAT3 in response to FGFR2. Consistent with these findings, lung metastases were limited by FGFR2 in orthotopic xenograft mouse models. To better understand the mechanisms underlying these actions we performed mammary morphogenesis assays. MCF-10A cells underwent proliferation arrest with development of 3D structures resembling breast acini in the presence of forced FGFR2 expression. As tumor stroma facilitates growth progression through crosstalk with neoplastic epithelial cells we performed heterologous orthotopic xenografts. We found that reduced tumor growth was not only due to the growth suppressive effect of FGFR2 on neoplastic breast epithelial cells but also due to the suppressive actions of FGFR2-IIIc on tumor-associated fibroblasts. Immunoprecipitates from FGFR2-IIIb and IIIc-expressing cells were subjected to Mass Spectrometry for identification of novel interacting partners. Current studies are underway to validate differentially-expressed proteins. **Conclusion & Future Prospect:** In contrast to the anticipated oncogenic actions of FGFR2, our studies provide strong evidence for the FGFR2-IIIb isoform as tumor protective. The extracellular splice variant FGFR2-IIIc displays a more significant effect on tumor stroma. Validation of the differential partners engaged by FGFR2 isoforms will be critical in deciphering this gene's functions in breast cancer initiation and progression. Sources of Research Support: Canadian Institutes of Health Research. Nothing to Disclose: CW, CC, WL, SLA, SE

Pub #	P2-87
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	Evidence That Leptin Induces HER2 Expression and Enhances the Stem Cell Population in Breast Cancer
Author String	C Giordano, D Vizza, I Barone, ML Panno, P Rizza, D Bonofiglio, S Ando, S Catalano University of Calabria, Arcavacata di Rende, Italy; University of Calabria, Arcavacata di Rende, Italy; University of Calabria, Arcavacata di Rende, Italy
Body	Obesity, a well known risk factor for postmenopausal breast cancer, is associated with development of more aggressive tumors. Several reports suggest that the hormone leptin, mainly secreted by adipocytes, acting independently or modulating other signaling pathways is involved in the etiopathogenetic mechanisms by which obesity influences breast cancer risk and prognosis. The HER2/Neu (ErbB2) receptor, a ligand-less receptor overexpressed in 25% to 30% of human breast cancers, has been clinically associated with aggressive metastatic diseases. Moreover, recent studies reported that HER2 overexpression modulates mammary tumorogenesis and invasion through its ability to increase malignant mammary stem cells population. Here, we evaluated, for the first time, whether leptin can modulate the expression of HER2 in MCF-7 human breast cancer cells, thus affecting the biology of breast cancer stem cells. Immunoblotting analysis revealed that treatment with leptin increased HER2 protein levels in a time and dose dependent manner. As expected, leptin treatment induced HER2 signaling pathway activation evidenced by an increase in the phosphorylation status of HER2 and its downstream effectors MAPK and AKT. However, leptin treatment was not able to modulate HER2 mRNA levels evaluated by real-time PCR at all times and doses investigated, suggesting that leptin-induced HER2 expression could be related to posttranscriptional mechanisms such as an enhanced protein stability or a reduced protein degradation. To investigate the long-term effect of leptin on MCF-7 breast cancer cells growth we performed anchorage-independent soft agar growth assays and found a significant increase in the colony numbers after 14 days of incubation with leptin. Finally, we tested for the effects of leptin on mammospheres formation efficency, as index of stem cells population, in consecutive passages. In the presence of leptin MCF-7 cells had a 1.86 fold higher spheres forming efficiency (%SFE) then vehicle treated cells. Upon passage, we found that leptin significantly increased secondary mammospheres formation as revealed by a 6-fold enhancement in relative %SFE. Collectively these data indicate that leptin singnaling induces HER2 expression and modulates the mammary stem-progenitor cell population, providing further insights into the crucial role of leptin in mammary tumorogenesis and tumor aggressiveness. Nothing to Disclose: CG, DV, IB, MLP, PR, DB, SA, SC

Pub #	P2-88
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	Embryonic Polyadenylation Binding Protein (ePABP) Mediates Nongenomic Steroid-Induced Oocyte Maturation by Interacting with Paxillin to Promote Activation of the MOS-MEK-Erk2 Pathway
Author String	F Caiazza, M Rasar Young, SR Hammes University of Rochester Medical Center, Rochester, NY; Yale School of Medicine, New Haven, CT
Body	Our laboratory uses *Xenopus laevis* oocytes as a model for studying transcription-independent steroid-mediated molecular signaling. Immature oocytes are arrested at the prophase I stage of meiosis until prior to ovulation, when gonadotropin-induced signals trigger them to re-enter the meiotic cycle and progress to metaphase II, at which point they are competent for ovulation and fertilization. Interestingly, steroids, specifically androgens, are the direct triggers of *Xenopus* oocyte maturation both *in vitro* and *in vivo*, acting through the classical androgen receptor (AR) via a [ldquo]release of inhibition[rdquo] mechanism whereby constitutive G protein signals that normally stimulate adenylyl cyclase are inhibited. This results in a rapid drop in intracellular cAMP, followed by increased expression of MOS, a constitutively active germ cell equivelent of Raf. MOS then activates the MAPK pathway, eventually leading to cyclin activation and meioti progression. How MOS expression is increased in response to steroid signaling is still unclear, but appears to involve polyadenylation of existing *Mos* mRNA, which somehow leads to increased translation of MOS protein. Our laboratory has made significant progress in this light, demonstrating that the scaffold molecule paxillin is required for MOS protein translation but not *Mos* mRNA polyadenylation. Here we propose that paxillin facilitates the binding of polyadenylation binding protein (PABP) to the 3' poly-A tail of mature *Mos* mRNAs to regulate their translation. In support of this hypothesis, paxillin binds to PABP in somatic cells, and PABP enhances translation in frog oocytes. Furthermore, we find that knockdown of PABP expression in *Xenopus* oocytes reproduces the phenotype observed for Paxillin knockdown, with normal androgen-triggered *Mos* mRNA polyadenylation but decreased MOS protein expression. Finally, we find that testosterone promotes binding of PABP to *Mos* mRNA in a time-dependent fashion. We propose a model whereby paxillir assists in targeting PABP to polyadenylated *Mos* mRNA, thus promoting MOS protein translation. MOS accumulation ultimately results in the activation of downstream kinase signaling and subsequent oocyte maturation. Nothing to Disclose: FC, MRY, SRH

Pub #	P2-89
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	May Tumor Microenvironment Cooperate with a Mutant ERα To Promote Breast Cancer Progression?
Author String	I Barone, L Gelsomino, S Panza, C Giordano, S Marsico, D Bonofiglio, SAW Fuqua, S Catalano, S Ando University of Calabria, Arcavacata di Rende, Italy; University of Calabria, Arcavacata di Rende, Italy; University of Calabria, Arcavacata di Rende, Italy; Baylor College of Medicine, Houston, TX
Body	Surrounding stromal cells and adipokines, as leptin, strongly influence the phenotypic behavior of malignant breast epithelial cells and elevated serum levels of leptin in obesity is a risk factor for breast cancer incidence, mortality and drug resistance. We identified a lysine to arginine transition at residue 303 (K303R) within estrogen receptor (ERα) in premalignant and invasive breast cancers, which confers estrogen hypersensitivity and resistance to hormone therapy. Microarray analysis showed increased leptin receptor mRNA levels in K303R-expressing MCF-7 breast cancer cells. We hypothesized that K303R ERα, as an amplified effector of leptin signaling, may potentiate tumor/stroma microenvironment stimulatory effects on breast cancer progression. We used as experimental models for breast cancer ERα-positive MCF-7 and ERα-negative SKBR3 cells stably transfected with wild-type (WT) or K303R ERα and for stromal cells primary fibroblasts isolated from human breast carcinoma (CAFs), that we showed secreting leptin. We found, in mutant cells, an increase in leptin receptor isoforms, and in its signalling activation. Leptin also enhanced phosphorylation and transcriptional activity of K303R ERα. Leptin treatment 100-1000ng/ml increased anchorage-dependent and independent cell growth, and in vitro cel motility and invasiveness, evaluated by scratch, migration and matrigel invasion assays, in WT and K303R clones, but in higher extent in mutant cells. Low physiological leptin doses had effects only in K303R clones. The JAK2/STAT3 inhibitor AG490 and the ER antagonist ICI 182,760 abrogated these effects. Conditioned medium (CM) isolated from CAFs was more effective in stimulating proliferation and migration of both MCF-7 and SKBR3 K303R ERα cells compared to WT cells. AG490 and ICI reversed these effects. To specifically define leptin contribution, we immunodepleted leptin from stroma-derived CM using specific antibodies and found a reduction on proliferation and migration especially in mutant cells. Understanding the molecular dynamics of stroma-tumor networks may help to improve decision-making for some ER-positive breast cancer patients. Since the K303R mutation was identified in 30% of typical hyperplasia, we could speculate that the pressure of the microenvironment in the presence of this mutation hypersensitive to leptin signaling may promote or accelerate the development of cancers from premalignant breast lesions, further increasing risk in obese women. Sources of Research Support: AIRC grants 2010 and 2011. Nothing to Disclose: IB, LG, SP, CG, SM, DB, SAWF, SC, SA

Pub #	P2-90
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	Ghrelin: Effects and Mechanisms of Action in Tumor-Induced Cachexia
Author String	MS Mendiratta, SM Patel, JM Garcia Baylor College of Medicine, Houston, TX; Baylor College of Medicine, Houston, TX; Ross University, North Brunswick, NJ
Body	**Background:** Cachexia is characterized by loss of adipose tissue and skeletal muscle. The current therapy is limited, associated with side effects and does not improve lean body mass (LBM). Cachexia in carcinoma is initiated by catabolic factors that cause muscle atrophy and breakdown of fat. Atrogin-1 and MuRF1 (muscle RING finger protein 1) are muscle specific ubiquitin ligases that are activated by TNF-α via activation of transcription factor NF[kappa]B. IGF-1 decreases proteolysis by down regulating atrogin-1 and MuRF1 expression via inhibition of FoxO but its levels are decreased in cachexia. Ghrelin is the natural ligand for GHS-R and thus stimulates GH release and indirectly increases IGF-1 levels. It has an orexigenic effect and suppresses the expression of cytokines. **Aim:** To determine the mechanism of action and the effects of ghrelin on muscle mass regulation in cancer-induced cachexia. **Methods:** Transgenic adult male mice (NGL WT) that express luciferase and GFP (green fluorescent protein) with NF[kappa]B activity were injected with LLC (Lewis lung carcinoma) cells or heat killed cells SQ. Once a tumor developed, Ghrelin or saline was injected intra-peritoneally twice daily and the mice were sacrificed after 3 weeks. Outcome measures were compared between three groups of mice: tumor + ghrelin (T+G), tumor + vehicle (T+V) and sham + vehicle(S+V). **Results:** Tumor-bearing mice developed significantly decreased fat mass, decreased gastrocnemius muscle mass and decreased grip strength. Ghrelin prevented the loss of fat mass in tumor mice and also helped preserve grip strength and maintain their gastrocnemius muscle mass. Lewis Lung carcinoma decreased AKT and p70s6k phosphorylation although the differences did not reach significance. Although FoxO1-3a was not affected by LLC, it was significantly increased by ghrelin. Ghrelin did not increase tumor size or time to progression in this model. **Conclusion:** Ghrelin prevents cancer-induced cachexia, muscle atrophy and weakness by preventing proteolysis and without affecting tumor growth. Sources of Research Support: Dr. Garcia is a consultant for Novo Nordisk and Bayer and receives research support from Aeterna Zentaris, Abbott and Helsinn therapeutics. Dr. Garcia's work is also supported by a MERIT grant from the Dept of VA and the Caroline Wiess Law Fund for Molecular Medicine. Nothing to Disclose: MSM, SMP, JMG

Pub #	P2-91
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	Cytokine Profiling in Type 2 Diabetes
Author String	S Gupta, A Maratha, A Natarajan, J Siednienko, T Gajanayake, S Hoashi, S Miggin Midland Regional Hospital, Mullingar, Ireland; National University of Ireland, Maynooth, Ireland; Midland Regional Hospital, Mullingar, Ireland
Body	Inflammation is a component of obesity-associated insulin resistance & type 2 diabetes (T2D) & significant increases in inflammatory mediators have been detected in serum of diabetics. We sought to determine the serum cytokines in 146 subjects: non diabetic controls (NGT: n=33, BMI 27.3±3.6 kg/m^2, HbA1c 5.5±0.2%) & compare them with diabetics with four different profiles: 1. T2D with good glycemic control & no complications (GC: n=30, BMI 32.5±6.2 kg/m^2, HbA1c 6.4±0.6%), 2. T2D with good glycemic control & complications (GCC: n=30, BMI 33.3±5.5 kg/m^2, HbA1c 6.8±0.6%), 3. T2D with poor glycemic control and no complications (PG: n=22, BMI 33.4±6.6 kg/m^2, HbA1c 10.2±0.1.5%), 4. T2D with poor glycaemic contro & complications (PGC: n=31, BMI 34.3±5.7 kg/m^2, HbA1c 9.9±0.1.2%), by multiplex cytokine profiling. Our data clearly shows that serum IL-12p70 levels are significantly suppressed in all four subgroups of T2D patients when compared to normal individuals. The units are expressed as (Mean (pg/ml) ± SEM) for the cytokine levels in the order of NGT (15.9± 3.9), GC(7.8±1.4), GCC (6.9± 2.2) , PG (6.6±1.5), PGC (7.2±1.1 & significant suppression denoted as NGT vs GC=*($p<0.03$), GCC= ** ($p<0.002$), PG= *($p<0.01$) & PGC= * ($p<0.02$). No difference was evident in IL-6, TNFα levels between the groups and a slight suppression is observed in the serum IL-1β levels in NGT vs GCC (NGT: 5.8±0.9, GCC: 3.6±0.6)*($p<0.03$). In addition PG showed significantly elevated IFNβ (334.6±157.9 *, $p<0.05$) and Rantes (282300±101300, **$p<0.004$) compared to NGT (IFNβ: 23.9±17.1*, Rantes: 186100±48330*). Interestingly IFNγ is significantly suppressed in all T2 diabetics only with complications when compared to NGT (PGC: 5.4±0.6; * $p<0.05$ & GCC: 4.9±0.6; * $p<0.02$) and no significant suppression observed in the T2D patients without complications (GC: 6.3±0.7; & PG: 5.7±1.0). Thus we conclude that 1. Low levels of IL12p70 in T2 diabetics may signify impaired immune state irrespective of glycaemic control 2. Low levels of IFNγ may also signify impairment in immune response in diabetics with established complications. 3. The higher levels of Rantes & IFNβ in diabetics with poor glycaemic control suggests a proinflammatory process which may be associated with diabetes related complications. Thus our study demonstrates that diabetics exhibit perturbations in cytokine and chemokine levels, supporting their role in infection, diabetes disease progression and complications. Sources of Research Support: Health Research Board of Ireland and Irish Endocrine Society. Nothing to Disclose: SG, AM, AN, JS, TG, SH, SM

Pub # P2-92

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)

Title Growth Hormone Secretagogues Improve Performance of Mouse Cardiomyocytes Isolated from *In Vitro* Ischemia/Reperfusion by Regulating Intracellular Calcium Homeostasis

Author String Y Ma, L Zhang, B Launikonis, JN Edwards, C Chen
University of Queensland, Brisbane, Australia; University of Auckland, Auckland, New Zealand

Body

Ischemic heart disease is the major cause of cardiac mortality around the world. Ischemia/reperfusion (I/R) model is widely used to mimic the process of a transient blockage and subsequent recovery of coronary blood flow. Endogenous ghrelin and its synthetic analogue hexarelin, are growth hormone secretagogues (GHS) that exert a protective effect on the cardiovascular system. We aimed to determine whether the presence of ghrelin or hexarelin at the start or end of ischemia *in vitro*, termed pre-treatment and post-treatment, would improve the performance of cardiomyocytes isolated from I/R injury and the underlying mechanisms.

The I/R hearts were isolated from adult male C57BL mice and stabilized for 20 min in perfusion with oxygenized medium, then subjected to 20 min of no-flow global ischemia and 30 min of reperfusion in ischemic group, whereas control group hearts were continually perfused. 10 nM ghrelin or 1 nM hexarelin was administered in the perfusion solution before or after ischemia for 10 min. Cardiomyocytes were isolated from the hearts at the end of perfusion with collagenase. Single cell shortening, intracellular calcium ($[Ca^{2+}]_i$) transients, sarcoplasmic reticulum (SR) calcium (Ca^{2+}) content and voltage-gated calcium currents (I_{Ca}) were measured.

The results showed that the cell shortenings and the corresponding $[Ca^{2+}]_i$ transients were significantly decreased by I/R, but were completely reversed in all GHS pre- or post-treated groups. The decreased amplitude of the $[Ca^{2+}]_i$ transient after I/R was due to a reduction in (1) I_{Ca} leading to decreased Ca^{2+} influx during action potential and (2) SR Ca^{2+} content leading to a decreased Ca^{2+} release from SR. Ghrelin or hexarelin pre- and post-treatment increased both I_{Ca} and SR Ca^{2+} content after I/R injury. GHS receptor type 1a (GHS-R1a) antagonist, [D-Lys3]-GHRP-6 (200 nM) or BIM28163 (100 nM), was introduced into the perfusion system 5 min before GHS post-treatment for 15 min, that completely blocked the effects of GHSs on cardiomyocyte sarcomere shortening and Ca^{2+} transients. In conclusion, both ghrelin and hexarelin activate GHS-R1a and produce a positive inotropic effect on cardiomyocytes from I/R injury via regulation of calcium homeostasis by increasing amplitude of $[Ca^{2+}]_i$ in response to electrical stimulation through increase in I_{Ca} and SR Ca^{2+} content.

Sources of Research Support: Australian National Health and Medical Research Council (NHMRC).

Nothing to Disclose: YM, LZ, BL, JNE, CC

Pub #	P2-93
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Factors, Cytokines & Intracellular Signaling (1:30 PM-3:30 PM)
Title	Guanylyl Cyclase/Natriuretic Peptide Receptor-A Antagonizes VEGF-Stimulated Mitogen-Activated Protein Kinases and Downstream Signaling Involving AP-1 and CREB
Author String	S Tripathi, KN Pandey Tulane University Health Sciences, New Orleans, LA
Body	The objective of this study was to determine the effect of atrial natriuretic peptide (ANP) and its receptor guanylyl cyclase/natriuretic peptide receptor-A (GC-A/NPRA) on mitogen-activated protein kinases (MAPKs) and the downstream proliferative transcription factors activating protein-1 (AP-1) and cAMP-response element binding protein (CREB) in agonist-stimulated mouse mesangial cells (MMCs). Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum under 95% O_2 /5% CO_2 at 37^0C. Cells were transfected with Lipofectamine -2000. ANP inhibited vascular endothelial growth factor (VEGF)-stimulated phosphorylation of MAPKs (Erk1, Erk2, JNK, and p38) to a greater extent in NPRA-transfected cells (50-60%) relative to vector-transfected cells (25-30%). The analyses of the phosphorylated transcription factors revealed that ANP inhibited VEGF-stimulated activation of CREB, and the AP-1 subunits (c-jun and c-fos). Gel shift assays demonstrated that ANP inhibited VEGF-stimulated AP-1 and CREB DNA-binding ability by 62 % and 67% respectively. The addition of the PKG inhibitor KT-5823 restored VEGF-stimulated activation of MAPKs, AP-1, and CREB, demonstrating the integral role of cGMP/PKG signaling in ANP/NPRA-dependent inhibition of MAPKs, AP-1, and CREB. Our results demonstrate that ANP-NPRA system exerts an inhibitory effect on MAPKs and down-stream AP-1 and CREB critical for cell growth and proliferation. Sources of Research Support: NIH Grant HL 57531. Nothing to Disclose: ST, KNP

Pub #	P2-94
Session Information	POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title	GPR98 Gene in the Regulation of Human and Mouse Bone Mineral Density
Author String	T Urano, M Shiraki, H Yagi, M Ito, M Sato, Y Ouchi, S Inoue The University of Tokyo, Tokyo, Tokyo, Japan; The University of Tokyo, Tokyo, Tokyo, Japan; Research Institute and Practice for Involutional Diseases, Nagano, Japan; University of Fukui, Fukui, Japan; Nagasaki University Hospital, Nagasaki, Japan
Body	Genetic factors are important in the development of osteoporosis. The present study includes 750 Japanese postmenopausal women with BMD data, and 57,244 single-nucleotide polymorphisms (SNPs) were analyzed for 251 subjects using the Affymetrix GeneChip Human Mapping 50K Hind SNP array. We have chosen 13 SNPs in the first-stage analysis, as having p values lower than the thresholds determined by quantile-quantile plots of p values in single SNP analyses of dominant and recessive models of inheritance with total body BMD ($p < 6.99E-6$ and $p < 1.60E-5$, respectively). Second-stage analysis for the remaining 499 subjects revealed that 5 SNPs had significant associations with low BMD phenotype, as having lower combined p values between the first- and the second- stage analyses than the value determined by Bonferroni's correction. One SNP near the GPR98 gene showed a significant p value for the combined analyses. We discovered that the SNP near the GPR98 gene were significantly associated with both total body and lumbar spine BMD in 750 Japanese postmenopausal women. The associations of the SNP with femoral neck and lumbar spine BMD were also replicated for Caucasian women in silico using the Framingham Heart Study database. These replicable associations of GPR98 SNPs with BMD in different races further encourage us to consider that this gene may contribute to the development of osteoporosis. Therefore, we have analyzed whether the deficiency of Gpr98 gene is involved in the control of BMD in the mouse model. Compared with 12 weeks-old wild-type mice (n=6), femur BMD in 12 weeks-old Gpr98 knockout mice (n=5) was significantly lower ($p < 0.005$). Micro-computed tomography analysis of femoral bones revealed that the three-dimensional bone volume fraction and cortical bone thickness of Gpr98 knockout mice was significantly reduced compared with that of WT mice. These data indicated that Gpr98 KO mice display osteopenic phenotype. Thus, genetic analyses in both human and mouse models uncovered the importance of GPR98 gene in the regulation of the BMD. Nothing to Disclose: TU, MS, HY, MI, MS, YO, SI

Pub #	P2-95
Session Information	POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title	Gene Expression Studies of Bone Biopsies Reveal Clusters of Correlated Gene Expression Based on Chromosomal Location
Author String	JP Berg, D Sachse, S Reppe, OK Olstad, KM Gautvik University of Oslo, Oslo, Norway; Oslo University Hospital, Oslo, Norway

Body

Context

Postmenopausal osteoporosis is a frequent disease leading to fragile bone and fractures. Approximately 40 % of Caucasian women will suffer at least one fragility fracture during their lifetime. Efficient prevention of osteoporotic fractures requires improved ability to identify those at risk.

Objective

To identify chromosomal clusters of correlated gene expression in bone biopsies and their relation to genes associated with bone mineral density in postmenopausal women. The correlations were based on data from a previous study from our group (1).

Participants

A total of 301 postmenopausal ethnic Norwegian women (50-86 years) were invited to the study, of which 178 were excluded due to medication or diseases. Of the 123 remaining, 23 later decided not to participate. One hundred women had trans-iliacal bone biopsies, and 84 contained adequate RNA for gene expression analysis.

Main outcome measures

Gene expression analysis was performed on HG-U133 plus 2.0 chips (Affymetrix). Probe sets containing more than 43 absent calls were eliminated in the cohort across the entire data set, which resulted in a reductio of informative probe sets from 54,675 to 22,815. The expression data was log-transformed and ordered by location before calculating one statistical correlation matrix per chromosome. Clusters of correlated gene expression were identified.

Results

The analysis revealed the clustering of 1241 genes in more than 200 clusters of three or more genetic neighbors with correlated expression pattern (r[ge]0.3) in bone biopsies from postmenopausal women. The largest cluster comprised 24 genes, but 2/3 of clusters consisted of only three to five genes. Among the top 60 genes previously associated with BMI adjusted bone mineral density Z-scores in trochanter and femoral neck in these women MEPE (chr 4), CLIC5 (chr 6), KIAA0368 (chr 9), PPP3CB (chr 10), OSBPL1A (chr 18), TMEM86B and BCL2L12 (both chr 19) were located in gene expression clusters. Ingenuity[reg] Systems pathway analysis showed that several of the largest clusters were associated with the NF-[kappa]B pathway and inflammation.

Conclusions

This large gene expression data set has revealed several chromosomal locations with highly correlated gene expression patterns in iliac crest biopsies. The information may reveal mechanisms governing regional regulation of gene transcription in bone.

(1) Reppe et al., Bone 2010; 46:604

Sources of Research Support: The South-Eastern Norway Regional Health Authority, and Ullevaal University Hospital, project #29750104; the Norwegian Osteoporosis Society; the Norwegian Research Council; Anders Jahre's Foundation for Promotion of Science; Rachel and Otto Bruuns Legate; and the Novo Nordisk Foundation. This work is part of the European Union project OSTEOGENE (no. FP6-502491).

Nothing to Disclose: JPB, DS, SR, OKO, KMG

Pub #	P2-96
Session Information	POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title	Characterization of Global Gene Expression Implicates Multiple Signaling Pathways Involved in the Regulation of Longitudinal Bone Growth by Perichondrium
Author String	SS Spath, AC de Andrade, M Chau, J Baron, O Nilsson Centre for Molecular Medicine and Karolinska Institutet and University Hospital, Stockholm, Sweden; Eunic Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD
Body	Longitudinal bone growth takes place at the growth plate, which is located at the end of long bones. At the growth plate, cartilage expansion through chondrocyte proliferation, hypertrophy and matrix production results in elongation of the bone. The growth plate is surrounded by perichondrium, a fibrous connective tissue sheet, proposed to be involved in the regulation of the growth plate. However, little is known about molecular mechanisms by which the perichondrium may regulate growth plate chondrogenesis. The aim of this study was to identify genes and signaling pathways that are expressed in the perichondrium and to identify potential new markers specifically expressed in perichondrium compared to growth plate. Using manual microdissection, microarray, and bioinformatic analysis, as well as real-time PCR confirmation, several genes and enriched pathways specifically in the perichondrium or growth plate of 1-week-old rats were identified. Regulatory genes that showed significantly greater expression ($P < 0.01$; >3 fold) in perichondrium compared to the growth plate included members of the IGF-system (Igf1, Igfbp3, Igfbp5), Wn signaling (Wnt9a, Wisp1, Wif1, Dkk3, β-catenin), BMP signaling (Bmp4, Bmp5), and FGF signaling (Fgf9, Fgfr1). Several extracellular matrix components were also detected at higher levels ($P<0.01$; >10 fold) in perichondrium than in growth plate, including collagen type 1a1, 3a1, 4a1, and 24a1. In addition, several stem cell markers, i.e. CD34, CD93, Notch1 and Nestin, were expressed at higher levels ($P < 0.01$) in perichondrium than in growth plate. Using quantitative RT-PCR, perichondrium-specific expression patterns identified by microarray have so far been verified for Igf1, Igfbp-3, and -5, pleiotrophin, periostin, dkk3 and Bmp5. In summary, microdissection and micoarray analysis have identified multiple regulatory genes that are differentially expressed in perichondrium, suggesting that IGF, Wnt, BMP, and FGF signaling serve to regulate the perichondrium itself and/or the adjacent growth plate. Sources of Research Support: Grants from the Swedish Research Council (K2007-52X-20316-01-4), the Stockholm County Council, the Swedish Society of Medicine, HKH kronprinsessan Lovisas F[ouml]rening f [ouml]r Barnasjukv[aring]rd, S[auml]llskapet Barnav[aring]rd, Stiftelsen Frimurare Barnhuset i Stockholm, and the Karolinska Institute. Nothing to Disclose: SSS, ACdA, MC, JB, ON

Pub #	P2-97
Session Information	POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title	Mutational Analyses of Genes in Korean Patients with Familial or Sporadic Forms of Isolated Hypoparathyroidism: A Series of *Korean Hypopara Registry Study*
Author String	YS Eom, B Choi, H-S Yi, Y' Chung, TS Jung, SY Park, S Lee, S Hong, H Jueppner, M Mannstadt, S Lee Gachon University School of Medicine, Incheon, Korea; Ajou University School of Medicine, Suwon, Korea; Gyeongsang National University College of Medicine, Jinju, Korea; Kwandong University College of Medicine, Seoul, Korea; Sungkyunkwan University, Suwon, Korea; Lee Gil Ya Cancer and Diabetes Institute Gachon University of Medicine and Science, Incheon, Korea; Massachusetts General Hospital and Harvard Medical School, Boston, MA; Massachusetts General Hospital and Harvard Medical School, Boston, MA
Body	**Background:** Hypoparathyroidism is characterized by hypocalcemia, hyperphosphatemia, and absent or markedly reduced serum levels of parathyroid hormone (PTH). Symptoms of hypocalcemia include muscle cramping, numbness, tetany, and seizures. The most common cause of hypoparathyroidism is neck surgery. Other causes include autoimmune destruction of the parathyroid glands and congenital, genetic disorders. Isolated hypoparathyroidism (IH) can be caused by mutations in the genes encoding PTH, CaSR and GCMB. Our objective was to analyze gene mutations in a large cohort of Korean patients with sporadic or familial forms of isolated hypoparathyroidism (IH). **Methods:** Two IH families and 17 patients with sporadic IH were identified and included in the analysis. All coding exons and exon-intron borders of *prepro-PTH, CaSR and GCMB* were sequenced using PCR-amplified DNA. One heterozygous *GCMB* mutation (C106R mutant) was further analyzed with functional studies, including electrophoretic mobility shift assays (EMSA) and luciferase-reporter assays to assess DNA-binding and transactivation ability. **Results:** In one family, we identified a novel heterozygous mutation in exon 2 of *GCMB* in an affected female and her son, but not in healthy members of this family and not in DNA from >50 healthy controls. The nucleotide changes amino acid residue 106 (C106R) that is located in the putative DNA-binding domain of GCMB and is conserved in numerous mammalian species. Functional studies revealed reduced binding of the mutant protein as determined by EMSA using a GCM binding motif and significantly reduced activity in luciferase assays using DF1 cells and 6xgbs Luc reporter. In two other patients with sporadic IH, we furthermore identified heterozygous CaSR mutations (D410E and P221L). In addition, one single nucleotide polymorphisms (SNP) was found in the *preproPTH* gene (c.247C>A), two SNPs in the *CaSR* gene (c.2956G>T, c2968A>G), and four SNPs in the *GCMB* gene (c.1-44T>C, c.91-242A>G, c.343+163G>A, c.583-72A>T). The R990G missense mutation in CaSR, previously identified in patients with hypercalciuria, was identified in three IH patients. **Conclusion:** We have identified a novel *GCMB* mutation that may explain AD-HP. Also, we have identified two heterozygous *CaSR* mutations (one novel and one known mutation), and several SNPs in the three candidate genes. Future functional studies of the CaSR mutations will help elucidate the molecular mechanism leading to idiopathic hypoparathyroidism. Sources of Research Support: Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology, Korea [2010-0005854 to S.L.]. Nothing to Disclose: YSE, BC, H-SY, Y-SC, TSJ, SYP, SL, SH, HJ, MM, SL

Pub #	P2-98
Session Information	POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title	KAI-4169, a Novel Calcimimetic for the Treatment of Secondary Hyperparathyroidism
Author String	A Baruch, D Maclean, K Yin, K Das, JE Tomlinson, E Sho, J Janes, S Alexander, S Walter, D Mendel, F Karim KAI Pharmaceuticals, South San Francisco, CA
Body	Secondary hyperparathyroidism (SHPT) is a frequent complication in patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD) in which the impairment of blood and bone mineral homeostasis (calcium and phosphate) and vitamin D metabolism lead to parathyroid gland hyperplasia and elevated parathyroid hormone (PTH) levels. Elevated PTH levels are linked to deleterious physiological effects including osteodystrophy, vascular calcification, left ventricular hypertrophy and increased risk for cardiovascular events, which is the leading cause of morbidity and mortality in these patients. We have identified a novel peptide calcimimetic, KAI-4169, that binds to and activates the calcium sensing receptor (CaSR) in vitro and inhibits secretion of PTH from the parathyroid gland in vivo. In vitro pull-down assays show that a tagged version of KAI-4169 is able to specifically bind to and co-immunoprecipitate the human CaSR protein. In vitro cell assays in which the human CaSR has been transfected into HEK293 cells have also shown that KAI-4169 can stimulate signaling via the CaSR as measured by IP1 production, and can activate p42/44 MAPK in a CaSR-dependent manner. In contrast, KAI-4169 did not produce these CaSR-dependent effects in untransfected HEK293 cells, which do not express the CaSR. In a preclinical rat model of acute renal insufficiency in which creatinine and baseline PTH levels become significantly elevated (baseline PTH in this model range from 450 pg/mL to over 1200 pg/mL) a single intravenous (IV) bolus administration of KAI-4169 resulted in dose-dependent reduction in PTH both in terms of the magnitude and duration of the effect, with a single IV bolus dose of 0.5 mg/kg KAI-4169 suppressing PTH by >50% from baseline for >24 hours. In a rat model of chronic renal insufficiency (the rat 5/6 nephrectomy model) in which animals were fed a high phosphate diet for 40 days, untreated animals developed elevated PTH (>1500 pg/mL), parathyroid gland hyperplasia (as measured by gland weight and BrdU staining) and significant vascular and soft tissue calcification. Repeat dosing with KAI-4169 (3 mg/kg) three times per week for 40 days significantly reduced PTH levels, parathyroid gland hyperplasia and vascular calcification. KAI-4169 is a long acting calcimimetic peptide that is being developed as an IV treatment for ESRD patients with SHPT. Nothing to Disclose: AB, DM, KY, KD, JET, ES, JJ, SA, SW, DM, FK

Pub #	P2-99
Session Information	POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title	Effect of Vitamin D Therapy on Insulin Resistance and Metabolic Control in Patients with Type 2 Diabetes Mellitus and Its Pharmacogenetic Analysis
Author String	F Strobel, E Klahold, J Reusch, E Ramos-Lopez, M Penna-Martinez, K Badenhoop Universtiy Hospital Frankfurt/Main, Frankfurt/Main, Germany
Body	The effects of vitamin D (VD) on the metabolism have been mainly studied in non-diabetics. We investigated the influence of a six-month VD supplementation of 2,000 IU/d in patients with non-insulin-requiring type 2 diabetes mellitus (T2DM) and the pharmacogenetic impact of Vitamin D system polymorphisms (VDR ApaI, TaqI, and CYP27B1). Patients with T2DM (n=86) were randomized in a double-blind, placebo-controlled study. During the first six months the patients received 20 drops Vigantol oil or placebo oil (medium chain triglycerides) once a week, followed by six months follow-up with measurements of 25D levels, PTH, calcium, phosphor, body weight, blood pressure, HbA1c and C-peptide.Vitamin D system gene polymorphisms were analysed by RFLP and PCR. After 6 months of therapy the verum group[acute]s (n = 40) 25D level had increased by a factor of 2.14 to a median of 28.4 ng/ml (71 nmol/l). The mean increase of 11.85 ng/ml ($p<0.001$) in the verum group was significantly higher than in the placebo group. The PTH tended ($p=0.08$) to decrease more in the verum group until the end of therapy. The verum group showed a non significant increase of calcium by 1.02 up to a median of 2.43 mmol/l but a significant increase of phosphor ($p=0,04$) by 1.06 up to a median of 3.6 mg/dl. In the placebo group no changes were seen, neither in calcium nor in phosphor. The changes in body weight and systolic blood pressure were not significant in any group. At baseline all patients with 25D levels > 20 ng/ml (52.5 nmol/l) (n=14) had significantly lower HbA1c. The HbA1c was 0.35 % ($p=0.01$) lower than in patients with VD [le] 20 ng/ml (n=71). After VD therapy all patients with 25D levels > 20 ng/ml (52.5 nmol/l) showed a higher C-peptide levels (by 0.95 ng/ml, $p=0.01$). Pharmacogenetic differences were significant for the relative increase of 25D in carriers of CYP27B1 CC ($p<0.001$) and AC ($p>0.001$, VDR TaqI TT ($p>0.001$) and Tt ($p>0.001$) and VDR ApaI AA ($p=0.005$) and Aa ($p>0.001$). The diffference in PTH suppression was significant for CYP27B1 AC ($p=0.047$) and for the increase of C-peptide with AA ($p=0.029$). This study shows lower HbA1c at baseline as a function of the 25D status in patients with T2DM without insulin. Furthermore C-peptide increases significantly after six months of VD therapy. Some effects of this pilot vitamin D therapy appear to be under the influence of pharmacogenetic variation. Sources of Research Support: EU-FP7 grant NAIMIT (grant agreement 241447). Nothing to Disclose: FS, EK, JR, ER-L, MP-M, KB

Pub #	P2-100
Session Information	POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title	Cytokine Dysregulation in Diabetic Foot Infection Relates to Severe Vitamin D Deficiency
Author String	S Tiwari, DV Pratyush, SK Singh Institute of Medical Sciences, Banaras Hindu University, Varanasi, India
Body	**Introduction:** Immune-regulatory role of vitamin D is well established. Diabetic Foot infections reflect the immune-compromised state of the patients and therefore it is speculative that vitamin D deficiency will be more common and severe in diabetic foot influencing their immune status. The aim of the study was to evaluate the vitamin D status and its effect on inflammatory cytokines in diabetic patients with foot infections **Method:** Serum 25 (OH) vitamin D concentrations was measured by RIA (Diasorin) in 112 diabetic foot cases and 107 diabetic controls. Serum level of IL6 from 100 cases & 73 controls, IL1 from 91 cases & 107 controls and TNF-α from 87 cases & 61 controls were also measured by ELISA (Diaclone). Data was presented as mean (±SE) unless otherwise indicated and was analyzed by SPSS using independent [ldquo]T [rdquo] test and non- parametric test wherever applicable. **Results:** Vitamin D deficiency (<20ng/ml) was found in 71.4% of cases & 61.6% of controls but severe deficiency (<10ng/ml) was more common in cases than controls (48.2% vs 20.5%). Cases had significantly higher level of IL6 (128.3±7.4 vs 87±9.0; p=0.001), IL1 (101.5±20.8 vs 49.3±10.8; p=0.02) and TNF-α (182.3±20.8 vs 96.5±22.9; p=0.006) than controls. There was a negative correlation of vitamin D concentration with inflammatory cytokines which was statistically significant for IL6 (-.154; p=0.04) and IL1 (-.323; p=0.000) but not for TNF-α (-.102; p=0.07). A significant difference in level of IL1 [113.5(±28.2) vs 50.6(±9.1); p=0.007] and IL6 [127.1(±9.3) vs 99.4(±7.6); p=0.02] in patients with severe vitamin D deficiency when compared with those having 25 (OH) vitamin D [ge]10ng/ml was noted. Inspite of remarkable elevation in TNF-α level [185.0(±26.1) vs 122.6(±19.2); p=0.05] the difference could not reach statistical significance in these subgroups. Such analysis did not show statistical difference in cytokine levels with vitamin D concentration cut offs >10ng/dl. There was no difference in age (years) [53.6 (±1.0) vs 51.9 (±1.0); p=0.2], duration of diabetes (years) [6.7 (±0.5) vs 6.5 (±0.7); p=0.8], glycemic status (HbA1c %) [9.7(±0.25) vs 9.0 (±0.28); p=0.054] and BMI (kg/m2) [23.6(±0.5) vs 24.4(±0.4); p=0.2] between cases & controls. **Conclusion:** Vitamin D deficiency was more prevalent in diabetic foot infection in our region. This could be one of the factors contributing to inflammatory cytokine dysregulation in such patients particularly with vitamin D concentration less than 10ng/ml. Sources of Research Support: Indian Council of Medical Research, New Delhi, India. Nothing to Disclose: ST, DVP, SKS

Pub #	P2-101
Session Information	POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title	Phosphaturic Tumors That Cause Tumor-Induced Osteomalacia Express Cell Surface Markers That Suggest They Derive from Skeletal Stem Cells
Author String	WH Chong, RI Gafni, AA Molinolo, MT Collins, N Bhattacharyya NIH, Bethesda, MD; NIH, Bethesda, MD
Body	Background: Tumor-induced osteomalacia (TIO) is a rare paraneoplastic syndrome characterized by hypophosphatemia and osteomalacia secondary to renal phosphate wasting. The observed phosphaturia is mediated by fibroblast growth factor 23 (FGF23), which is produced in abundance by tumors commonly referred to as phosphaturic mesenchymal tumors (PMT) - mixed connective tissue variant. PMTs are typically benign and can occur from head to toe, in bone or soft tissue. While the histologic features of the tumors have been well-described, little is known about their developmental or cellular origin. We hypothesized that PMTs may be derived from pericytes in different tissues that in a non-bone setting can be induced to an osteogenic pathway. This hypothesis is based on several observations: 1) osteocytes, the primary physiologic source of FGF23, differentiate from skeletal stem cells, 2) like several cells in the osteogenic lineage, PMTs commonly support osteoclastogenesis, and 3) features of skeletal tissues, including chondroid matrix and even lamellar bone, are commonly observed in PMTs. To investigate this, we studied a group of PMTs for evidence of their relatedness to skeletal cells. Methods: Tumors from 10 patients with TIO, all with histopathological findings consistent with PMTs, were analyzed. Immunohistochemical staining for FGF23 and bone-related markers was performed. Results: FGF23 staining was seen in all tumors, confirming its role in causing the disease. CD146, a marker for a pericytes, which can become osteogenic in the appropriate milieu, was present on pericytes and endothelial cells within and extraneous to the tumor as well as FGF23-positive tumor cells. Tumor cells also stained positively for receptor activator of nuclear factor ?B ligand (RANKL), a protein central in osteoclastogenesis and a marker for skeletal cells at many stages of differentiation. Conclusion: PMTs stained positively for cell surface markers of pericytes and skeletal cells at various stages of osteogenic differentiation, supporting the hypothesis that these tumors are derived from pericytes induced to become osteogenic by an as yet unidentified factor. The fact that these tumors stain positively for the pericyte marker, CD146, may explain their ability to arise in multiple tissues throughout the body. Insight derived from the study of the origins of these tumors may enhance our understanding of FGF23 physiology and lead to new diagnostic and/or therapeutic options for TIO. Nothing to Disclose: WHC, RIG, AAM, MTC, NB

Pub #	P2-102
Session Information	POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title	Delayed Osteoblast Differentiation and Reduced Osteoprotegerin Expression in Cystic Fibrosis Bone Disease
Author String	CR McKibbin, LK Dunn, KL Clines, WJ Chung, S Gabriel, GA Clines University of Virginia, Charlottesville, VA; University of Alabama at Birmingham, Birmingham, AL; University of North Carolina at Chapel Hill, Chapel Hill, NC; Birmingham VA Medical Center, Birmingham, AL
Body	Cystic fibrosis is associated with low bone mineral density, increased fracture risk and uncoupled bone turnover--impaired osteoblastic bone formation and enhanced osteoclastic bone resorption. Intestinal malabsorption, vitamin D deficiency and inflammatory cytokines contribute to CF bone disease, but epidemiological investigations and animal models support a direct causal link between CFTR inactivation in bone and CF bone disease. We investigated the role of CFTR in bone using expression and functional analyses. CFTR mRNA was detected in murine calvarial osteoblasts at a concentration similar to pancreas, a tissue also affected by CF. Immunohistochemical staining of murine calvarial bone revealed expression in osteoblasts and osteocytes. CFTR expression was not detected in osteoclasts. Subsequent studies were therefore focused on CFTR function in the osteoblast. In the calvarial organ culture assay of osteoblast activity, CFTR genetic inactivation reduced bone formation (6430 [micro]m2 vs. 16440 [micro]m2, p=0.0023) and osteoblast numbers. However CFTRinh-172, a small-molecule inhibitor that blocks CFTR-specific Cl- cell membrane conductance, did not alter osteoblast activity in this assay. Fluorescent immunostaining revealed that CFTR was excluded from the cell membrane but present in the cytoplasmic compartment. These data suggested a role for CFTR in osteoblasts dissimilar to its classic role as a Cl- channel found on the apical membrane of secretory epithelial cells. Mechanisms for uncoupled bone turnover with CFTR inactivation were investigated. Cftr null calvarial osteoblasts expressed significantly less Osterix, Collagen 1a1 and Osteocalcin, but not the early differentiatio marker Runx2, than WT osteoblasts indicating delayed osteoblast differentiation. The lack of osteoclast CFTF expression suggested dysfunction in osteoblast-derived RANKL/OPG as a cause for enhanced bone resorptio of CF. Cftr null and WT calvarial osteoblasts expressed equivalent amounts of Rankl mRNA, but Opg mRNA was reduced by 65% (p=0.002) in Cftr null osteoblasts. Overall, the Rankl:Opg ratio was 2.7X higher in Cftr KO vs. WT osteoblasts translating into higher osteoclast activity and bone resorption. We present a novel role for CFTR in bone and provide evidence that uncoupled bone turnover of CF bone disease is a direct consequence of osteoblast CFTR inactivation by delaying osteoblast differentiation and enhancing osteoclastogenesis via reduced osteoblast Opg expression. Sources of Research Support: ASBMR Career Development Award; NIH Grant AR056826. Nothing to Disclose: CRM, LKD, KLC, WJC, SG, GAC

Pub #	P2-103
Session Information	POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title	Long-Term Urinary Potential Renal Acid Load and Nitrogen Excretion Are Associated with Bone Modeling and Remodeling in Healthy Children
Author String	L Shi, A Ute, S Eckhard, S Wudy, T Remer Research Institute of Child Nutrition, Dortmund, Germany; University of Cologne, Cologne, Germany; Justus-Liebig-University, Giessen, Germany
Body	Background: In diet studies, both long-term anabolic effects of protein and catabolic effects of dietary acid load on bone strength in healthy children have been observed. Until now, prospective studies in children using reliable biomarkers are lacking. Objective: To examine whether the association of long term dietary protein intake and dietary acid load with bone status in healthy children can be confirmed using specific, validated 24-h urinary biomarkers of them after considering the potential bone-anabolic effect of the direct DHEA metabolite androstenediol (ADIOL). Method: Nitrogen excretion (marker of protein intake) and urinary potential renal acid load (uPRAL) were quantified in 24-h urine samples of 162 healthy children, who had [ge]3 urine collections during the 4 y preceding proximal forearm bone analyses by peripheral quantitative computed topography. uPRAL was determined by the sum of measured nonbicarbonate anions (chloride + phosphorus + sulfate) minus the sum of measured mineral cations (sodium+ potassium + calcium + magnesium). Urinary ADIOL was quantified by GC-MS. Results: Using multiple regression analysis adjusted for bone age, sex, pubertal stage, forearm muscle area and length, urinary calcium and ADIOL excretion, we observed that urinary nitrogen was significantly positively associated with cortical area (P=0.002), bone mineral content (P=0.003), and polar strength strain index (P=0.03), which reflected a combination of modeling and remodeling. Children with a higher uPRAL had significantly less cortical area (P=0.04) and bone mineral content (P=0.03). ADIOL was significant ($p<0.05$) for all bone outcomes in regression models if forearm length was not adjusted for. Conclusions: In line with dietary assessment findings, urinary biomarker analyses confirmed the long-term positive effect of protein intake and concomitant negative effect of higher dietary acid load on bone status of children even after considering ADIOL. Sources of Research Support: German Research Foundation (DFG), RE 753/7-1). Nothing to Disclose: LS, AU, SE, SW, TR

Pub #	P2-104
Session Information	POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title	Marrow Fat Decreases with Recovery from Anorexia Nervosa
Author String	PK Fazeli, MA Bredella, LM Freedman, BJ Thomas, AC Breggia, E Meenaghan, CJ Rosen, A Klibanski Massachusetts General Hospital and Harvard Medical School, Boston, MA; Masssachusetts General Hospital and Harvard Medical School, Boston, MA; Maine Medical Center Research Institute, Scarborough, ME
Body	Anorexia nervosa (AN), a psychiatric disorder characterized by self-induced starvation, affects 0.5-1% of college-aged women in the US and results in significant loss of bone mineral density (BMD). Although women with AN have severe depletion of visceral and subcutaneous adipose tissue, we reported increased marrow adipose tissue (MAT) compared to normal subjects-- inversely associated with BMD. Because adipocytes and osteoblasts differentiate from a common progenitor mesenchymal stem cell, understanding factors that regulate MAT may provide insight into bone loss in AN. Whether MAT normalizes in women who have recovered from AN (AN-R)-- defined as women weighing >85% IBW with regular menses for at least 3 months-- compared to women with active AN is unknown. We studied 29 women, 8 AN (mean age: 33.4 $\pm$ 2.4 years $\pm$ 1 SEM), 6 AN-R, and 15 healthy controls (HC) of comparable age. We measured MAT of the L4 vertebra and femur by ^{1}H-magnetic resonance spectroscopy, BMD of the spine and hip by DXA, leptin by RIA, and Pref-1, a regulator of osteoblast and adipocyte differentiation, by ELISA. By design, the BMI of the AN group (18.3 $\pm$ 0.6kg/m^2) was significantly lower than that of AN-R (22.5 $\pm$ 1.4kg/m^2; p=0.002) and HC (22 $\pm$ 0.4kg/m; p=0.001). BMD of the spine and hip were significantly lower in AN as compared to AN-R and HC. MAT of the L4 vertebra was significantly greater in AN (1.006 $\pm$ 0.195AU) compared to both AN-R (0.478 $\pm$ 0.075AU; p=0.03) and HC (0.583 $\pm$ 0.073AU; p=0.03). Pref-1 levels were significantly higher in AN (0.61 $\pm$ 0.09ng/mL) as compared to HC (0.36 $\pm$ 0.02ng/mL; p=0.009). There was a trend toward decreased Pref-1 in AN-R compared to AN (p=0.09). Log Pref-1 was inversely associated with BMD of the hip (R=-0.48; p=0.01), lumbar spine (R= -0.42; p=0.03) and lateral spine (R= -0.42; p=0.04) in the entire group. In the individual groups, Pref-1 was positively associated with L4 MAT in women with AN (R=0.89; p=0.003) and inversely associated with L4 MAT in HC (R= -0.71; p=0.004). Therefore, with recovery from AN, there is a decrease in MAT and an increase in BMD. Pref-1 is a positive predictor of MAT in AN and inversely associated with BMD. In conclusion, MAT and Pref-1 approach normal levels in conjunction with BMD in women who have recovered from AN. This suggests that MAT plays an important role in mineral metabolism in states of nutritional deprivation and understanding this role may provide insight into the mechanisms of bone loss in AN. Nothing to Disclose: PKF, MAB, LMF, BJT, ACB, EM, CJR, AK

Pub #	P2-105
Session Information	POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title	Chronic Mild Hyponatremia Causes Bone Loss
Author String	J Barsony, Q Xu, T Sandberg, JG Verbalis Georgetown University, Washington, DC
Body	Chronic hyponatremia is common disorder that is often neglected: 2.4 % of hospitalized patients have pronounced hyponatremia (serum [Na+] <130 mmol/L) and 15-28 % of inpatients have mild hyponatremia (serum [Na+] = 130-135 mmol/L). We recently reported that pronounced chronic hyponatremia (PCH) causes loss of bone mineral density (BMD) in a rat model of the syndrome of inappropriate antidiuretic hormone secretion (SIADH), and that even moderate lowering of [Na+] in cell culture increased osteocalstogenesis and osteoclastic resorption. In these studies, we explored the possibility that mild chronic hyponatremia (MCH) also might cause bone loss. Twelve-month-old female Sprague-Dawley rats (n=8 per group) were studied for 3 months: one group was infused with desmopressin (DDAVP, 5 ng/h) and fed a liquid diet to generate PCH (serum [Na+]= 114.5±8 mmol/L); a second group was infused with a lower dose of DDAVP (0.5 ng/h) and pair-fed the same liquid diet to generate MCH (serum [Na+] = 130.9±2.7 mmol/L); a third group of normonatremic controls (NN) were pair-fed the same liquid diet but without infusing DDAVP (serum [Na+] = 146.6±1.3 mmol/L). Body weight increased by 10 % every month in all three groups. BMD was measured using serial in vivo densitometry, and ex vivo densitometry in excised femurs and L4 vertebras at the end of the study. Serum and urine parameters of calcium homeostasis were also measured throughout the study. PCH caused marked progressive decrease of BMD both in the spine ($p<0.01$) and femur ($p<0.01$) compared to NN. MCH caused a moderate decrease of BMD in the spine (in vivo: 24.1±6.7% of baseline; ex vivo: L4 MCH = 0.1179±0.0049, NN = 0.1271±0.007 g/cm2, $p<0.01$) and femur (in vivo: 7.3±1.8 % of baseline; ex vivo: MCH = 0.1600±0.0031, NN = 0.1686±0.0025 g/cm2, $p<0.05$). Consistent with increased bone resorption, urinary calcium and phosphorus were both increased in PCH compared to NN (both $p<0.001$), and to a lesser extent in MCH (calcium/creatinine ratio 0.55±0.14 versus 0.44±0.08; phosphorus 17.1±1.0 versus 7.8±0.3 mg/24h). Serum FGF23 was unaffected by either PCH or MCH. These results demonstrate that even mild hyponatremia causes significant bone loss over time, likely due to increased bone resorption directed toward liberating stored sodium from bone crystalloid matrix. In conclusion, even mild hyponatremia due to SIADH, when prolonged, likely represents a significant risk to bone health. Sources of Research Support: Extramural grant NIH/NIA R01-AG029477. Disclosures: JGV: Consultant, Astellas; Cardiokine; Otsuka; Sanofi-Aventis. Nothing to Disclose: JB, QX, TS

Pub #	P2-106
Session Information	POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title	Adolescents with Polycystic Ovary Syndrome (PCOS) Have Similar Bone Mineral Density (BMD) and Bone Mineral Content (BMC) to Controls (C)
Author String	AT Gerken, SE Oberfield, IB Libby, SJ Silverberg, DJ McMahon, D Gallagher, LR Allen, A Hassoun, AB Sopher Columbia University Medical Center, New York, NY; Columbia University Medical Center, New York, NY; St Luke's Roosevelt Hospital Center, New York, NY
Body	**Background:**PCOS is characterized by oligo/amenorrhea and hyperandrogenism. Those with PCOS may have low estradiol (E_2) and are at risk of developing insulin resistance (IR). Few adult studies have addressed BMD in PCOS, with mixed results. None has addressed BMD and BMC in PCOS adolescents. Hyperinsulinemia and hyperandrogenemia may affect BMD and BMC positively, while low E_2 may affect them negatively. **Objective:**To compare BMD and BMC in non-obese adolescent PCOS and C subjects, and to assess relationships with bone turnover markers (BTM), reproductive and adrenal (R/A) hormones and IR. We hypothesize that PCOS will have lower BMD and BMC compared to C, and that E_2, androgens, and IR will be positively related to BMD and BMC. **Design/Methods:**15 adolescent girls (13-21yr): 6 PCOS (menstrual age(MA): 3.1±1.3 yr; BMIz:0.26±1.13), 9 C (MA:6.6±2.5 yr; BMIz:0.26±0.59) had fasting blood for BTM, R/A hormones, glucose (G) and insulin (I). G and I were obtained at 30,60,90,120min after 75g OGTT. HOMA, FGIR and WBIS were calculated to determine IR. DXA (GE Lunar Prodigy) for total-body BMD and BMC was performed. BMD and BMI z-scores were calculated. Baseline characteristics were evaluated by t-test; OGTT differences were analyzed by weight-adjusted ANCOVA and bone differences by MA-adjusted ANCOVA. Relationships between BMD and BMC and BTM, R/A hormones and IR were evaluated by Pearson correlations. **Results:** As expected, PCOS had higher androgens (androstenedione, DHEAS, free testosterone (T), free androgen index), lower sex hormone binding globulin, greater IR (I_0, HOMA, c-peptide) ($P<0.05$ for all) and trend for lower E_2 ($P=0.06$) than C. PCOS had lower MA compared to C($P=0.007$). BTM (carboxy-terminal telopeptide of type I collagen, osteocalcin) were lower in PCOS ($P<0.05$). Despite these findings, BMD, BMDz, and BMC did not differ between the groups. BMD correlated with IR in both groups:WBIS ($r=-0.83, p<0.05$) and FGIR($r=-0.81, p<0.06$) in PCOS and I_0($r=0.76$, $p<0.02$), HOMA($r=0.77, p<0.02$) and c-peptide($r=0.77, p<0.01$) in C. BMDz correlated with E_2($r=0.86, p<0.03$) and DHEAS($r=0.83, p<0.05$) in PCOS, but did not correlate with BTM or R/A hormones in C. **Conclusions:**In this pilot study of non-obese girls with and without PCOS, BMD was similar in both groups despite lower E_2 in PCOS. IR measures correlated with BMD in PCOS and C. Our findings suggest that in PCOS, the bone-enhancing effects of hyperinsulinemia and hyperandrogenemia may offset the detrimental effects of low E_2 on BMD. Nothing to Disclose: ATG, SEO, IBL, SJS, DJM, DG, LRA, AH, ABS

Pub # P2-107

Session Information POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Rapidly Assessing Changes in Bone Mineral Balance Using Natural Stable Ca Isotope Composition of Urine

Author String JLL Morgan, GW Gordon, JL Skulan, AD Anbar
Arizona State University, Tempe, AZ; Arizona State University, Tempe, AZ; University of Wisconsin, Madison, WI

Body

Rapid changes in bone mineral balance can be detected using a Ca isotope biomarker based on measurements of natural, biologically induced variations in the abundances of the stable Ca isotopes in urine. Ca isotope variations are a result of the six naturally occurring Ca isotopes (^{40}Ca, ^{42}Ca, ^{43}Ca, ^{44}Ca, ^{46}Ca and ^{48}Ca) reacting at different rates depending on mass. In soft tissue, these variations exist because bone formation depletes soft tissue of light Ca isotopes. Bone resorption releases that isotopically light Ca back into soft tissue. In a previously published study, we observed variations in Ca isotope abundance consistent with net bone resorption after 4 weeks in a 90-day bed rest study (data collected at 0, 4, 8 and 12 weeks) (1). Our new 30-day bed rest study involved 12 patients on controlled diet, monitored for 12 days prior to bed rest and 7 days post bed rest. Samples of urine were collected as either per void or daily-pooled samples throughout the study to examine short-term variations. Ca isotope abundances in the samples were measured by multiple collector inductively couple plasma mass spectrometry (MC-ICP-MS) following chemical separation and purification of Ca using newly developed procedures. This technique is both sensitive and precise; the abundance ratio of ^{44}Ca/^{42}Ca can be measured using < 100 [mu]g of Ca with a typical precision of ± 0.1 (± 2 [sigma]) parts per thousand (permil; [permil]).

After 7 days of bed rest, the mean value of ^{44}Ca/^{42}Ca decreases by approximately 0.3 [permil] for all 12 patients. We interpret this decrease as reflecting the onset of negative bone mineral balance. The ^{44}Ca/^{42}Ca remains low during the remainder of bed rest and into the post bed rest period. Day-to-day variations for individual patients were approximately 0.14 [permil], possibly reflecting changes in diet. For the 12 days prio to bed rest the average ^{44}Ca/^{42}Ca remains high with no systematic change in the average of all patients. These results demonstrate that Ca isotopes can serve as a biomarker that provides significant information about changes in bone mineral balance after just 7 days of bed rest, long before detectible changes in bone mineral density occur. The ability to rapidly assess changes in bone mineral balance may permit the effectiveness of countermeasures to bone loss to be evaluated much more quickly than currently is possible, thus accelerating the pace of discovery of new treatments for metabolic bone disease.

(1) Skulan et al., Clinical Chemistry 2007; 53(6); 1155-1158

Sources of Research Support: NASA Grant NNX08AQ36G awarded to ADA.

Nothing to Disclose: JLLM, GWG, JLS, ADA

Pub #	P2-108
Session Information	POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title	Facilitation of Osteogenic Bone Marker Release in Postmenopausal Women by Single, Rather Than Spaced, Mechanical Loading or Anabolic Hormones
Author String	KT Borer, Q Zheng, AI Daoud, T Kernozek, MM Gross, BJ Roessler The University of Michigan, Ann Arbor, MI; East China Normal University, Shanghai, China; Harvard University, Cambridge, MA; University of Wisconsin, La Crosse, WI; The University of Michigan, Ann Arbor, MI
Body	AIM. To examine the relative role of hormones, mechanical loading and its timing on the osteogenic response (ratio of markers of bone formation, osteocalcin (OS) or C terminal propeptide of type 1 procollagen (CICP) over CTX (C-terminal telopeptide of type 1 collagen), a marker of bone resorption. METHODS. Forty postmenopausal women, 58 y old, were assigned to walk on either an uphill (slope 8 to 12 ^{o}) or -6^{o} downhill treadmill during either one 40-min bout (40 UP, 40 Down) or two 20-min bouts (20 UP, 20 Down) separated by 7 h, or to remain sedentary. Hourly measurements of anabolic growth (GH) and parathyroid (PTH) hormones and catabolic cortisol served to measure the anabolic endocrine index. Downhill slope increased, and uphill slope decreased, ground reaction force (GRF) as measured with mechanosensitive Novel Pedar shoe inserts. Timing of loading was manipulated with a single 40 min bout or two 20 min bouts of exercise separated by 7 h. CICP, OS (Quidel) and CTX (Immunodiagnostic Systems) were measured in serum. RESULTS. Peak GRFs were significantly higher in downhill (1097 N) than uphill (814 N) exercise. Relative effort was significantly higher during uphill than downhill (74.6% vs 46.6% VO2 max, respectively) exercise. The osteogenic CICP/CTX ratio, expressed as percent change, exhibited a 55% greater area under the curve (AUC) in the 40 Down trials, and a 44% reduction in 20 Up group following the mid-day meal compared to the other three groups. The osteogenic OS/CTX ratio changed in a similar way. There was a highly significant correlation between the CICP/CTX and the GRFs in all four groups (r^2=0.708, F=15.55, p<0.0009), as was the case for the OS/CTX ratio. The PTH/cortisol ratio was elevated between 16 and 22 h in the 40 Up compared to the other four groups. The GH/cortisol ratio was elevated between 15 and 20 h in 20 Up, 40 Up and 20 Down groups compared to the other two groups. There was no correlation between the osteogenic and anabolic endocrine indices.CONCLUSIONS. Greater mechanical loading of downhill exercise increases the osteogenic index in postmenopausal women when consolidated in one bout than when divided into two bouts separated by 7 hours. The osteogenic response is seen after the mid-day meal. The favorable increase in the osteogenic index in postmenopausal women is correlated with increased mechanical loading during downhill exercise while the anabolic hormonal response to uphill exercise shows no such association. Sources of Research Support: NIH R15DK066286 and R15DK082800. Nothing to Disclose: KTB, QZ, AID, TK, MMG, BJR

Pub #	P2-109
Session Information	POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title	CNP Forms in Adult Hyperthyroidism: Correlation with Thyroid Hormones and Markers of Bone Turnover
Author String	BJ Schouten, TC Prickett, PJ Hunt, M Richards, EA Espiner University of Otago, Christchurch, New Zealand
Body	C-type natriuretic peptide (CNP) is a paracrine hormone that is essential for normal endochondral bone growth. Although in vitro studies also show that CNP increases osteoblastogenesis (1), its role in bone remodelling is unclear. Using assays of amino terminal proCNP (NTproCNP), a bio-inactive product of CNP gene expression readily measurable in plasma, we have shown that levels strongly correlate with linear growth velocity in children (2), and with growth plate width in rodent pups. Consistent with these findings, plasma NTproCNP correlates with growth velocity and with change in thyroid hormone levels (3) during corrective treatment of children with acquired thyroid disorders. Whether CNP production is responsive to change in thyroid status, or change in bone turnover rates, after growth plate closure has not been studied. Accordingly we have studied changes in CNP production in adult subjects presenting with hyperthyroidism- a condition where both bone formation and resorption rates are enhanced. In 20 (19 female) subjects (mean age 43yr), plasma concentrations of thyroid hormones (T3 and T4), CNP and NTproCNP, and markers of bone resorption (CTx) and formation (bALP, osteocalcin and PINP) were measured at regular intervals over 24 weeks as euthyroid status was restored by thionamide treatment. At presentation (baseline), plasma NTproCNP was strongly correlated with T3 ($r=0.48$, $p=0.03$) and free T4 index ($r=0.54$, $p=0.01$). For all time points, plasma CNP and NTproCNP were highly correlated ($r=0.7$, $p<0.0001$) and both peptides correlated strongly with CTx ($r=0.53$, $p<0.0001$ and $r=0.64$, $p<0.0001$ respectively). During treatment, plasma NTproCNP declined progressively and was positively correlated with change in T3 ($r=0.68$, $p=0.001$). Associations of NTproCNP, T3 and CTx with other bone markers (PINP, osteocalcin, bALP) were weaker and reflected the latter's variable temporal responses after normal levels of thyroid hormones were restored (ie after 4-6 weeks). We conclude that, as in the immature skeleton, CNP production is linked to thyroid hormone levels in adults. The close coupling of T3, NTproCNP and CTx during changes in bone turnover suggests that CNP acts to maintain skeletal integrity in the face of T3- dependent bone resorption but participates less in later phases involving bone mineral accrual. (1) Hagiwara H et al., Am J Physiol 1996; 270:C1311-1318 (2) Prickett TC et al., Pediatr Res 2005; 58:334-340 (3) Reh CS et al., Program of the 91st Annual Meeting of the Endocrine Society, Washington, DC, 2009; Poster 03-342 Nothing to Disclose: BJS, TCP, PJH, MR, EAE

Pub #	P2-110
Session Information	POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title	Bone Mass, Bone Geometry and Body Composition in Female-to-Male Transsexual Persons
Author String	E Van Caenegem, K Wierckx, D Dedecker, F Van de Peer, K Toye, Y Taes, J-M Kaufman, G T'Sjoen Ghent University Hospital, Ghent, Belgium
Body	**Context:** Sex steroids have an important impact on gender differences in bone geometry acquired during puberty. Female-to-male (FM) transsexual persons undergo extreme hormonal changes due to ovariectomy and testosterone substitution, allowing further insight into sex steroid effects on bone physiology in the adult. **Objective:** To examine the effects of cross-gender sex steroid exposure on bone geometry and volumetric bone mineral density (vBMD), using peripheral quantitative computed tomography, on bone turnover markers, and body composition (DXA) in FM-transsexual persons. **Design:** A cross-sectional study, with 50 FM-transsexual persons at least 9 months after sex reassignment surgery and in 50 age-matched control women. **Results:** The FM-transsexual persons have a higher body lean mass and muscle area at dominant proximal forearm and calf and a greater grip strength, compared to female controls (all $p<0.001$). In addition, they have a lower body fat mass, smaller subcutaneous fat area (dominant forearm and calf) and a larger waist and smaller hip circumference (all $p<0.001$). Biochemical, markers of bone turnover (i.e. serum CTX and PINP) were higher in FM-transsexuals (both p[le]0.01), but IGF-1 was comparable to controls. At the distal radius, we observed a higher trabecular vBMD in FM-transsexuals ($p<0.02$). A larger cortical bone size (periostal and endosteal circumference) was found at the radius and tibia (all $p<0.001$). At the radius cortical vBMD was lower, cortical bone area and BMC were higher, and cortical thickness was smaller compared to controls (all $p<0.03$). At the tibia, cortical bone size was positively associated with physical activity ($p<0.001$) interacting with serum testosterone ($p=0.03$), whereas LH was an independent negative predictor of cortical bone size at both radius and tibia in the FM-transsexual group (all $p<0.04$). An inverse relationship was found between markers of bone turnover and cortical vBMD (all $p<0.04$). **Conclusions:** FM-transsexual persons presented with a different body composition with more muscle mass and strength and less fat mass, as well as an altered bone geometry with larger bones compared to age-matched female controls. The latter bone geometry differences might result from the combined effects of androgen action, direct and indirect through increased muscle mass, and of diminished estrogen exposure on cortical bone. Disclosures: GT: Clinician, Lilly USA, LLC; Novartis Pharmaceuticals; Ipsen; Investigator, Bayer, Inc.; Coinvestigator, Novartis Pharmaceuticals. Nothing to Disclose: EVC, KW, DD, FVdP, KT, YT, J-MK

Pub #	P2-111
Session Information	POSTER SESSION: TRANSLATIONAL - Bone, Calciotropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title	The Temporal and Spatial Regulation of Bone Acquisition by Serum IGF-I and Ovarian Hormones
Author String	HW Courtland, Y Wu, H Sun, S Yakar Mount Sinai School of Medicine, New York, NY
Body	Our study was aimed to understand the consequences of diminished ovarian hormones and decreased serum IGF-1 levels on bone. To avoid confounding effects of constitutive serum IGF-1 deficiency (liver IGF-1 deficient, LID model) on skeletal acquisition, we used an inducible liver IGF-1 deficient (iLID) model (on C57BL6/J background), in which a single injection of tamoxifen (0.3 mg/mouse) reduces serum IGF-1 by 60% at the time of interest. Mice were ovariectomized (OVX) at 12 weeks and depleted of serum IGF-1 (by tamoxifen injection) at the same day. Serum IGF-1 levels in controls did not differ significantly between sham and ovx mice (Control sham 264+15 ng/ml, Control OVX 271+/-28ng/ml). Similarly, iLID mice showed no difference in serum IGF-1 levels in response to ovariectomy, but had significantly lower serum IGF-1 levels when compared to controls (iLID sham 96+/-18 ng/ml, iLID OVX 79+/-11ng/ml). The structural consequences of serum IGF-1 and estrogen deficiencies on cortical bone of the femoral mid-diaphysis were revealed by [micro]CT 8 weeks following surgery (sham/OVX) and/or IGF-1 depletion (animals were 20 weeks old). We found that 8 weeks post OVX resulted in significant decrease in cortical bone thickness (Ct.Th) in control mice but did not affect other traits at the cortical envelope nor the trabecular bone fraction. Depletion of both IGF-1 and ovarian hormones (iLID/OVX) did not reveal any further deterioration of bone architecture. As such, we did not detect significant differences between sham and OVX iLID mice in bone length, total cross-sectional area (Tt.Ar), cortical area (Ct.Ar), cortical thickness (Ct.Th), marrow area (Ma.Ar), or trabecular bone fraction (BV/TV). In conclusion, we found that depletion of ovarian hormones in the adult mouse results in loss of cortical bone, however reductions in serum IGF-1 do not show additive effects. Nonetheless, it may be possible that elevated GH levels (in the iLID mice) protect against loss of cortical bone mass after ovariectomy. Sources of Research Support: NIH Grants AR054919 & AR055141 to SY. Nothing to Disclose: HWC, YW, HS, SY

Pub #	P2-112
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Utility of FGF23 Venous Sampling in a Child with Hypophosphatemic Rickets
Author String	GB Kletter, MC Dales, SS Shorr, A Boyce, WH Chong, F Wodajo, R Chang, C Chen, MT Collins Swedish Hospital, Seattle, WA; Renton Pediatric Associates, Renton, WA; CSBD, NIDCR, Bethesda, MD; NICHD, Bethesda, MD; NIH, Bethesda, MD; Inova Fairfax Hospital, Fairfax, VA
Body	Background: Tumors secreting FGF23 have been recognized recently as a cause for hypophosphatemic ricket (tumor-induced osteomalacia, TIO). Clinical case: A now 14-year young lady presented at age 11 years with bone pain and fractures. Prior to presentation, she had been healthy and growing well. Assessment revealed hypophosphatemic rickets, which proved difficult to control with progressive worsening of the hypophosphatemia. At initial presentation, significant laboratory tests were: phosphorus 2.7 mg/dL (normal 3.0 to 5.6), alkaline phosphatase 701 U/L (normal < 350), intact PTH 43.3 pg/mL (normal 10-65), and tubular reabsorption of phosphate 55% (normal >85). Her phosphorous declined despite supplemental therapy to a nadir of 1.2 mg/dL. Genetic causes of rickets were excluded by no evidence of mutations in *PHEX* and *DMP-1*. *FGF23* had a c.716C>T in the heterozygote form (p.THR239MET). This was initially reported as a change that was previously unreported. However, her unaffected parents were found to have the same change, which is now believed to be a polymorphism without significance to the protein function. Her C-terminus FGF23 was 418 RU/mL (normal <230). Imaging studies, including whole body PET/CT, octreotide scan and MRI, were all normal. She required increasing doses of phosphate, but even with these her serum phosphate declined, her bone pain increased, and she was more limited in her daily activities. A slowing of her linear growth was noted. Further imaging studies done at the NIH revealed a small suspicious areas by PET/CT in her right proximal tibia, as well as the lung, jaw, feet and multiple long bones. Venous sampling with FGF23 determination confirmed the source of her FGF23 to be coming from the right proximal tibia area, which was confirmed by MRI. A 5 mm tumor with features consistent with a phosphaturic mesenchymal tumor was removed from the area. Following the surgery phosphorus normalized (5.2 mg/dL one day post surgery), and 1-25 dihydroxy vitamin D increased. Now, at 3 months post the surgery, she has regained full activity, bone pain has resolved, and there have been no further fractures. Conclusion: This case demonstrates the difficulties in reaching the diagnosis in patients, especially children, with tumor-induced osteomalacia, and the utility of venous sampling in locating the tumors. Nothing to Disclose: GBK, MCD, SSS, AB, WHC, FW, RC, CC, MTC

Pub #	P2-113
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Response in Vitamin D Status after Vitamin D_3 Supplements in Relation to Vitamin D Binding Protein Genotype
Author String	S Saetung, H Nimitphong, S Chanprasertyothin, L-o Chailurkit, B Ongphiphadhanakul Ramathibodi Hospital Mahidol University, Bangkok, Thailand; Ramathibodi Hospital Mahidol University, Bangkok, Thailand
Body	Vitamin D insufficiency is highly prevalent and some individuals with vitamin D insufficiency need vitamin D supplementation. Vitamin D and its metabolites circulate binding to vitamin D binding protein (DBP) and genetic variation in DBP is associated with 25-hydroxyvitamin D levels. It is currently unclear how the responsiveness in vitamin D status will be affected by DBP genetic variation. In the present study, we investigated the change in serum 25OHD levels after supplementation with vitamin D3 for 6 months. Twenty healthy volunteers were recruited in this study. The volunteers were given multivitamin containing vitamin D3 400 IU/day for 6 months. 25(OH)D2 and 25(OH)D3 were measured by liquid chromatography/mass spectrometry. DBP genotype based on the rs416572 in DBP was determined by real-time PCR. Data were presented as mean±SE. After vitamin D3 supplementation, 25(OH)D3 increased significantly at both 3 months (+ 6.5±1.7 ng/mL, $P < 0.001$) and 6 months (+ 5.9±1.5, $P < 0.001$). Subjects were then divided into 2 groups, those homozygous for the C allele in rs416572 (n =12) and the rest (n= 8). There was not difference in age, gender, body mass index (BMI) and baseline 25D2, 25D3 and total 25D between the two groups. The increases in 25D3, however, wer significantly higher in group 1 compared to group 2 at both 3 months (+9.2±2.4 vs. +2.4±1.2, $P < 0.05$) and 6 months (+8.3±2.2 vs. +2.0±1.4, $P < 0.05$) after vitamin D supplementation. The changes in serum parathyroid hormone, however, were not different between the 2 groups both at 3 months and 6 months after vitamin D supplements. Conclusions Vitamin D binding protein genotype affects response in serum 25(OH)D after vitamin D3 supplementation without significantly influencing response in parathyroid hormone. Nothing to Disclose: SS, HN, SC, L-OC, BO

Pub #	P2-114
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Prevalence and Predictors of Vitamin D Deficiency in Healthy Young Adults
Author String	DM Mitchell, MP Henao, JS Finkelstein, S-AM Burnett-Bowie Massachusetts General Hospital, Boston, MA
Body	Vitamin D deficiency is associated with impaired bone mineralization and may be a risk factor for other healt conditions. The prevalence of vitamin D deficiency in healthy young adults is not well described. **Methods**: We measured serum 25-hydroxyvitamin D (25OHD) by chemiluminescent immunoassay (*DiaSorin*, Stillwater, MN) in an ethnically diverse cohort of 634 adults aged 18-50. Age, sex, race, and ethnicity data were obtained. A subset (n=406) was assessed for multivitamin (MVI) use. Subject recruitment occurred in Boston, MA, year-round, from January 2006 through April, 2008. Subjects had no known health conditions that might affect vitamin D absorption or metabolism and were excluded if taking >2000 international units o vitamin D daily. **Results**: 39% of subjects had vitamin D deficiency (serum 25OHD [le] 20 ng/mL), and 7% had severe deficiency (25OHD [le] 10 ng/mL). Predictors of lower serum 25OHD values in univariate analyses included male sex; black, Asian, or [ldquo]other[rdquo] race; and lack of MVI use (p<0.001 for all predictors). 16% of MVI users had deficiency compared with 42% of non-users (RR 2.6, p<0.001). Serum 25OHD levels varied by age in females (p<0.001) but there was no variation by age in males (p=0.91). There was significant seasonal variation in serum 25OHD with highest values in summer and lowest values in spring (p<0.001). The magnitude of variation was less prominent in Asian subjects as compared to white subjects and was not significant in black subjects. Seasonal variation was also not seen among MVI users. Using logistic regression, we developed a clinical score consisting of non-white race (8 points), absence of MVI use (6 points), male sex (3 points), and age ((age in years - 18)*0.1) with a higher score predicting a higher likelihood of deficiency. A score of [ge] 6 had a sensitivity of detecting deficiency of 95% and a negative predictive value (NPV) of 92%. A more lenient cutoff of [ge] 7 had a sensitivity of 88% and a NPV of 88%. 78% and 65% of our cohort had scores of [ge] 6 and [ge] 7 respectively. **Conclusions**: Vitamin D deficiency (25OHD [le] 20 ng/mL) is prevalent in healthy young adults in the Boston area irrespective of race and ethnicity. Black and Asian adults are at highest risk of deficiency and have less seasonal variation in 25OHD levels. Routine use of multivitamins appears to be protective. We present a clinical score that may guide decisions regarding screening for deficiency. This score awaits validation in future prospective studies. Sources of Research Support: NIH grants K23-DK-073356 to SMB and M01-RR-01066; the Massachusetts General Hospital Physician-Scientist Development Award; and the Boston Area Diabetes and Endocrinology Research Center Grant. Nothing to Disclose: DMM, MPH, JSF, S-AMB-B

Pub #	P2-115
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Associations between 25(OH)D and Clinical Indicators of Vascular Function among Apparently Healthy African-American and European-American Adults: Relationships May Differ with Ethnicity
Author String	JA Alvarez, BA Gower, DA Calhoun, SE Judd, Y Dong, T Dudenbostel, AP Ashraf University of Alabama at Birmingham, Birmingham, AL; University of Alabama at Birmingham, Birmingham, AL; University of Alabama at Birmingham, Birmingham, AL; Medical College of Georgia, Augusta, GA; University of Alabama at Birmingham, Birmingham, AL
Body	**Background:** Vitamin D may influence vascular function. The purpose of this study was to determine if serum 25-hydroxyvitamin D [25(OH)D] is associated with various clinical indicators of vascular function, and to determine if lower 25(OH)D among African Americans (AAs) compared to European Americans (EAs) contributes to ethnic disparities in vascular function. **Subjects and methods:** Subjects were healthy, AA (n = 23) and EA (n = 22) adults ages 18-50 years with no apparent vascular dysfunction. The main outcomes were augmentation index adjusted for a heart rate of 75 beats/min (AIX75), carotid-femoral pulse wave velocity (PWV), and central aortic pressure (CAP) determined using applanation tonometry, as well as flow-mediated dilation (FMD) determined using ultrasound. Seated and supine blood pressures were also examined. Percent body fat was determined with dual energy X-ray absorptiometry. **Results:** Mean 25(OH)D was 15.2 ± 5.1 ng/ml in AAs and 28.8 ± 9.4 ng/ml in EAs ($p<0.001$). Serum 25(OH)D was significantly associated with AIX75 (standardized $\beta = -0.29$, $p = 0.01$), supine SBP (standardized $\beta = -0.32$, $p = 0.02$), central aortic SBP (standardized $\beta = -0.33$,$p = 0.01$) and central aortic DBP (standardized $\beta = -0.43$, $p = 0.004$), independent of age, sex, and percent body fat. In similar analyses within each ethnic group, 25(OH)D was associated with AIX75 (partial $r = -0.44$, $p = 0.05$), PWV (partial $r = -0.42$, $p = 0.07$), and FMD (partial $r = 0.42$, $p = 0.08$) among AAs, but not EAs ($p = 0.44$ to 0.96). The relationships of 25(OH)D with AIX75 and PWV were independent of supine and central aortic SBP. Relative to EAs, AAs had 58% greater AIX75 (9.37 ± 1.73 vs. 3.92 ± 1.89, $p = 0.06$) and 13% lower FMD (8.97% vs 10.25%, $p = 0.16$) after adjusting for age, percent body fat, and TV hrs/wk. Further adjustment for 25(OH)D reduced the ethnic differences in AIX75 to 41% (8.46 ± 1.94 vs. 4.98 ± 2.15, $p = 0.31$) and FMD to 7% (9.23 ± 0.64 vs. 9.97 ± 0.66, $p = 0.50$). **Conclusions:** Among all subjects, serum 25(OH)D was independently, inversely associated with AIX75, supine SBP, and CAP. Ethnic differences in the relationships of 25(OH)D with vascular outcomes emerged, such that 25(OH)D was associated with AIX75, PWV, and FMD among AAs, but not EAs. Furthermore, lower 25(OH)D among AAs may contribute to their greater AIX75 and lower FMD compared to EAs. Sources of Research Support: UAB Diabetes Research Training Center P60 DK-079626 and Child Health Research Center Grant K12 HD043397 (T0909180013) awarded to APA; American Heart Association (Greater Southeast Affiliate)Predoctoral Fellowship awarded to JAA. Nothing to Disclose: JAA, BAG, DAC, SEJ, YD, TD, APA

Pub #	P2-116
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	A Case of Hereditary Vitamin D-Resistant Rickets (HVDRR) without Alopecia: A Novel Mutation
Author String	KE Huang, PJ Malloy, D Jeandron, D Feldman, P Pitukcheewanont Children's Hospital Los Angeles, Los Angeles, CA; Stanford University School of Medicine, Stanford, CA
Body	Background: We report a novel mutation in a case of HVDRR without alopecia. Clinical Case: A 22 month old Hispanic male (length -3.4 SDS; weight -2.1 SDS) presented with failure to thrive, short stature, and gross motor delay. He did not have alopecia. Family history was negative for consanguinity. Initial history suggested vitamin D deficiency with a diet predominantly breast milk, limited sun exposure, and a maternal history of vitamin D deficiency. Initial blood tests showed severe hypocalcemia and secondary hyperparathyroidism: calcium 5.1 mg/dL, (8.8-10.8); phosphorous 4.1 mg/dL, (4.5-5.5); alkaline phosphatase 1481 U/L, (80-220); intact PTH 537.1 pg/mL, (10-71). A skeletal survey x-ray revealed severe osteopenia, metaphyseal changes consistent with rickets, and multiple fractures of the long bones. Treatment was initiated with intravenous calcium gluconate and enteral calcium glubionate, high dose ergocalciferol, and high dose calcitriol. He was noted to have significant [ldquo]hungry bones syndrome [rdquo] with persistent and profound hypocalcemia despite escalating doses of enteral calcium therapy, and eventually required continuous intravenous calcium gluconate therapy. Subsequently, vitamin D studies returned as follows: 25-hydroxyvitamin D 34 ng/mL, (20-100); 1,25-dihydroxyvitamin D 507 pg/mL, (31-87) This suggested vitamin D resistant rickets, and this diagnosis was confirmed by DNA sequencing. His subsequent hospital course was complicated by the fact that his calcium levels could not be well controlled on enteral calcium citrate or enteral calcium glubionate therapy. Eventually, using calcium gluconate (intravenous formulation) enterally, we were able to maintain calcium levels above 7 mg/dL. Genetic Studies: A unique homozygous T to C base substitution was found in exon 6 in the VDR gene. This mutation converts leucine to proline at position 227 in helix 3 in the ligand binding domain (LBD). This mutation would be expected to render the vitamin D LBD non-functional, leading to HVDRR, without alopecia. The LBD mutations that do not interfere with retinoid X receptor (RXR) dimerization are usually no associated with alopecia. Conclusion: We found a novel LBD mutation of the VDR gene in a child with HVDRR. Despite the absence of alopecia, HDVRR should be considered in a patient with profound hypocalcemia that is refractory to conventional therapy of vitamin D deficiency rickets. Nothing to Disclose: KEH, PJM, DJ, DF, PP

Pub #	P2-117
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Four Cases of Hypervitaminosis D Following Treatment for Vitamin D Deficiency
Author String	MB Vanstone, SE Oberfield, TO Carpenter Yale University School of Medicine, New Haven, CT; Columbia University Medical Center, New York, NY
Body	Background: Pharmacologic vitamin D (D) is reserved for D deficiency rickets or hypocalcemia; recommended dosing has been assumed to be safe. Clinical Case(s) (1) An infant girl was treated for craniotabes with 1000-1400IU of D daily during her first 2 mos of life. 25OHD at 2 wks was 21 ng/mL; at 2 mos, hypercalcemia (10.7 mg/dl) was present. 25OHD was 84 ng/mL. D was reduced to 400IU daily. At 5 mos, hypercalcemia persisted (11.0 mg/dL); 25OHD was 78 ng/mL. D was discontinued. Serum calcium (Ca) was normal by 6 mos. (2) An exclusively breastfed 4 mo-old African-American boy presented with seizures and rickets. Serum Ca was 5.5 mg/dL, alkaline phosphatase activity (AP) was 1110U/L, PTH was twofold elevated, 25OHD was < 5ng/ml, and 1,25(OH)2D was 7 pg/mL. After correction of serum Ca, he received 100 mg/kg/day of oral Ca, 4000 IU of D/day, and calcitriol (0.5 mcg/day). 1 wk later, serum Ca was 10.3 mg/dL and 25OHD was 33 ng/ml. Ca and D were decreased by half, and calcitriol was discontinued. 6 wks later hypercalcemia (10.4 mg/dL) persisted and 25OHD was 79 ng/mL. All supplementation was stopped and hypercalcemia resolved 6 wks later (Ca 10.1 mg/dL, 25-OHD 40 ng/mL). (3) A 2-9/12 yr-old African-American girl presented with rickets; she had been exclusively breast-fed until 5 mos of age without D. She now drank 12-18 oz of milk/day. Biochemical findings (Ca 9.9 mg/dL, AP 661 U/L, PTH 102 pg/mL, 25OHD 5 ng/mL) indicated D deficiency and 2000IU/day of D was begun. 3 mos later hypercalcemia (10.9 mg/dL) was present and 25OHD was 102 ng/mL. D was discontinued with normalization of serum Ca (9.9 mg/dL) 3 mos later; 25OHD was 24 ng/ml. (4) A 3-4/12 yr-old previously healthy girl presented with several wks of fatigue, vomiting, constipation, headaches, and recent polydipsia and nocturia. She was given oral D (600,000 IU/vial) in another country, receiving 6 vials (3,600,000 IU) over 3 wks. Serum Ca was 17.4 mg/dL and 25OHD was 300 ng/mL. A sonogram revealed mild medullary nephrocalcinosis. Acute hydration, furosemide, and dietary Ca restriction, with the later use of a single pamidronate dose corrected the serum Ca. Conclusion: Hypervitaminosis / hypercalcemia can be unpredictable in small children. D therapy in this age group must be closely monitored as hypercalcemia occurred in 3 of our cases using dosages within well-recognized recommendations. Treatment guidelines for D deficiency may require modification for this age group. Disclosures: TOC: Consultant, Kyowa Hakko Kirin Pharma. Nothing to Disclose: MBV, SEO

Pub #	P2-118
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Vitamin D_2 and D_3 Replacement Effectiveness in Patients with Liver Disease
Author String	DA Krajewski, NJ Jackson, J Barsony Georgetown University Hospital, Washington, DC; Georgetown University, Washington, DC
Body	High prevalences of vitamin D deficiency and osteoporosis have been reported in patients with chronic liver disease (LD), and a previous study suggested that ergocalciferol (D2) may not be as effective to restore vitamin D sufficiency. Even in patients without liver disease, the effectiveness of D2 versus cholecalciferol (D3) is uncertain. We performed a retrospective analysis of the medical records of outpatients to determine potential differences between the D2 and D3 doses required to normalize vitamin 25-OHD levels in vitamin D deficient patients with normal liver function (controls: CD2, n=28; CD3, n=31) and with chronic liver disease (LD2, n=12; LD3, n=14). Data are presented as means ± SD, and were analyzed by ANOVA and Holm-Sidak test for multiple pairwise comparisons, and t-tests, as applicable. Patients with renal insufficiency, hypercalcemia, or taking medications known to alter vitamin D metabolism were excluded. There were no significant differences between the four groups in age (58.8±13.4 years), race (patients with dark skin 43.8±4.3%), baseline serum 25-OHD (20.9±6.9 ng/mL), PTH levels (43.2±29.9 pg/mL), BMI (27.2±5.1 kg/m [sup2]), and duration of treatment until reaching vitamin D sufficiency (120.2±79.2 days). Results showed that the LD2 group had a significantly lower endpoint 25-OHD than the LD3 group (38.1±6.1 vs. 65.0±20.8 ng/mL, $p<0.001$), and therefore a lower increment of 25-OHD ($p<0.01$). Both controls and LD patients required higher cumulative doses of D2 (CD2: 767,143±503,954 IU; LD2: 1,051,667±487,327 IU), than D3 (CD3: 334,456±210,165 IU; LD3: 563,028±314,918 IU)($p<0.01$), whereas the difference between control and LD groups in cumulative D3 doses was less pronounced ($p=0.135$, NS). The daily D2 dose required to raise 25-OHD level by 1 ng/mL was much higher in patients with LD than in controls (LD2: 450.3±350.5 IU; CD2 196.5±116.8 IU, $p<0.01$), whereas the difference in the daily D3 dose required to raise 25-OHD by 1ng/mL was less pronounced (LD3: 195.2±151.6 IU; CD3: 124.2±117.5 IU, $p=0.392$, NS). Our results indicate that D3 is a more effective treatment for vitamin D deficiency in patients with chronic LD, and that the D3 requirement is higher in LD patients than in controls. These findings indicate that vitamin D metabolism, is impaired in LD, likely due to accelerated rate of catabolism or excretion. Abnormal vitamin D metabolism in LD likely contributes to the known higher osteoporosis, fracture and mortality rates in LD patients. Nothing to Disclose: DAK, NJJ, JB

Pub #	P2-119
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Serum 25-Hydroxyvitamin D Is Associated with Reduced Risk of Metabolic Syndrome at 5 Years: Results from a National, Population-Based Prospective Study (The Australian Diabetes, Obesity and Lifestyle Study -- AusDiab)
Author String	C Gagnon, ZX Lu, DJ Magliano, DW Dunstan, JE Shaw, PZ Zimmet, K Sikaris, N Grantham, PR Ebeling, RM Daly NorthWest Academic Centre, The University of Melbourne, Melbourne, Australia; Faculty of Medicine and Health Sciences, The University of Sherbrooke, Sherbrooke, Canada; Melbourne Pathology Services, Melbourne, Australia; Monash Medical Centre, Melbourne, Australia; Baker IDI Heart and Diabetes Institute, Melbourne, Australia; School of Population Health, University of Queensland, Brisbane, Australia; Edith Cowan University, Perth, Australia; Deakin University, Melbourne, Australia
Body	[Bold]Background[/Bold]: Cross-sectional epidemiological studies suggest serum 25-hydroxyvitamin D (25OHD) levels are associated with metabolic syndrome (MetS). However, whether serum 25OHD predicts incident MetS remains unclear. [Bold]Objective[/Bold]: To evaluate whether serum 25OHD was associated with incident MetS in a large national, population-based prospective cohort of adults aged [ge]25 years. [Bold]Participants and Methods[/Bold]: 6,537 of the 11,247 adults evaluated in 1999-2000 in the AusDiab study returned for follow-up in 2004-2005. We studied those without MetS who had complete data including serum 25OHD at baseline (n=3,981; mean age 50; 57% women; 93% Europids). Associations between serum 25OHD and 5-year incidence of MetS [modified 2009 criteria (1), at least 3/5 of the following: waist circumference (WC) [ge]102 cm for men and [ge]88 cm women of European origin or [ge]90 cm for men and [ge]80 cm for women of Non-European origin; triglycerides [ge]1.7 mmol/L; HDL-C <1.0 mmol/L for men and <1.3 mmol/L for women; systolic blood pressure [ge]130 and/or diastolic blood pressure [ge]85 or on treatment; fasting glucose [ge]5.6 mmol/L or known diabetes] and its components were assessed using logistic and linear regression analyses, respectively, after adjusting for multiple confounders. [Bold]Results[/Bold]: 509 incident cases of MetS were diagnosed. Those who developed MetS had lower serum 25OHD (median 60 vs. 65 nmol/L; P<0.001) than those who did not. The risk of developing MetS decreased with increasing quartiles of serum 25OHD (P for trend <0.001). Compared with those in the lowest quartile (<50 nmol/L), the risk of MetS was 39% lower [OR, 95% CI: 0.61 (0.45-0.83)] in those in the highest quartile ([ge]81 nmol/L) after adjusting for age, sex, ethnicity, season, latitude, smoking, physical activity, family history of diabetes, education and dietary calcium intake (model 1). Linear regression analysis showed that serum 25OHD was inversely associated with the following components of MetS at follow-up after adjusting for confounders in model 1, WC and the baseline outcome variables: WC (P<0.01), fasting glucose (P=0.04) and 2h plasma glucose (P=0.02). Serum 25OHD was not associated with fasting triglycerides (P=0.07), HDL-C levels (P=0.23), systolic (P=0.86) or diastolic blood pressure (P=0.053). [Bold]Conclusion[/Bold]: In Australian men and women, higher serum 25OHD levels were associated with a reduced risk of MetS, lower WC, fasting and 2h plasma glucose at 5 years. (1) Alberti KG et al., Harmonizing the Metabolic syndrome: A joint interim statement of the International Diabetes Federation task force on epidemiology and prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and Internationa Association for the Study of Obesity. Circulation 2009; 120:1640-45. Nothing to Disclose: CG, ZXL, DJM, DWD, JES, PZZ, KS, NG, PRE, RMD

Pub #	P2-120
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Vitamin D Toxicity: Too Much of a Good Thing
Author String	A Gore, BJ Hull Medical University of South Carolina, Charleston, SC
Body	Introduction: Vitamin D (Vit D) is a fat-soluble vitamin essential for bone mineralization and calcium homeostasis. Growing evidence also suggests that it has multi-systemic, extra-skeletal benefits (1). Over the counter (OTC) Vit D supplements, although not FDA approved, are commonly recommended for treatment o Vit D deficiency. We present a case of severe and protracted hypercalcemia in a woman who took massive amounts of cholecalciferol for 2 months. Case Report: A 63yo female with morbid obesity, HTN, DM2, CAD, COPD, fibromyalgia and depression was admitted with acute confusion. History was notable for ankle fracture two months prior. Physical exam revealed an obese, chronically ill appearing female, with a left leg cast. Blood work showed elevated Calcium of 13.6 mg/dl (8.4-10.2 mg/dl), Corrected Ca 14.6mg/dl, BUN 47mg/dl (8-20 mg/dl) ,Cr 2.9mg/dl (0.4-1 mg/dl), iPTH <3pg/ml (14-72 pg/ml). Patient was admitted for altered mental status (AMS) and acute renal failure and had a prolonged hospital stay. Workup for non-PTH mediated hypercalcemia revealed no offending medications, granulomatous diseases or malignancy. Serum 1, 25 dihydroxy Vit D was 79 pg/ml (15-75 pg/ml) and 25OH Vit D was markedly elevated at 218 ng/ml (25-80 ng/ml). Patient was treated with IV fluids, Furosemide, Calcitonin, Pamidronate and Prednisone. Mental status and renal failure improved to baseline. Patient reported taking OTC Vit D3 (50,000 IU TID) for 2 months prior to admission. Her PCP had recommended taking OTC supplements for treatment of Vit D deficiency. DISCUSSION: Vit D toxicity is a well described albeit extremely rare complication. The handful of reported cases have mostly involved accidental overdose (2). There is a recent trend towards aggressive supplementation to maintain 'optimal levels'. Our case illustrates that Vit D toxicity, though rare, can cause significant morbidity and life-threatening complications. Elderly patients and those with renal failure may be at higher risk. We would like to heighten awareness in the medical community about the easy availability of OTC D3-50 (Cholecalciferol 50,000IU). We caution against the unsupervised use of this, as well as other vitamin supplements. OTC vitamins, minerals and herbal supplements should be closely reviewed during medication reconciliation. (1) Holick MF,Vitamin D: extraskeletal health.Endocrinol Metab Clin North Am. 2010 Jun;39(2):381-400 (2) Vieth R, Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. Am J Clin Nutr 1999; 69:842-856. Nothing to Disclose: AG, BJH

Pub #	P2-121
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Vitamin D Status and Nonalcoholic Fatty Liver Disease in Koreans: Korean Genome Epidemiologic Study (KoGES)
Author String	JA Seo, CR Eun, H Cho, YJ Kim, MJ Cho, JH Kim, HY Choi, SJ Yang, HJ Yoo, HY Kim, SG Kim, KM Choi, SH Baik, DS Choi, HJ Yim, C Shin, NH Kim College of Medicine, Korea University, Seoul, Republic of Korea; College of Medicine, Korea University, Seoul, Republic of Korea; College of Medicine, Korea University, Seoul, Republic of Korea
Body	Although circulating vitamin D level has an inverse relationship with body fat content, the association of circulating vitamin D levels and nonalcoholic fatty liver disease (NAFLD) has not been known in population-based studies. Therefore, we examined whether vitamin D levels have an association with NAFLD and body composition in apparently healthy Koreans. 25-hydroxyvitamin D [(25(OH)D] levels were measured in the cross-sectional samples of 488 men and 725 women (mean age 57.0±7.4 years) from an ongoing, prospective, population-based cohort study after excluding subjects with viral hepatitis or [ge]140 g/week of alcohol drinking. Visceral fat area (VFA) and liver minus spleen attenuation (LSA), which has inverse relationship with hepatic fat accumulation were measured by abdominal CT scanning. LSA <5 was defined as NAFLD, and 25(OH)D level <20 ng/ml was defined as vitamin D deficiency. About 27.2% of men and 17.6% of women had NAFLD and 69.1% of men and 83.2% of women had vitamin D deficiency. Low 25(OH)D levels were strongly associated with high liver fat accumulation, VFA and triglycerides levels in men after adjusting for age, season, and multivitamin intake (p<.0001, 0.001, and <.0001, respectively), but no significant association was found in women. In men, hepatic fat accumulation and triglyceride levels were significantly increased in subjects with lowest quartile of month-matched 25(OH)D compared to those with the highest quartile (p for trend 0.0009 and 0.002, respectively) after adjusting for age, body mass index, exercise and vitamin intake. Adjusted odds ratios (95% confidence interval) for the presence of NAFLD in the lowest quartile compared with the highest quartile of 25(OH)D was 3.56 (95% CI=1.44-8.75) in men and 1.88 (95% CI=0.98-3.61) in women. Vitamin D deficiency is common and highly correlated with NAFLD in Korean men. Nothing to Disclose: JAS, CRE, HC, YJK, MJC, JHK, HYC, SJY, HJY, HYK, SGK, KMC, SHB, DSC, HJY , CS, NHK

Pub #	P2-122
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Double-Blind Randomized Study To Determine the Efficacy of Intramuscular Vitamin D_3 Supplementation ir Tropical Chronic Pancreatitis
Author String	E Bhatia, SVB Reddy SGPGIMS, Lucknow, India
Body	**Background:** Tropical calcific pancreatitis (TCP) is an idiopathic chronic pancreatitis prevalent in developing regions of the world. As with other forms of chronic pancreatitis, vitamin D deficiency is common in TCP patients. The optimal route and dose of vitamin D supplementation in chronic pancreatitis are not known. **Aims:** To evaluate the efficacy of 2 different dose schedules of intramuscular (i.m.) vitamin D3 supplementation in normalizing serum 25-hydroxyvitamin D (25OHD) in TCP patients. **Study design:** Double blind randomized placebo controlled study **Patients and methods:** Forty TCP patients (age mean$\pm$SD 33$\pm$9 years, baseline 25OHD 10.8$\pm$ 5.8 ng/ml) with vitamin D insufficiency (25OHD <30 ng/ml) were randomized into 3 groups: Group 1: single injection o 600,000 IU i.m. vitamin D3, Group 2: single injection of 300,000 IU i.m. vitamin D3 and Group 3: placebo injection. All 3 groups received 1 gm calcium and 500 IU vitamin D3 orally daily and were followed for 9 months. Serum 25OHD, corrected calcium, phosphorus and alkaline phosphatase were measured at baseline, 1, 3 and 6 months after injection. Thirty-four patients who completed the study (Group 1, 2 and 3: 13, 11 and 10 patients respectively) were included for per-protocol analysis. The primary outcome measure was the proportion of patients in each group with 25OHD >30 ng/ml at 6 months after treatment. **Results:** Baseline 25OHD were similar in all 3 groups. The primary end-point was achieved in 85% in Group 1, 27% in Group 2 and 0% in Group 3 ($p < 0.01$). The proportion of patients with 25OD>30 ng/ml in Group 1 was significantly higher than Groups 2 ($p=0.01$) or 3 ($p < 0.01$). 25OHD at 0, 3 and 6 months in Group 1 (12.3 $\pm$ 5.6, 49.6 $\pm$ 16.5 and 37.9 $\pm$ 9.9 ng/ml) and Group 2 (10.2 $\pm$ 7.1, 33.2 $\pm$ 9.5 and 26.3 $\pm$ 7.4 ng/ml) increased significantly ($p<0.01$ at all time points compared to baseline). In the placebo group, 25OHD remained in deficient range throughout. Serum alkaline phosphatase at 6 months was significantly reduced as compared to baseline in Group 1 (230 $\pm$ 20 vs. 165 $\pm$ 11 IU/L, $p < 0.05$), but not in Groups 2 or 3. No patient in any group developed hypervitaminosis D or hypercalcemia. **Conclusions:** In TCP, a single dose of i.m. vitamin D3 (600,000 IU) is an effective and safe form of vitamin D supplementation over a period of 6 months. Clinical Trials Number NCT00956839 Nothing to Disclose: EB, SVBR

Pub #	P2-123
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Only Cholecalciferol on Top of Ongoing Calcitriol and Calcium Treatment Allowed Restoration of Severe Hypocalcemia in a Patient with CKD: Is 25(OH)D Status More Important for Intestinal Calcium Absorption Than We Think It Is?
Author String	K Amrein, H Worm, G Schilcher, P Krisper, H Dobnig Medical University Graz, Graz, Austria; Medical University Graz, Graz, Austria; Medical University Graz, Graz, Austria
Body	**Background** Calcitriol deficiency parallels the loss of renal function; current guidelines include replacement therapy in chronic kidney disease (CKD). It is, however, under debate whether 25-hydroxyvitamin D [25(OH)D] deficiency should also be treated. We report a patient with severe hypocalcemia unresponsive to regular oral calcitriol and cholecalciferol therapy. **Case Report** A 49-year-old man with CKD stage IV presented with diffuse bone pain and pronounced muscle weakness. Furthermore, he complained of diarrhea and had lost 16 kg during the past year. He received oral calcitriol (1 [micro]g per day) and calcium (2000 mg). Laboratory results were remarkable for severe hypocalcemia (ionized serum calcium, 0.77 mmol/l, normal 1.15-1.35) and hyperparathyroidism (parathyroid hormone [PTH], 780 pg/ml, normal 15-65) despite normal calcitriol levels (62pmol/l, normal 48-110). His serum 25 (OH)D level was low (10 ng/ml, normal 28-58). A full medical examination including magnetic resonance cholangiopancreaticography led to a diagnosis of exocrine pancreatic insufficiency. The calcitriol dose was increased to 2 [micro]g and oral vitamin D3 was added (4000 IU daily). Additionally, lipase/amylase replacement therapy was started. Despite boosted serum calcitriol (163 pmol/l), hypocalcaemia (total serum calcium, 1.76 mmol/l), hyperparathyroidism (1009 pg/l) and vitamin D deficiency (6 ng/ml) persisted. A transiliac crest biopsy showed a remarkable degree of osteomalacia. The patient received five im injections of 100.000 IU cholecalciferol each at short intervals of a few weeks. Several months later, the patient was doing considerably better. His 25(OH)D serum level and his calcitriol serum level had increased to 95 ng/mL and 199 pmol/ml, respectively, whereas PTH had decreased to 371 pg/ml and ionized serum calcium levels almos normalized (1.14 mmol/l). Taken together, the remarkable rise in serum calcium levels in a previously severe hypocalcemic patient with CKD and malabsorption who was on constant calcitriol and calcium supplementation could only be achieved by correction of low 25(OH)D status through intramuscular injections. **Conclusion** This case demonstrates that 25(OH)D also confers important regulative activity of intestinal calcium absorption. It is likely that the increase of 25(OH)D substrate within the intestinal wall was a prerequisite to ensure sufficiently high local concentrations of 1,25(OH2)D that could not be achieved by calcitriol supplementation alone. Nothing to Disclose: KA, HW, GS, PK, HD

Pub #	P2-124
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	A Case of Calcitriol-Associated Hypercalcemia from Aesthetic Surgery Injections
Author String	S Trikudanathan, CB Becker Brigham and Women's Hospital, Boston, MA
Body	Background: Injections of foreign substances into the skin can result in chronic inflammatory reactions and calcitriol-mediated hypercalcemia. Clinical case: A 52 year old woman was transferred to our hospital with symptoms of fatigue, generalized weakness, dry mouth and constipation along with newly diagnosed hypercalcemia (serum calcium -17 mg/dL). She denied change in weight or appetite, back pain, kidney stones, or previous history of hypercalcemia. Her past medical history was significant for hypertension and elective breast/body contour augmentation with subcutaneous injections of an unknown substance 10 years earlier in Mexico. Medications included furosemide and multivitamins. Exam on admission was notable for blood pressure of 190/110, dry mucous membranes, and extensive, hard nodular nontender lesions in breasts, buttocks and thighs. Laboratory studies revealed serum calcium (corrected for albumin) 14.6mg/dL (normal, 8.7-10.4), phosphorus -3.0 mg/dL (normal, 2.4-4.3) and creatinine of 1.57 mg/dL (normal, 0.5-1.3). Ionized calcium was elevated at 1.79 mmol/L (normal, 1.13-1.32). Intact PTH was low at 10.5 pg/mL (normal, 15-65), SPEP was normal and PTH related peptide was undetectable. 25 hydroxyvitamin D was low at 9.6 ng/mL (normal, 25-80) and 1, 25 hydroxyvitamin D was 55 pg/mL (normal, 18-78). Bone marrow aspirate was negative for lambda and kappa light chain staining. CT scans of chest, abdomen and pelvis were negative for malignancy. PET-CT scanning did not reveal any bony lesions or FDG avid tumor. However diffuse soft tissue abnormalities indicative of chronic inflammation were noted in the breasts, buttocks and thighs. Punch biopsy from a thigh lesion revealed lymphohistiocytic inflammation at a site of previous aesthetic injections. She was treated with intravenous fluids, a single dose of palmidronate and oral prednisone at 40mg daily. On subsequent follow up her serum calcium normalized to 10.1 mg/dL and 1, 25 hydroxyvitamin D decreased to 35 pg/mL. We believe the most likely cause for the hypercalcemia was chronic inflammation from foreign body injections (most likely silicone) with increased production of 1, 25 hydroxyvitamin D from activated macrophages. Conclusion: Injections of foreign substances for aesthetic purposes can cause inflammatory infiltrates and calcitriol-dependent, PTH-independent hypercalcemia. Nothing to Disclose: ST, CBB

Pub #	P2-125
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Fetal Rickets in a Full-Term African-American Male Born in the City of Buffalo
Author String	M Mogri, C Albini Women and Children's Hospital of Buffalo, Buffalo, NY

Body

Introduction

Clinical rickets and fractures are common in premature or low birth weight infants. In term babies,Vit-D deficiency and nutritional rickets in first couple of months of life usually presents with hypocalcemic seizures or respiratory compromise. We present an asymptomatic term African American male, appropriate for gestational age (GA) with florid clinical signs of rickets at birth.

Case

DW was born in April to a 17 year old mother G1P1 at 37-4/7 weeks GA, birth weight 3.03 kg and length 20.5 inches in Buffalo. Mother was on prenatal vitamins with 400 IU Vit-D during pregnancy. Pregnancy was uncomplicated except maternal hypertension, a known complication of Vit-D deficiency. At birth DW was asymptomatic with no respiratory distress, tremors or seizures but on physical exam was found to have craniotabes, widen wrists and rachitic rosary. Skeletal survey showed widening of metaphyseal zones, cupping of the distal femur, proximal tibia, distal radius and ulna. Electrolytes showed calcium 8.8 mg/dl (8-10.4), elevated phosphate 7.9 mg/dl(3.1-7.5),alkaline phosphatase 269 unit/l(85-330), 1,25-OH Vit-D 22pg/ml (15-25), low 25-OH Vit-D 7ng/dl (30-100) and elevated PTH 259 pg/ml (12-72).Maternal workup showed low calcium 8.4mg/dl (9-10.8) but normal ionized calcium 4.9 mg/dl (4.7-5.3), phosphate 3.7mg/dl (2-5.4), high alkaline phosphatase 248 unit/l (30-150), low 25 OH Vit-D 13ng/dl (32-100) , 1,25 OH vitamin D 63ng/dl (21-65) and PTH 22pg/ml (12-72). DW was started on calcitriol 0.05 mcg/kg/day and 400 IU/day of 25-OH Vit-D. At 3 weeks of age calcitriol was discontinued and 25-OH Vit-D was increased to 3000 IU/day. At 6 months DW's 25-Vit-D level was 56pg/dl and 1,25-OH Vit-D level was 108pg/dl. Repeat X-rays showed healed rickets.

Conclusion

To our knowledge this is the first case report of clinical rickets in a full term appropriate for gestational age infant presenting at birth. African American race and birth in late winter month in Buffalo were the only noted risk factors for the development of Vit-D deficiency in our patient. Current guidelines recommend 200-400IU/day Vit-D supplementation during pregnancy. We suggest screening all pregnant women for Vit-D deficiency and increasing Vit-D supplementation during pregnancy. Further all infants should be screened for clinical signs of rickets at birth allowing early diagnosis and treatment thereby preventing complications of hypocalcemia including seizures, hypotonia, poor growth and fractures.

Nothing to Disclose: MM, CA

Pub #	P2-126
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	25-Hydroxyvitamin D Delayed Response to Seasonal Ultraviolet Radiation Type B Variation: The São Paulo Vitamin D Evaluation Study (SPADES)
Author String	SS Maeda, GL Saraiva, LF Hayashi, MS Cenderoglo, LR Ramos, MP Correa, CH Mesquita, M Lazaretti-Castro Federal University of São Paulo, São Paulo, Brazil; Federal University of São Paulo, São Paulo, Brazil; Federal University of Itajubá, Itajubá, Brazil; University of São Paulo, São Paulo, Brazil
Body	Introduction: Vitamin D synthesis is stimulated by ultraviolet radiation (UVR), which varies seasonally along the year, and is also dependant on the latitude, time of the day and atmospheric compounds. Objective: Evaluate the 25-hydroxyvitamin D levels (25OHD) in subjects in the city of São Paulo (23[deg]34'S), belonging to different age groups and presenting specific behavioral characteristics, and correlate it with UVR. Case: 591 individuals were included, distributed as follows: 177 were living in institutions (NURSING, 76.2±9.0 years old), 243 were part of the community elderly (COMMUNITY, 79.6±5.3 yo), 99 were enrolled in a physical activity program directed to the elderly (ACTIVE, 67.6±5.4 yo) and 72 were young (YOUNG, 23.9±2.8 yo). Methods: 25OHD was measured by a radioimmunoassay. The daily doses of UVR (mJ/cm2) accumulated between 7 am and 5 pm were determined using a sunlight biometer properly calibrated and adjusted every 10 minutes. Results: UVR values vary along the year, following a sinusoidal-like pattern. Because of Earth[acute]s orbit, we thought about a cyclic repetition for 25OHD and UVR values every 12 months. The sinusoidal formulas which predict UVR and 25OHD values according to the month of the year were created using Origin 5.0. The non-linear fitting method was used to fit the UVR intensity and the 25OHD levels to the sinusoidal model P1+P2.sin[-2[pi]/12.(t-P3)]. It was possible to find statistically significant differences in relation to UVR between the four seasons, with UVR being higher during summer and lower during winter ($p<0.001$). All the groups showed significant differences in 25OHD levels, according to the seasons of the year. 25OHD mean levels and amplitude were significantly higher for the groups YOUNG and ACTIVE, when compared to COMMUNITY and NURSING groups. It was possible to observe that while the lowest value for the UVR is measured in June, the lowest 25OHD level is measured in October, corresponding to an average delay of 3.0±0.5 months. For the group COMMUNITY, no correlations were found between 25OHD and the UVR from the same season ($r=-0.03$). However, a strong correlation was found between current 25OHD level and UVR from the previous season ($r=+0.98$, $p<0.001$). Conclusions: A seasonal variation of 25OHD for all the groups studied was demonstrated. The amplitude of variation was higher for young and physically active people, possibly due to the more significant sunlight exposure in these groups. Sources of Research Support: São Paulo Research Foundation Grant 03/13194-6. Nothing to Disclose: SSM, GLS, LFH, MSC, LRR, MPC, CHM, ML-C

Pub #	P2-127
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Evaluation of a Standard Vitamin D Repletion Regimen in Diabetes Mellitus
Author String	N Khoury, KA Carmichael Washington University School of Medicine, Saint Louis, MO
Body	**Background:** Vitamin D may play an important role in diabetes mellitus. Co-morbidities including obesity, lack of sun exposure, renal insufficiency, non-alcoholic fatty liver disease (NAFLD), and diet predipose to hypovitaminosis D. Since adequate vitamin D stores may improve diabetes outcomes, it is important validate replacement regimens. It is not known whether commonly used regimens replete vitamin D stores in this population. **Methods:** In a retrospective chart review at a single large diabetes center, we assessed adequacy of a standard vitamin D repletion regimen of 50,000 IU of ergocalciferol weekly. All subjects were above 18 years of age, non-pregnant, and without significant renal insufficiency (CKD stage 1 or less), hyperparathyroidism, or cystic fibrosis. Those with diabetes mellitus related to corticosteroids, pancreatic insufficiency, or solid organ or bone marrow transplantation were excluded. Anthropometric data, hemoglobin A1c (HbA1c), and presence of diabetic complications and NAFLD were determined. **Results:** 148 patients (mean age 53.5 years) were identified with appropriate 25-hydroxy-vitamin D levels before and after repletion. Mean baseline vitamin D level was 16.8 ng/mL. 62.1% reached a level greater than 35 ng/mL over a mean of 171.6 days. 10% of patients had NAFLD. Age, sex, body mass index (BMI), HbA1c, and presence of NAFLD had no statistically significant effect on baseline vitamin D levels. Only BM had a significant negative predictive value on change in Vitamin D level with repletion. Presence of NAFLD did not affect vitamin D responses. **Conclusions:** A standard vitamin D repletion regimen does not adequately achieve goal vitamin D levels in more than 30% of diabetics. Higher BMI predicts less correction of vitamin D levels with repletion while presence of non-alcohol fatty liver disease appears to have no impact on repletion success. Disclosures: KAC: Speaker Bureau Member, Merck & Co., Bristol-Myers Squibb, Amylin Pharmaceuticals, Lilly USA, LLC. Nothing to Disclose: NK

Pub #	P2-128
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Late-Onset Neonatal Hypocalcemia Due to High Dietary Phosphorus Load in the Setting of Profound Vitamin D Deficiency: Should High-Risk Mothers Be Screened?
Author String	NS Larson, KS Vogt Walter Reed Army Medical Center, Washington, DC; USUHS, Bethesda, MD
Body	Background: Symptomatic neonatal hypocalcemia is a well described clinical entity that may require emergency intervention. Clinical Case: A 5 day old, 37 week Black male presented in December with a 1 day history of intermittent left arm and leg twitching. He was afebrile and acting well, waking for feeds of standard cow's milk formula every 2 hours. His urine and stool output were appropriate. The pregnancy was diagnosed late so precise due date was unsure; prenatal course was otherwise unremarkable. Birth weight and length were 50% for 37 weeks gestation and he had lost <10% of birth weight. He was non-dysmorphic with normal tone, but hyperreflexic on exam. Initial laboratories showed corrected serum calcium of 5.98mg/dL (7.6-10.4mg/dL), phosphorus 10.2mg/dL (4.5-9mg/dL) and magnesium 0.9 mEq/L (1.3-2mEq/L). After difficult intravenous access, ionized calcium of 0.7mmol/L (1.1-1.42mmol/L) was obtained, Chvostek and Trousseau signs were positive and movements progressed to tetany and clinical seizures. Serum iPTH level was elevated at 71pg/mL (<10-65pg/mL); 25-OH vitamin D level was 4ng/mL (5-42ng/mL). Tubular reabsorption of phosphate (TRP) was high at 93%. FISH for 22q11 deletion was negative. Maternal laboratories showed normal serum calcium 10.1mg/dL (8.4-10.2mg/dL), phosphorous 5.6mg/dL (2.5-4.5mg/dL) and iPTH 26 pg/mL (<10-65pg/mL); her 25-OH vitamin D was 14ng/mL (>20ng/dL). She endorsed low dairy intake and was taking a daily multivitamin with 400 international units of vitamin D. The infant was treated initially with intravenous calcium gluconate and magnesium. He was started on oral calcitriol, ergocalciferol and calcium carbonate and weaned from parenteral calcium by day 3. No further magnesium supplementation was required. Calcitriol and calcium were discontinued after 2 weeks with normal follow-up labs. He was maintained on oral ergocalciferol for repletion. Conclusions: Factors contributing to this infant's hypocalcemia include vitamin D deficiency (secondary to maternal vitamin D deficiency), the high phosphorous load of standard formula coupled with relative PTH resistance (age-related and known effect of vitamin D deficiency), and hypomagnesemia. The etiology of his hypomagnesemia remains unclear. Maternal vitamin D deficiency is an easily modifiable risk factor that might have been predicted in this case of a Black infant born in the winter at 39*91'N latitude to a mother with low dairy intake. Stephanie C Hsu, Michael A Levine. Perinatal calcium metabolism: physiology and pathophysiology. Seminars in Neonatology (2004) 9, 23-36 Nothing to Disclose: NSL, KSV

Pub #	P2-129
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Serum 25-Hydroxyvitamin D Status from 2004 to 2010 among Middle-Aged and Elderly Koreans: Hallym Aging Study
Author String	OH Ryu, M-G Choi, D-M Kim, HJ Yoo, DH Kim Hallym University College of Medicine, Chun-cheon, Republic of Korea; Hallym University College of Medicine, Chun-cheon, Republic of Korea
Body	Background: Vitamin D deficiency has become a health problem globally due to its increasing prevalence and potential health risks. But there are little data in the status and trend of vitamin D deficiency in Korea. This study was conducted to investigate the severity and trend of vitamin D deficiency in middle-aged and elderly Korean populations. Methods: Hallym aging study (HAS) is a cohort study to evaluate the quality of life in aged population (aged 45 years or older) in Chuncheon city, South Korea since 2003. Data from a comprehensive questionnaire, a physical examination, and blood tests were obtained from the participants in HAS from 2004 to 2010 every 3 years. Serum 25-hydroxyvitamin D [25(OH) D] was measured using a serum preserved at -80[deg]C by chemiluminescence immunoassay in 324 participants (male=148, female=176) completed all the 3 survey. The data of 25 (OH) D in 2004 were compared with that of 2010 by paired t-test. Results: The mean age was 68.8±7.2 (men) and 66.1±7.8 (women) at 2004. The serum 25(OH)D concentrations of the male participants were significantly decreased from 16.1±7.1 ng/mL in HAS 2004 to 12.9 ±6.9 ng/mL in HAS 2010 (P<0.001). The concentrations of 25(OH) D in the female participants were non-significantly decreased from 12.8 ±5.9 ng/mL to 11.7±7.0 ng/mL during the same study period (p=0.133) The age-adjusted prevalence of 25 (OH) D deficiency (<20 ng/mL) was increased from 63.6.5% to 78.4%% during study period in men. In women, the prevalence was increased from 78.4% to 83.3%. Conclusions: Vitamin D deficiency was very severe in middle-aged and elderly Koreans. In middle-aged and elderly men, the decline of vitamin D concentration was steeper than that of women. Nothing to Disclose: OHR, M-GC, D-MK, HJY, DHK

Pub #	P2-130
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	25-Hydroxyvitamin D Levels in Healthy Adults in Hawaii
Author String	JR Kothapally, L Armas, M Akhter, JA Chang Creighton University, Omaha, NE; Pulmonary Associates, Aiea, HI
Body	Vitamin D deficiency has been reported in 30% - 50% of adults and children in the lower contiguous United States. Latitude, altitude, season, skin pigmentation, and age are recognized factors that influence how much vitamin D can be made by solar exposure. The aim of this study was to quantify how much sun and skin exposure influence vitamin D status. We report here 25(OH)D levels in healthy adults residing in and around Honolulu, Hawaii (latitude 21.3[ordm]N). The subjects (n=90, age 20-59 yr, females = 63 , males = 27) were a convenient sample of healthy, community-dwelling adults with limited non-solar sources of vitamin D. They reported their race as: 25 Asian/Pacific Islander, 1 Black, not Hispanic, 1 Hispanic, 56 White, not Hispanic, 4 reported more than one. Data were gathered during March and August. We determined BMI, 25(OH) D levels, sun exposure history and skin exposure history. We used a portable colorimeter that utilizes the CIE L*a*b* color system to measure constitutive skin color of the upper hip and facultative skin color of the dorsal surface of the hand. The mean (± SD) 25(OH)D levels were 96.9 nmol/L (50.7) with a range from 41-351 nmol/L. 39 (43%) of the study population had 25(OH)D levels less than 80 nmol/L. The subjects reported a mean (SD) of 34 (±21) hours of sun exposure during a typical week with 41% of skin exposed (equivalent of wearing shorts and T shirt). A sun exposure index was calculated by multiplying the average weekly duration of sun exposure by the percentage of skin exposed during weekdays. There was a correlation between 25(OH)D levels and sun exposure index (Pearson correlation 0.590, P<.0001). The point at which 95% of the subjects were above 80 nmol/L occurred at a sun index of 15, the equivalent of 16 hours of sun exposure per week in a bikini (90% skin exposure). There was also a correlation between 25(OH)D levels and skin tone and an inverse correlation between 25(OH)D levels and age (Pearson correlation 0.264 and -0.278 respectively, P<0.05). In conclusion, even in the tropics vitamin D status depends on a certain level of sun and skin exposure and casual exposure of hands and face does not appear to be enough. This study gives data to quantify how much sun exposure our skin needs to reach a certain 25(OH)D level. Nothing to Disclose: JRK, LA, MA, JAC

Pub #	P2-131
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Determinants of Circulating Vitamin D and Bone Density in a Young Adult Physician Population
Author String	B Manickam, NE Villagranan, A Benjamin, T Washington, S Kukreja, E Barengolts University of Illinois, Chicago, IL

Body

Introduction: In this prospective cohort study, we examined the determinants of serum 25-hydroxyvitamin D (25OHD) level and bone mineral density (BMD) in apparently healthy resident physicians and medical students working at an urban hospital.

Materials and Methods: We evaluated a lifestyle questionnaire, serum 25OHD and parathyroid hormone (PTH) levels, and BMD by dual-energy X-ray absorptiometry.

Results: Among 104 participants (mean age 28.1 yrs), there were 25% males and 75% females, 42% whites and 58% non-whites. Prevalence of 25OHD deficiency (25OHD<20 ng/ml), insufficiency (25OHD 20-29 ng/ml), and sufficiency (25OHD[ge]30 ng/ml) was 48%, 30%, and 22%, respectively. Prevalence of normal BMD, osteopenia, and osteoporosis was 63%, 31%, and 6%.

We compared participants with (LowD;<20 ng/ml) and without (Non-LowD) vitamin D deficiency. Compare to Non-LowD, LowD group had lower BMD in femoral trochanter (TR, p=0.02), and a trend towards lower BMD in total hip (Hip, p=0.07), and femoral neck (FN, p=0.08). LowD group was taking less calcium (p=0.01) and more vit D (p=0.006) supplements, drank less coffee (p=0.03), performed more weight bearing exercise (p=0.03), and had a lower prevalence of family history of osteoporosis (p=0.047).

In Multivariate analysis, the determinants of 25OHD level for the whole group included gender (p=0.045), race (p<0.0001), vit D supplements (p=0.032), and calcium supplements (p=0.026). These factors explained 45% of the variation in 25OHD level.

The determinants of BMD were analyzed for the whole group. In univariate analysis Hip BMD determinants included age (p=0.027), race (p=0.017), and BMI (p<0.0001), and there was a trend to significance for gender (p=0.052) and exercise (p=0.074). Similarly, determinants of femoral neck BMD included age (p=0.008), race (p=0.043), and BMI (p=0.0007), and potential determinants of LS BMD included race (p=0.082), BMI (0.0009), and PTH (p=0.094). These factors explained 35% of BMD variability for Hip and FN, and 28% of variability for LS.

In multivariate regression analysis only BMI (p<0.0005) remained an independent determinant of BMD for Hip, FN, and LS. There was a trend towards significance for age (p=0.084) for FN BMD, and for 25OHD (p=0.077) and PTH level (p=0.094) for LS BMD.

Conclusion: Young resident physicians and medical students have a relatively high prevalence of vitamin D deficiency and low BMD. Both 25OHD and BMD in this population are determined in part by modifiable risk factors.

1. Multani SK et al.,J Postgrad Med. 2010 Apr-Jun; 56(2):65-70.
2. Tokar K et al.,J Ark Med Soc. 2003 Nov; 100(5):170-5.
3. Reyes MO et al.,Arch Intern Med. 2004 Mar 22;164(6):603-14.

Sources of Research Support: University of Illinois at Chicago (UIC) Center for Clinical and Translational Science (CCTS), Award Number UL1RR029879 from the National Center for Research Resources.

Nothing to Disclose: BM, NEV, AB, TW, SK, EB

Pub #	P2-132
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Vitamin D Deficiency, Lipid Profile, Adipokines, Intrahepatic Lipids and Intima-Media Thickness in Men with HIV Infection
Author String	O Moreno-Perez, C Serna-Candel, R Sanchez-Ortiga, C Escoin, J Portilla, V Boix, E Merino, S Reus, R Alfayate, M Mauri, AM Pico Hospital General Universitario de Alicante, Alicante, Spain; Hospital Clínico San Carlos, Madrid, Spain; Hospital General Universitario de Alicante, Alicante, Spain; Hospital General Universitario de Alicante, Alicante, Spain
Body	Objectives: Recent epidemiological studies have demonstrated an association between vitamin D deficiency (DVD) and cardiovascular risk in the general population. Our goal was to analyze the association between DVD, lipid profile, markers of systemic inflammation (hsCRP, PAI-I, TNF-alpha and its soluble receptors, IL-6, adiponectin), intrahepatic lipids (LFC) and a marker subclinical atherosclerosis [intima-media thickness (IMT)]. Methods: An observational, transversal, in HIV-infected men, naive to ART (naive) or current TAR with an enhanced PI (PI) or current TAR 2-3 NRTIs plus one NNRTI (NN). DVD was defined as 25-OH-vitamin D concentration (DVC) <30 ng / ml (RIA Coat-A-Count, Siemens); hsCRP (turbidimetry kinetics; IMMAGE, Beckmann Coulter, Inc.); adipokines (enzyme immunoassay, Quantikine, R & D Systems; Adiponectin ELISA , Mediagnost). LFC was measured by (1)H magnetic resonance spectroscopy (1.5 T Gyroscan Intera, Philips Medical Systems). IMT, Hitachi EUB-5500HV, Mannheim criteria. Analysis: Pearson / Spearman Rho correlation and linear regression for quantitative variables, T-student / U-Mann Whtiney test[acute]s for DVC differences in qualitative variable evaluated. Results: 89 patients (14 naive), age 42 ± 8 years, years since HIV diagnosis 7.8 ± 5.6 years, 19.2% stage C. The DVD was associated to higher concentrations of median triglycerides 161 [123-217] vs 88 [72-126] (p 0.001) and mean non-HDL-Cholesterol 143.4 ± 39.6 vs 121.2 ± 40.8 (p 0.04). The hsCRP was negatively correlated with DVC [-0.22, p 0.04; B -6.3 (-11.9--0.67), p 0.02]; there was no association between DVC and adipokines. The DVD-patients also had a higher percentage of fat in both hepatic lobes. In the neurosonology study DVD was associated with a higher: mean IMT (mm) in right common carotid (CC) 0.62 ± 0.13 vs. 0.55 ± 0.05 (0.01), maximum IMT in right bifurcation 0.88 ± 0.17 vs 0.77 ± 0.14 (0.03), maximum IMT in left CC 0.75 [0.67 to 0.85] vs 0.66 [0.64 to 0.82] (0.03) and mean IMT in left CC 0.61 [0.56 to 0.7] vs 0.52 [0.5 to 0.6] (0008). Patients with DVC Sources of Research Support: Spanish Foundation for Research and Prevention of AIDS (FIPSE). Nothing to Disclose: OM-P, CS-C, RS-O, CE, JP, VB, EM, SR, RA, MM, AMP

Pub #	P2-133
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Regional Variation and Determinants of Vitamin D Status in Thailand
Author String	L-o Chailurkit, W Aekplakorn, B Ongphiphadhanakul Ramathibodi Hospital, Bangkok, Thailand; Ramathibodi Hospital, Bangkok, Thailand
Body	Vitamin D insufficiency is highly prevalent. Most of the studies concerning vitamin D status were generated from countries situated at temperate latitudes. It is less clear what the extent of vitamin D insufficiency is in countries situated in the tropics and how geographical regions within country would affect vitamin D status. I the present study, we investigated adults in the 4th National Health Examination Survey (2008) cohort (NHES 2008) the vitamin D status in Thais according to geographical regions and other risk factors. Subjects consisted of 2,641 adults, aged 14 - 98 years, randomly selected from NHES 2008 according to geographical regions. Serum 25 hydroxyvitamin D2 [25(OH)D2] and 25(OH)D3 were measured by liquid chromatography tandem mass spectrometry and summed to reflect vitamin D status. Vitamin D insufficiency was defined as total 25(OH)D levels less than 30 ng/mL. Data were expressed as mean ± SD. Subjects residing in Bangkok had lower 25(OH)D levels than other parts of the country (Bangkok, central, northern, northeastern and southern regions: 27.5 ± 7.7, 32.4 ± 8.8, 33.3 ± 8.9, 34.3 ± 8.9 and 32.5 ± 9.3 ng/mL respectively; $p < 0.001$). Within each region except for the northeastern part of the country, subjects living inside municipal area had lower circulating 25(OH)D (central, 30.8 ± 8.4 ng/mL vs 34.1 ± 8.9 ng/mL, $p < 0.001$; north 31.8 ± 8.8 ng/mL vs 34.8 ± 8.7 ng/mL, $p < 0.001$; northeast 33.7 ± 9.3 ng/mL vs 35.0 ± 8.4 ng/mL, $p = 0.09$; south, 30.7 ± 8.2 ng/mL vs 34.1 ± 9.9 ng/mL, $p < 0.001$). When only municipal areas were analyzed, subjects in Bangkok still had significant lower 25(OH)D levels than the rest of the country. Overall, the prevalence of vitamin D insufficiency was 64.5 %, 46.7 %, and 33.5 % in Bangkok, municipal areas except Bangkok, and outside municipal area in other parts of the country, respectively. In addition, the prevalence of vitamin D insufficiency according to geographical regions was 43.1%, 39.1%, 34.2% and 43.8% in the central, north, northeast and south, respectively. Besides geographical region, there were significant associations between lower serum 25(OH)D levels and female gender ($r = 0.28$, $p < 0.001$), younger age ($r = 0.25$, $p < 0.001$), living in municipal area ($r = 0.20$, $p < 0.001$), higher BMI ($r = 0.18$, $p < 0.001$), and religion ($r = 0.07$, $p < 0.001$). Conclusions: Vitamin D insufficiency is common and varies across geographical regions in Thailand. Sources of Research Support: Thailand Research Fund and Health Systems Research. Nothing to Disclose: L-OC, WA, BO

Pub #	P2-134
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Osteomalacia from Severe Vitamin D Deficiency in a 25-Year-Old Filipino Male
Author String	MV Holgado-Galicia, IT Isip Tan Philippine General Hospital, Manila, Philippines
Body	Background: Osteomalacia is a severe form of vitamin D deficiency, which is unusual in the adult population and in tropical countries due to adequate sun exposure. However, vitamin D deficiency has been shown to be paradoxically prevalent in the Asian region. Summary: A 25-year-old Filipino male was admitted for work-up of recurrent lower extremity weakness occurring for 11 years. Over the past two years, he noted a gradual decrease in height, associated with difficulty in ambulating. He denied any history of trauma or fall. Family history revealed similar symptoms of periodic paralysis and gradual decrease in height in the mother, older brother, and maternal uncles, none of whom were worked up or treated. Laboratory tests done showed hypokalemia (2.3 mmol/L, NV: 3.5-5.10), hypophosphatemia (0.46 mmol/L, NV: 0.81-1.58), elevated alkaline phosphatase (484 U/L, NV: 38-126), normal serum calcium and magnesium, normal urine calcium and phosphorus, metabolic alkalosis, low Vitamin D levels (40.69 nmol/L, NV: 47.7-144.0), and elevated intact PTH (67.15 pg/ml, NV: 15-65). Skeletal survey showed generalized osteopenia with multiple bilateral symmetric fractures on the fibula, ulna, femoral neck, and scapula. Bone densitometry showed a Z-score of -5.0, -6.2, and -5.8 at the lumbar spine, right and left femurs, respectively, indicating osteoporosis. The patient was diagnosed with osteomalacia secondary to Vitamin D deficiency with secondary osteoporosis and secondary hyperparathyroidism, pathologic fractures, and hypokalemic periodic paralysis probably secondary to an inherited renal tubular disorder. Parenteral and oral potassium replacements were given, which normalized potassium levels. The patient was also started on Calcitriol, calcium and Vitamin D supplements and was kept on close follow-up. Conclusion: Vitamin D deficiency results in bone loss, muscle weakness, and increase risk of fractures in adults. Routine screening has not been shown to be cost-effective, even in regions where prevalence rate is high. A high index of suspicion is required to identify individuals at high risk for vitamin D deficiency who may benefit from such screening. Treatment of osteomalacia, the end-stage of severe Vitamin D deficiency, is simple, widely available, and has been shown to reverse bone density loss and improve musculoskeletal status. Nothing to Disclose: MVH-G, ITIT

Pub #	P2-135
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Vitamin D Levels among Patients with Severe Mental Illness: Cardiometabolic Risk Correlates
Author String	RCY Chen, E Dent, J Snars, TJ Lambert Concord Hospital, Concord, Australia; Concord Hospital, Concord, Australia; University of Sydney, Sydney, Australia
Body	BACKGROUND: Vitamin D deficiency is common and there is great interest in the role of vitamin D, not only in skeletal health but also in chronic diseases. Vitamin D deficiency usually has to be treated with oral supplementation as the main source is through exposure to sunlight, and insufficient amounts are found in food. A recent Australian study found that 58% of psychiatric inpatients were Vitamin D insufficient, mainly in mood disorders (1). AIMS: To examine the relationship between Vit D status and cardiometabolic risks for patients with a range of psychiatric diagnoses. METHODS: Vit D levels in addition to other cardiometabolic risk factors were assessed for 226 patients receiving short-term inpatient care who were reviewed in our multidisciplinary clinic. Normal VitD levels are above 50 nmol/L, a mild insufficiency is 25-50 nmol/L, moderate is 12.5-25 nmol/L, and severe insufficiency is < 12.5 nmol/L. RESULTS: Three patients (1.1%) were severely deficient of Vitamin D, 18(6.9%) had moderate deficiency, 103(39.3%) mild, and 52.7% had normal Vit D levels. When controlling for age, gender, ethnic risk for diabetes, smoking status, exercise levels, and IDF MetS; mental health diagnosis did not predict low Vitamin D. Lower Vitamin D levels were recorded in winter (p=0.001). Previous and current smokers had significantly higher Vitamin D levels than those who reported never smoking (p=0.000). CONCLUSION: About half of psychiatric patients had some degree of insufficient Vit D, and this was not significantly increased among those with psychosis compared to other disorders. As expected, Vitamin D levels were lowest in wintertime. The finding that smoking predicted higher Vitamin D levels was in contradiction to previous studies in the general population demonstrating lower Vitamin D levels and impaired calcium metabolism. While the pathogenesis of this interaction requires further investigation, we hypothesise these findings may relate to current hospital smoking policy, where those who smoke must leave the hospital grounds, thus spending more time in the sun. For patients with mental illness, we advise following general consensus guidelines for bone health as part of routine medical care. Screening of Vitamin D levels, along with oral supplementation for insufficiency is universally recommended for patients with mental illness In addition, specific exercise programs to increase time outdoors are likely to be of benefit for patients with mental illness. (1) Berk M et al., Aust NZ J Psychiatry 2008; 42(10): 874-878 Sources of Research Support: NSW Department of Health. Nothing to Disclose: RCYC, ED, JS, TJL

Pub #	P2-136
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Evaluation of 25(OH) Vitamin D Status in Chronic Kidney Disease Patients of North Queensland, Australia
Author String	SK Chaubey, G Kan, V Manickam, R Muller, L Roberts, KS Sangla Cairns Base Hospital, Cairns, Australia; The Townsville Hospital, Townsville, Australia; James Cook University, Townsville, Australia; The Townsville Hospital, Townsville, Australia; The Townsville Hospital, Townsville, Australia
Body	**Background.** Vitamin D insufficiency and deficiency is known to be associated with chronic kidney disease.There has been new interest in the role of 25 hydorxy vitamin D { 25(OH)- D)} which may be separate from 1,25 $(OH)_2$ vitamin D. There are very few studies to assess vitamin D status in selected chronic kidney disease (CKD) patients in Australia specifically in north Queensland and the Australian Indigineous population. **Objective:** To assess 25(OH)-D status in patients with chronic kidney disease availing outpatient and in patient services of a tertiary level regional hospital in the North Queensland. **Methods:** Data was collected from 190 CKD patients aged 22 years to 90 years.133 patients were Caucasian, 53 patients were Aboriginal and Torres State Islander (ATSI) and 4 patients were from Asian background. It included patients from all the stages of CKD. Among them a significant proportion of patients (82/ 190) were in stage 5 CKD while 43 /190 were in stage 4 CKD and 50/190 in stage 3 CKD. Only 15 patients were in stage 1 or 2 CKD. In the 82 stage 5 CKD patients, 59 and 17 patients were on hemodialysis and peritoneal dialysis respectively as renal replacement therapy. According to the Kidney Disease Outcomes and Quality Initiative guidelines, patients were assigned to 3 groups: group 1, with a sufficient 25(OH)-D serum level (>7: nmol/L); group 2, with an insufficient level (50 to 74 nmol/L); and group 3, with deficiency ($\leq$ 49 nmol/L). The variables included were age, sex, indigenous status, stage of CKD, renal replacement therapy (RRT) , albumin-corrected calcium, phosphate, parathyroid hormone (PTH) , alkaline phosphatase (ALP), albumin in plasma and proteinuria. **Results:** 82 /190 patients (43.15%) had abnormaly low 25(OH)-D status. It included 39/ 53 (73.58%) patients from ATSI, 42/ 133 (31.57%) patients from Caucasian and 1/4 (25.0%) patient from Asian background. All measured variables except sex, albumin-corrected calcium and proteinuria showed statistically significant correlation with 25(OH)-D insufficiency or deficiency, but after general logistic regression only ATSI ethnicity and hypoalbuminemia remained statistically significant. **Conclusions:** Our study revealed that 25(OH)-D insufficiency and deficiency were very common in CKD population. Lower levels were associated with ATSI ethnicity and low albumin. Further studies are needed to find out reasons and implications of 25(OH)-D insufficiency and deficiency in this setting. Nothing to Disclose: SKC, GK, VM, RM, LR, KSS

Pub #	P2-137
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Effects of Skin Type on Serum 25-Hydroxyvitamin D Response to Ultraviolet Irradiation
Author String	P Pramyothin, NS Dabai, MF Holick Boston University, Boston, MA
Body	**Background:** Up to 10 million Americans are affected by fat malabsorption syndromes and are unable to absorb vitamin D efficiently. An alternative to oral supplementation is to expose the skin to ultraviolet radiation (UVR). The Sperti lamp (Sperti, Crescent Springs, KY) is an indoor portable fluorescent device sanctioned by the FDA that can generate vitamin D in the skin. Exposure to UVR from Sperti lamp has been shown to result in conversion of 7-dehydrocholesterol to previtamin D3 in an n-hexane solvent system and in human skin. The goal of the study is to evaluate the effects of UVR on serum 25-hydroxyvitamin D [25(OH) D] in healthy volunteers, and to determine the effects that Fitzpatrick skin type has on serum 25(OH)D response to UVR. **Methods:** Healthy volunteers with Fitzpatrick skin types II-V were enrolled into the study. Subjects were exposed to UVR 3 times weekly for 4 weeks. Each subject was exposed to UVR at an amount equivalent to 75% of the minimal erythemal dose (0.75MED). The exposure time was increased based on skin type (to achieve 0.75MED in every subject) and ranged from 4.1-6.6 minutes. Serum 25(OH)D was obtained at baseline and weekly. **Results:** Eight subjects were enrolled into the study (4 with skin type II, 2 with skin type III, 1 with skin type IV, and 1 with skin type V). Mean baseline 25(OH)D level was 20.1±8.4 ng/ml. Overall, there was a significant increase in mean 25(OH)D of 36% at 4 weeks compared to baseline (27.3±7.6 ng/ml, $p<0.01$). Subjects with skin types II and III (n=6) showed a significant increase in mean 25(OH)D of 47.5% (from 18.4±8.2 ng/ml to 27.1±7.8 ng/ml, $p =0.01$). Among these six subjects, three had a robust response to UVR (with a 25(OH)D increase of 92.9%, 92.4%, and 100.0% from baseline). Subjects with skin types IV and V had a minimal 25(OH)D increase of 9.0% and 9.3%. None of the subjects reported any adverse effects. **Conclusion:** Overall, significant increase in mean 25(OH)D was observed after a 4-week UVR exposure in healthy adult volunteers. Those with skin types IV and V who did not show significant increase in serum 25 (OH)D when exposed to UVR may have required longer exposure time due to the sunscreening effect of their skin pigment. Nothing to Disclose: PP, NSD, MFH

Pub #	P2-138
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Prevalence of 25-Hydroxyvitamin D Deficiency in Korean Adolescents: Association with Age, Season and Parental Vitamin D Status
Author String	SH Kim, MK Oh, MJ Park Inje University Sanggye Paik Hospital, Seoul, Republic of Korea; Busan Paik Hospital, Inje University College of Medicine, Busan, Republic of Korea
Body	**Objectives:** To assess the prevalence and associated factors of vitamin D deficiency in healthy Korean adolescents. **Study Design:** Data from Korean National Health and Nutrition Examination Surveys 2008 were analyzed. A total of 1004 adolescents (543 boys, 401 girls; aged 10-19 yrs) were enrolled in this study. **Results:** Serum 25(OH)D values were (mean ± SD) 18.5 ± 6.7 ng/mL, which were higher in boys than in girls [19.4 ± 6.8 (boys) vs. 17.5 ± 6.4 ng/mL (girls), $p < 0.0001$]. Overall, 13.8% of adolescents (boys 11.6%, girls 16.5%) were 25(OH)D deficient (<11 ng/mL) and 80.6 % were 25(OH)D insufficient (11~29 ng/mL). There was a higher prevalence of vitamin D deficiency during winter and spring than during summer and fall. Those who had lower physical activity and those who had lower calcium intake were more likely to have lower 25(OH)D levels. On logistic regression analysis, [Odds Ratio(95% Confidence Interval)], senior high school students [4.8(2.1-8.0)], girls [1.5(1.0-2.2)], winter / spring season [16.0(7.4-34.9) / 15.8(7.4-33.6)], paternal vitamin D deficiency [3.9(1.1-13.9)] and maternal vitamin D deficiency [5.2(2.9-9.3)] were significantly associated with vitamin D deficiency. **Conclusions:** Vitamin D deficiency is common in Korean adolescents. Senior high school age, winter/spring season, and parental vitamin D deficiency were the main risk factors for vitamin D deficiency. Nothing to Disclose: SHK, MKO, MJP

Pub #	P2-139
Session Information	POSTER SESSION: CLINICAL - Vitamin D (1:30 PM-3:30 PM)
Title	Type of Dietary Fat Is Associated with 25-Hydroxyvitamin D Increment in Healthy Adults Older Than 65 Years
Author String	S Niramitmahapanya, SS Harris, B Dawson-Hughes Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA
Body	**Background**: Mono- and polyunsaturated fats may have opposing effects on vitamin D absorption (1). The purpose of this study was to determine whether intakes of different dietary fats are associated with the increase in serum 25-hydroxyvitamin D (25OHD) following supplementation with vitamin D3. **Methods**: Participants, 152 healthy men and women age 65 and older, had serum 25OHD measured at baseline and after two years of taking 700 IU/d of vitamin D3 along with 500 mg of calcium(2). Intake of monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs) and saturated fat (SAT) was estimated from a food frequency questionnaire(3) at 18 months. **Results**: Mean±SD intake of MUFA was 22.4±10.3 g, PUFA was 11.6±5.2 g and SAT was 21.5±11.9 g. Afte adjustment for baseline 25OHD, mean change in 25OHD, nmol/l, was positively associated with MUFA, g (ß 2.33±0.9, p=0.015), and negatively associated with PUFA, g (ß -2.77±1.1, p=0.01) and SAT, g (ß -1.24±0.6, p=0.031). Further adjustment for total caloric intake and sex had litle effect on the magnitude or statistical significance of these results. **Conclusion**: The fat composition of the diet appears to influence the 25OHD response to supplemental vitamin D3. Diets rich in MUFA may improve absorption of vitamin D3 supplements in healthy older adults. More studies are needed to confirm these findings. (1) Hollander D, Muralidhara KS, Zimmerman A. Vitamin D-3 intestinal absorption in vivo: influence of fatty acids, bile salts, and perfusate pH on absorption. Gut. 1978 Apr;19(4):267-72. (2) Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. NEngl J Med. 1997 Sep 4;337(10):670-6. (3) Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51-65. Nothing to Disclose: SN, SSH, BD-H

Pub #	P2-140
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	A Case of I-Cell Disease (Mucolipidosis Type II) Masquerading as Rickets
Author String	MH Lin, P Pitukcheewanont Children's Hospital of Los Angeles, Los Angeles, CA
Body	Introduction/Background: I-cell disease (mucolipidosis type II) is a rare disease and the diagnosis is often missed. This disorder and rickets both can present with fractures, kyphoscoliosis, and similar biochemical studies. Their similarities often lead to delayed diagnosis and treatment for patients with I-cell disease. Clinical Case: A 6-month-old Asian male, ex-36-6/7-week, with mild coarse facies and lumbar gibbus was incidentally found to have a non-displaced right humerus fracture. Ensuing laboratory studies were consistent with rickets: low calcium (8.3 mg/dL, n>9 mg/dL), low phosphorus (2.8 mg/dL, n>4.5 mg/dL), elevated alkaline phosphatase (1352 U/L, n<270 U/L), elevated intact PTH (197 pg/mL, n<71 pg/mL), and mildly low 25-OH vitamin D (19 ng/mL, n>20 ng/mL). Skeletal survey X-rays revealed diffuse osteopenia, multiple fractures, metaphyseal fraying with extensive periosteal elevation of the long bones, lumbar kyphosis, and bullet-shaped metacarpal bones. These findings were suggestive of I-cell disease and further diagnostic studies were initiated. Diagnostic evaluation: DNA testing of GNPTAB gene sequencing showed c.3565 C>T and c.3663delG mutation. Plasma enzyme studies showed elevations of multiple serum enzymes (β-galactosidase 178.1 nmol/hr/ml, β-mannosidase 2325 nmol/hr/ml, α-mannosidase 2550 nmol/hr/ml, β-glucuronidase 5550 nmol/hr/ml, β-hexosaminidase A 918.8 nmol/hr/ml, and α-glucosaminidase 290 nmol/hr/ml, n<50 nmol/hr/ml). These laboratory findings confirmed a diagnosis of I-cell disease. In light of early diagnosis of this patient, bone marrow transplantation has been proposed. Conclusion: Patients with I-cell disease may present with the biochemical profiles and radiographic images suggestive of rickets. However, clinical abnormalities such as coarse facies, delayed development, lumbar gibbus and spinal abnormalities should prompt further workup for I-cell disease. A high index of suspicion fo I-cell disease can allow early diagnosis and potentially improve prognosis. (1) Unger S et al., Eur J Pediatr 2005; 164:236-243. Nothing to Disclose: MH-CL, PP

Pub # P2-141

Session Information POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)

Title Effects of Dietary Phosphate Manipulation and Ergocalciferol Administration on Circulating Soluble α-Klotho in Healthy Men and Women

Author String DM Mitchell, CM Baldwin, BZ Leder, S-AM Burnett-Bowie
Massachusetts General Hospital, Boston, MA

Body

Fibroblast growth factor 23 (FGF23) is a novel phosphate (P_i)- and vitamin D regulating hormone. Transmembrane Klotho is a critical co-factor for FGF23 action. It is unknown, however, if circulating α-Klotho (formed through post-translational changes) is important in FGF23 physiology or whether it is affected by physiologic states known to stimulate FGF23. **Methods:** We measured soluble α-Klotho using an ELISA kit (*IBL Co. Ltd*, Minneapolis, MN) in 45 men and women aged 18-45 who had previously undergone either 5 days of P_i loading with 2.5g/day dietary P_i (n=20), 5 days of P_i deprivation with 0.5g/day dietary P_i plus 2.2g $Al(OH)_2$ and $Mg(OH)_2$ QID (n=10), or 12 weeks of ergocalciferol 50,000 international units weekly (n=15). These 45 subjects had experienced significant changes in FGF23 (*Kainos Co.*, Tokyo, Japan) with the three physiologic manipulations when paired t-tests were used to compare their post- to pre-intervention FGF23 levels. **Results:** With dietary P_i loading, from baseline to day 5, FGF23 increased from 32±9 to 56±15 pg/mL ($p<0.0001$), serum P_i was unchanged, urinary P_i excretion (F_eP_i) increased ($p<0.0001$), 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) decreased ($p=0.03$), and soluble α-Klotho was unchanged (970±330 to 1015±330 pg/mL $p=0.31$). With dietary P_i deprivation, from baseline to day 5, FGF23 decreased from 47±18 to 22±8 pg/mL ($p<0.001$), serum P_i and F_eP_i decreased ($p=0.04$ and 0.0002 respectively), $1,25(OH)_2D$ increased ($p=0.09$), and soluble α-Klotho was unchanged (971±280 to 977±275 pg/mL $p=0.83$). With ergocalciferol administration, from baseline to week 12, FGF23 increased from 46±14 to 109±40 pg/mL ($p<0.001$), serum P_i, F_eP_i, $1,25(OH)_2D$ and soluble α-Klotho were unchanged (1200±396 to 1249±416 pg/mL $p=0.47$ for α-Klotho). There was a trend that FGF23 and α-Klotho were positively associated ($R=0.26$, $p=0.08$). α-Klotho was not, however, associated with serum P_i, F_eP_i, or $1,25(OH)_2D$. **Conclusions:** Despite regulating FGF23, dietary P_i and ergocalciferol do not appear to regulate soluble α-Klotho. Given the weak association between α-Klotho and FGF23 and the lack of change in soluble α-Klotho with physiologic manipulations that affect FGF23, it is unclear if soluble α-Klotho is important for FGF23 physiology.

Sources of Research Support: NIH grants K23-DK-073356 to SMB and M01-RR-01066; the Massachusetts General Hospital Physician-Scientist Development Award; and the Boston Area Diabetes and Endocrinology Research Center Grant.

Nothing to Disclose: DMM, CMB, BZL, S-AMB-B

Pub #	P2-142
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Structural Bone Deficits and Body Composition Abnormalities after Treatment for Childhood Acute Lymphoblastic Leukemia
Author String	JL Brodsky, J Ginsberg, J Shults, MB Leonard The Children's Hospital of Philadelphia, Philadelphia, PA; University of Pennsylvania School of Medicine, Philadelphia, PA
Body	BACKGROUND: Childhood acute lymphoblastic leukemia (ALL) 5 year cure rates now exceed 80%, and this growing population has numerous risk factors for impaired bone accrual. The long-term effects on volumetric bone mineral density (vBMD), bone structure, and body composition have not been established. The objective of this cross-sectional study was to use tibia peripheral quantitative computed tomography (pQCT) to assess musculoskeletal outcomes in children and adolescent survivors after treatment for ALL. METHODS: Tibia pQCT was performed in 38 ALL subjects (ages 5-19 yrs) a median of 12 months (range 0-24) after completion of chemotherapy, and >650 controls. Sex- and race- specific Z-scores were generated for vBMD relative to age, and for cortical dimensions, fat area and muscle area relative to tibia length, based on the control data. Multivariate linear regression models were used to compare Z-scores in ALL subjects and controls. RESULTS: ALL survivors had greater BMI (+0.87; 95% CI +0.60;+1.15 $p<0.0001$) and fat area Z-scores (+0.75; 95% CI +0.42;+1.08 $p=0.0001$), compared with controls; muscle area and height Z-scores did not differ significantly. Trabecular vBMD (-1.04; 95% CI -1.46;-0.62 $p<0.0001$) and cortical vBMD (-0.56; 95% CI -0.9;-0.21 $p=0.002$) Z-scores were lower in ALL, compared with controls. Overall, 26% of ALL subjects had trabecular vBMD Z-scores < -1.64 (5^{th}%tile). Cortical dimensions (endosteal and periosteal circumference) did not differ between groups. However, stress strain index (estimate of overall bone strength) was lower in ALL (-0.41; 95% CI -0.79;-0.03 $p=0.03$), compared with controls. CONCLUSIONS: Substantial deficits in trabecular and cortical vBMD and SSI were observed in ALL survivors. Longitudinal measures are underway in the ALL subjects to investigate changes in these deficits and determinants of change. Intervention studies may be needed to promote bone health in this high-risk population. Sources of Research Support: Jill L. Brodsky is a Fellow of the Pediatric Scientist Development Program. The project described was supported by Award Number K12-HD000850 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development.; Jill L. Brodsky is a recipient of The Children's Hospital of Philadelphia Institutional Clinical and Translational Science Award Research Center Grant UL1 RR024134. Nothing to Disclose: JLB, JG, JS, MBL

Pub #	P2-143
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Circulating Osteoprotegerin and Sclerostin Do Not Display Any Episodic (Pulsatile) or Periodic Pattern of Secretion
Author String	KN Aronis, JP Chamberland, CS Mantzoros Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; Harvard School of Public Health, Boston, MA; Boston VA Healthcare System, Boston, MA
Body	**Introduction**: Osteoprotegerin (OPG) and sclerostin (SOST) are novel key regulators of bone homeostasis and remodeling with opposing effects. OPG acts as a decoy receptor for the Receptor Activator of the NF-[kappa]B exerting anabolic actions on the bone, while SOST, a wnt inhibitor, decreases bone mineral mass. Furthermore, OPG has been proposed to be a marker for coronary artery disease. It is well established that there is a high preanalystical variability in measuring all bone markers and this is partially attributed to the fact that bone remodeling follows a day/night pattern. However, there is no consensus on whether these two novel molecules follow any day/night pattern and whether these molecules are secreted in a pulsatile (episodic) fashion or not. **Methods**: Serum samples collected every 15 minutes over a 24 hour period from 6 healthy male individuals (23.0 ±3.6 years old) studied in the isocaloric fed state were used to evaluate the presence of any day/night variation or pulsatile pattern in circulating OPG and SOST levels. Both analytes were measured using MSD multiplex elcetrochemiluminescent assay. The presence of any potential day night pattern was evaluated using non - linear OLS regression cosinor regression while the potential pulsatile pattern was analyzed using the peak detection algorithm CLUSTER8. The latter utilizes the assay's measurement error, as expressed in SD between duplicate measurements, to detect clusters of statistically significant peaks or nadirs as compared to the preceding and following values using a pooled t statistic. **Results**: Cosinor non-linear analysis revealed no day night pattern for both OPG and SOST. Pulse detection algorithm revealed no pulsatile pattern in circulating levels of OPG and SOST. **Conclusions**: This is the first study examining periodic/episodic secretion patterns of OPG and SOST with a temporal resolution of 15minutes. We demonstrate that there is no day night variability or pulsatile secretion pattern in circulating levels of OPG and SOST. Sources of Research Support: Grant Number M01-RR-01032-328840 to the Harvard Clinical and Translational Science Center, from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health. Funding was also received from the National Institute of Diabetes and Digestive and Kidney Diseases (grants DK058785, DK079929 and DK081913), and the National Institute on Aging (grant AG032030). Nothing to Disclose: KNA, JPC, CSM

Pub #	P2-144
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Severe Symptomatic Hypocalcemia after Oral Ibandronate in a Patient with Metastatic Bone Disease
Author String	DLT Chen, A Goldrick, JR Greenfield St Vincent's Hospital, Sydney, Australia; St Vincent's Hospital, Sydney, Australia; Garvan Research Institute, Sydney, Australia
Body	Background: Ibandronate is used in managing bony metastatic disease and hypercalcaemia of malignancy. Symptomatic hypocalcaemia have been described with oral alendronate [1,2]. This is the first case of severe hypocalcaemia following oral ibandronate. Clinical case: A 65-year-old woman with widespread sclerotic bony metastases due to breast cancer and a baseline 25-OH vitamin D level of 87 nmol/L (35-150 nmol/L) was commenced on oral ibandronate (50mg daily). The corrected calcium level decreased from 2.11 mmol/L to 1.38 mmol/L (2.1-2.6 mmol/L) accompanied by lower leg muscle cramps 3 weeks after initiation of ibandronate therapy. She had an inappropriately normal PTH level of 6.8 pmol/L (1-7 pmol/L) with normal 25-OH vitamin D level of 59 nmol/L, 1-25 OH vitamin D level of 174 pmol/L (36-120 pmol/L) (patient on calcitriol) and normal magnesium level of 0.88 mmol/L (0.7-1.05 mmol/L). There was a history of previous total thyroidectomy for multinodular goitre. Intermittent calcium infusion was required to maintain the corrected calcium level aroun 1.9-2 mmol/L over the next 3 weeks. The patient was discharged on calcium citrate 250 mg tds and calcitriol 0.5 mcg qid. Serum corrected calcium was 1.96 mmol/L on discharge. Clinical lessons: Oral bisphosphonates are widely used in malignancy but rarely associated with symptomatic severe hypocalcaemia which has been more commonly associated with intravenous bisphosphonate therapy. Possible mechanisms in this case include hypoparathyroidism and metastatic bone disease with widespread sclerosis. This case demonstrates that patients unable to mount a compensatory parathyroid response may be at high risk of symptomatic hypocalcaemia when treated with oral bisphosphonates. It may be prudent to check calcium level several days after initiation of oral bisphosphonates in this clinical setting and to initiate calcium and vitamin D supplementation prior to initiation of bisphosphonates in patients with recognised risk factors for hypocalcaemia. 1. Maalouf NM. et al. Bisphosphonate-induced hypocalcemia: report of 3 cases and review of literature. Endocrine practice. 2006;12(1):48-53. 2. Schussheim DH, et al. Hypocalcaemia associated with alendronate. Ann Intern Med. 1999;130:329. Nothing to Disclose: DLTC, AG, JRG

Pub #	P2-145
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Levetiracetam *vs.* Older Antiepileptic Drugs: A Randomized Prospective Bone Health Study
Author String	T Hakami, T O'Brien, S Petty, M Sakellarides, J Christie, M Todaro, JD Wark University of Melbourne, Parkville, Australia; Royal Melbourne Hospital, Parkville, Australia; Royal Melbourne Hospital, Parkville, Australia
Body	Introduction: Antiepileptic drug (AED) therapy is associated with bone disease (as indicated by reduced bone mineral density, BMD) and increased fracture risk but the mechanism of this important adverse effect is unclear and it is uncertain whether newer AEDs carry a similar risk of bone disease to older AEDs. Aim: The aim of the present trial was to compare bone mineral measures, bone turnover markers and relevant hormones in patients who had substitution monotherapy with a new-generation AED (levetiracetam, LEV) versus one of two older AEDs, carbamazepine (CBZ) or sodium valproate (VPA). Methods: Patients with partial epilepsy on monotherapy with an older AED who had [ldquo]failed treatment [rdquo], either due to lack of efficacy or due to adverse effects, were randomized to change to either LEV or VPA/CBZ. Areal bone mineral density (aBMD) of the lumbar spine (LS), total hip (TH) and forearm, total body bone mineral content (TBBMC) and peripheral quantitative computed tomography (pQCT) of the radius and tibia were obtained at baseline and 1 year. Univariate and multiple regression analyses (SPSS) were performed seeking associations between change in bone measures and treatment group, age, sex, baseline height and weight, and follow-up interval. Results: 84 subjects (33 female, 51 male) aged 18 - 82 (median 39) years at baseline were enrolled; they had received AED therapy for 0 - 44.6 (median 0.85) years. In most, their first AED treatment change was within 3 months prior to baseline bone measures. Bone measures were obtained in 70 to 83 subjects at baseline and 1 year. Baseline bone mineral measures did not differ between treatment groups. There was no significant bone loss at the LS or TH nor in TBBMC in the whole study population nor in either treatment group and there was no difference in change in bone mineral measures between treatment groups. Other outcome variables are currently under analysis. Conclusion: These unique data show no evidence of rapid bone change following a change in AED monotherapy in patients previously treated for epilepsy, nor do they show any difference in the early bone response to treatment with LEV compared with CBZ or VPA. Longer-term follow-up studies appear to be required to better characterize effects of specific AEDs on measures of bone health. Sources of Research Support: UCB Pharma. Disclosures: JDW: Clinician, Servier; Consultant, Amgen; Sanofi-Aventis; Novartis Pharmaceuticals; Servier GlaxoSmithKline; Investigator, Eli Lilly & Company; Sanofi-Aventis; GlaxoSmithKline. Nothing to Disclose: TH, TO, SP, MS, JC, MT

Pub #	P2-146
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Clearance of Bone Turnover Markers with Hemodialysis in Subjects with End-Stage Renal Disease
Author String	S Yaturu, R Mathew, WJ Ritz, J Finn, K Pheleps Stratton VAMC, Albany, NY; Stratton VAMC, Albany, NY; Stratton VAMC, Albany, NY
Body	Although parathyroid hormone (PTH) levels are very high in end stage renal disease (ESRD), bone disease is mostly considered to be adynamic bone disease based on bone biopsies and bone turnover markers. Persistent elevation of PTH levels can down regulate PTH receptors in the bone, resulting in decreased response to PTH This can explain to a certain extent that. Osteoprotegerin (OPG), a marker of bone remodeling, correlates with decreased bone mineral density (BMD) in dialysis patients, suggesting the bone is active metabolically. We hypothesized that dialysis may be clearing the circulating bone turnover markers, leading to falsely low levels compared to the normal range (with normal kidney function). The aim of this study is to determine whether the bone turnover markers are cleared during dialysis. Our clinical, observational pilot study measured the bone markers pre and post dialysis and repeated at pre-dialysis at the follow up visit. We measured serum OPG, RANKL, osteocalcin, ostase, cross laps and sclerostin. PTH levels were measured as part of routine care. Osteocalcin decreased from 65.2 ng / ml to 15.2 ng/ml after dialysis and with recovery to 42.1 ng/ ml prior to next dialysis. Cross laps decreased decreased from 1.51 ng/ ml to 0.65 ng/ml after dialysis and with recovery to 1.50 ng/ ml prior to next dialysis. Osteoprotegerin decreased from 19 pmol/ L to 14.2 pmol/ L after dialysis and with recovery to 42.1 pmol/ L prior to next dialysis.There is no significant change in sRankl and ostase. PTH levels correlates with Cross laps pre, post and follow up pre dialysis levels ($r = 0.4$; $r = 0.44$; $r = 0.53$; $p < 0.05$) indicating the active turnover of bone secondary to PTH levels. We conclude that serum cross laps is a good marker of osteoclast activity and the goals of PTH should be much lower to prevent bone loss. Cross laps and osteocalcin levels should be measured pre dialysis sample to reflect the metabolic activity of the bone. Nothing to Disclose: SY, RM, WJR, JF, KP

Pub #	P2-147
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Evaluation of Parathyroid Hormone, 25-Hydroxyvitamin D and Bone Turnover Markers in Progressive Renal Failure
Author String	J Ahlan, T Cantor, C Albert, P D'Amour CRCHUM - H[ocirc]pital Saint-Luc, Montréal, Canada; Scantibodies Laboratory Inc, Santee, CA
Body	To evaluate the role of parathyroid hormone (PTH) levels on 25-hydroxy-vitamin D (25(OH)D) concentrations and bone turnover markers levels (osteocalcin and C-telopeptide) in progressive renal failure, we have compared a group of young normal individuals (n = 29) with 3 older groups of renal failure patients defined by their serum creatinine concentration (< 250 [micro]mol/L, n = 21; > 250 < 500 [micro]mol/L, n = 19; > 500 [micro]mol/L, n = 27). All specimens were obtained anonymously through collaboration of the biochemistry laboratory of our institution as required by the ethic committee of our centre. PTH was measured with a 3rd generation cyclase activating (CA-) PTH assay and a 2nd generation Total (T) PTH assay from Scantibodies Laboratory Inc. and by an in house carboxyl-terminal (C), 1st generation RIA. 25(OH)D was measured by mass spectrometry, osteocalcin and C-telopeptide by ECLIA, total and ionized calcium, phosphate and creatinine by standard colorimetric methods. An ANOVA was used to compare groups. Univariate analysis were performed to study correlations. As serum creatinine increased from 70±14 to 912±181 [micro]mol/L ($p<0.000$), total calcium (2.35±0.06 to 2.24±0.18; $p<0.000$), and 25(OH)D levels (89±28 to 46±23 nmol/L; $p<0.077$) decreased while CA-PTH (2.0 ± 0.7 to 15.1±7.7 pmol/L; $p<0.000$), T-PTH (3.5±1.2 to 24.2±14.2 pmol/L; $p<0.000$), C-PTH (8.9±2.1 to 160±104 pmol/L; $p<0.000$), phosphate (1.18±0.17 to 1.77±0.47 mmol/L; $p<0.000$), osteocalcin (19±5 to 222±124 ng/ml; $p<0.000$) and C-telopeptide (0.263±0.168 to 1.941±0.853 ng/ml; $p<0.000$) all increased to supraphysiological levels. 25(OH)D levels were negatively related to PTH levels ($r = -481$, $p<0.000$), creatinine ($r = -453$, $p<0.000$), phosphate ($r = -358$, $p<0.000$), osteocalcin ($r = -298$, $p<0.000$) and C-telopeptide ($r = -298$, $p<0.007$). Similarly, osteocalcin and C-telopeptide levels were positively influenced by PTH levels ($r = 0.689$, $p<0.000$), creatinine ($r = 745$, $p<0.000$), phosphate ($r = 562$, $p<0.000$). These results suggest that 2ary hyperparathyroidism may play an important role in the control of of 25(OH)D levels by impairing liver formation of 25(OH)D or by increasing 25(OH)D turnover into inactive metabolites. Bone turnover on the other hand is positively influenced by PTH levels with limitations related to an impaired renal clearance of osteocalcin and C-telopeptide in renal failure patients. Sources of Research Support: Scantibodies Laboratory Inc., Santee, CA, USA. Disclosures: TC: Owner, Scantibodies Laboratory Inc. PD: Research Funding, Scantibodies Laboratory Inc. Nothing to Disclose: JA, CA

Pub #	P2-148
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Clinical Response to Oral Ibandronate in Paget Disease
Author String	L Voss, D Fontan, E Diniz, C Lucena, F Bandeira Agamenon Magalhães Hospital, University of Pernambuco Medical School, Recife, Brazil
Body	Introduction:The treatment goals of Paget's disease of bone (PDB) are to restore normal bone metabolism, relieve bone pain and prevent future complications. Bisphosphonates (BP) have been used for several decades as the class of choice for the treatment of PDB. Oral alendronate and risedronate are used in higher doses than those used for osteoporosis, that is 30mg/day and 20 to 40mg/day for 2 and 6 months respectively. Pamidronate (60-180mg), ibandronate (2mg) and zolendronato 5mg) can also be used intravenously. There is no data on the treatment of PDB with oral ibandronate. Objective: To evaluate the clinical and laboratory response before and 6 months after treatment with oral ibandronate (150mg/month) in 12 patients with PDB of whom 10 were previously treated with other BP. Materials and Methods: Patients were 7 males and 5 females, 9 with poliostotic desease with mean age 68.6 ±11,2 years-old. All had bone pain, 58% with pelvic, 50% lower extremity, 41,6% skull and 8,3% upper extremity involvement on X-Ray and bone TC-MDP scan. Mean serum 25(OH)D were 36,18 ± 10,9 ng/ml. We evaluated the intensity of bone pain, serum alkaline phosphatase activity (SAP) (normal: 38-126U/l) and serum ß-C telopeptide (sCTX) by electrochemiluminescence (normal: 50-450pg/ml). sAP was elevated in 7 patients, with a mean 1.49 ±0,98 times the upper limit of normal (ULN), while sCTX was elevated in 10 patients (mean 2,13±1,2 times ULN). Before treatment mean sCTX was 969,5±555,2 pg/ml. Results: After 6 months of treatment, the mean reduction was 65,24±28,9 % for sCTX, reaching 80% in 7 patients. There was one patient with normal values sCTX which showed 97.5% reduction at the end of treatment period, corresponding to the largest redution after treatment. The mean reduction of sAP was 49,21 ±37,9%, with all patients having normal values in sAP after treatment. There was a significant clinical response in all patients, with marked improvement in bone pain. Conclusion: Our data demonstrates a high efficacy of oral ibandronate in the treatment of PDB at equivalent doses used in the treatment of osteoporosis. Nothing to Disclose: LV, DF, ED, CL, FB

Pub #	P2-149
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	A Unique Case of Adult T-Cell Leukemia/Lymphoma Presenting with Hypercalcemia and a Low PTHrP Level
Author String	SS Bursheh, Y Wang, M Hawkins Albert Einstein College of Medicine, Bronx, NY; Montefiore Medical Center, Bronx, NY
Body	Background: Adult T-cell Leukemia/Lymphoma (ATLL) is an aggressive malignancy caused by infection with human T-cell lymphotrophic virus type 1 (HTLV-1). Patients usually present with lymphocytosis, lymphadenopathy (LAD), and hypercalcemia. The latter has been attributed to enhanced osteoclast activity, potentially due to expression of RANKL and PTHrP mRNA by HTLV-1 infected T-cells. Clinical Case: We report a case of a 65 year old Jamaican woman who had multiple admissions for mental status changes and hypercalcemia. Along with classic symptoms of hypercalcemia, she complained of left leg pain, fatigue and weight loss over several months. She had no other significant past history. Physical exam was benign except for bilateral lower extremity weakness. Calcium levels ranged between 9.9-19.8mg/dl (n 8.5-10.5), phosphorous 1.3-3.7 mg/dl (n 2.5-4.5) and creatinine 0.7-1.8 mg/dl (0.5-1.5). 25 hydroxy vitamin D level was 39 ng/ml (n 20-100), intact PTH consistently <3 pg/ml (n 10-65), 1,25 dihydroxy vitamin D <8 pg/ml (n 15-60), PTHrP was 6pg/ml (n 14-27), alkaline phosphatase 200 U/L (n 43-160) U/L, LDH 237 U/L (110-210), and hemoglobin was 10.8 g/dl (n 12.3-15.3). There was no leukocytosis or lymphocytosis. SPEP, UPEP, and tumor markers were negative. CT scan chest/abdomen/pelvis did not reveal any LAD. Positron Emission Test (PET) and a bone survey revealed extensive lytic lesions involving the entire skeleton. Diagnosis of ATLL was established by bone biopsy, revealing fibrosis and atypical lymphocytic infiltrate, and positive HTLV-1 serology. Hypercalcemia was refractory to initial therapy including hydration, calcitonin, and diuretics. She eventually required repeated IV bisphosphonate therapy every 10days. After the diagnosis was confirmed, CHOP chemotherapy and intrathecal methotrexate were initiated. The calcium level declined rapidly and was maintained in the low normal range. No further bisphosphonate therapy was required. Follow up imaging showed marked improvement in the lytic bone lesions. Conclusion: This case is unique in multiple respects. The patient had ATLL with normal WBC counts and no LAD. She had severe hypercalcemia and lytic bone lesions despite a low PTHrP level, suggesting local effects of infected T-cells. This case highlights the need for a high clinical suspicion for ATLL in patients with severe hypercalcemia originating from HTLV-1 endemic areas, even in the absence of typical features. Nothing to Disclose: SSB, YW, MH

Pub #	P2-150
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Recurrent Hypercalcemia in a Patient with Autoimmune Polyglandular Syndrome Type I (APS-I)
Author String	J Cella, M Charitou, H Narula, TA Wilson Stony Brook University Medical Center, Stony Brook, NY; Stony Brook University Medical Center, Stony Brook, NY
Body	**Background:** Hypercalcemia is most often PTH mediated with the most common cause being primary hyperparathyroidism. Therefore, it is rarely seen in patients with hypoparathyroidism. Adrenal insufficiency is a less common cause of hypercalcemia and the mechanism is poorly understood. **Case:** A 26 year old woman presented with serum calcium 13.4 mg/dL (8.6-10.2) and ionized calcium 6.4 mg/dL (4.2-5.2). She has a history of APS-I manifested by hypoparathyroidism, Addison's disease, and hypogonadism, and is maintained on cortisone acetate, fludrocortisone, calcium carbonate, vitamin D, calcitriol, and Ortho Evra[reg]. Due to poor compliance, her serum calcium is usually 6.5-8 mg/dL, and she has had multiple prior admissions for hypocalcemia. In the previous month, she was seen at an outside hospital three times for hypercalcemia (serum calcium 15, 11, and 12 mg/dL), treated each time with IV fluids. Reportedly, she stopped taking calcium carbonate and calcitriol after the first episode, one month prior to this presentation. During this admission she was treated with IV fluids and stress doses of hydrocortisone (as she presented with hypotension) with resolution of the hypercalcemia. Workup showed iPTH <2.5 pg/mL (14-72), PTHrP <1.1 pmol/L, 1,25 $(OH)_2$ Vitamin D 13 pg/mL (15-75), 25-OH Vitamin D 13 ng/mL (30-80), angiotensin converting enzyme 52 U/L (9-67). Chest CT showed no evidence of sarcoidosis or malignancy. Calcitriol and calcium carbonate were restarted and she was discharged with serum calcium 7.2 mg/dL. Follow-up serum calcium levels were initially 8.5-9 mg/dL, then increased to 13 mg/dL two weeks after discharge. She was again admitted to the hospital with hypotension (systolic BP 80), serum calcium 14.5 mg/dL, ionized calcium 6.7 mg/d, cortisol <0.2 mcg/dL, and ACTH 496 pg/mL (6-58). Hypercalcemia was thought to be due to adrenal insufficiency secondary to non-compliance with cortisone acetate. She was again treated with IV fluids and hydrocortisone, and discharged on calcitriol, calcium carbonate, cortisone acetate, and fludrocortisone. Non-compliance was confirmed with her pharmacist based on prescription filling patterns. **Conclusion:** In patients with APS-I, it is important to remember that the multiple endocrinopathies can have opposing clinical manifestations. Our case illustrates that hypercalcemia, while rare in patients with APS-I or any form of hypoparathyroidism, can occur as a result of acute adrenal insufficiency, and this is not mediated by PTH. Nothing to Disclose: JC, MC, HN, TAW

Pub #	P2-151
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	An Unusual Case of Acquired and Rapid Osteosclerosis
Author String	EM Alford, WA Murphy, J Shah, C Jimenez, B Lee, MI Hu The University of Texas MD Anderson Cancer Center, Houston, TX; Baylor College of Medicine, Houston, TX; The University of Texas MD Anderson Cancer Center, Houston, TX; The University of Texas MD Anderson Cancer Center, Houston, TX; Baylor College of Medicine, Houston, TX

Body

Introduction:

Osteosclerosis is extremely rare and can be due to genetic or acquired diseases, such as sclerosing metastasis, myeloma, myelofibrosis, secondary hyperparathyroidism, hepatitis C, fluorosis or bisphosphonate therapy. We present an unusual case of a patient with an alarmingly rapid rate of bone formation, consistent with osteosclerosis of unknown etiology.

Clinical Case:

A 58 year old male was diagnosed with Waldenstr[ouml]m's macroglobulinemia and treated with bortezomib/rituximab from 09/2008 to 02/2009 and remains in remission. In 09/2008, prior to chemotherapy, a bone density (DXA) found minimally elevated bone density. After initiation of chemotherapy, he developed symptomatic hypocalcemia, hypophosphatemia and vitamin D deficiency requiring significant calcium and vitamin D replacement for 10 months. Successive DXAs show progressive, excessive bone formation. In 08/2010, the lumbar spine and total hip T-scores were 13.1 and 9.9, respectively, compared with 2.9 and 2.1 i 09/2008. During this time, his intact parathyroid hormone was high, 25-OH vitamin D was low, calcitriol level was high, and bone turnover markers were extremely elevated [CTX 3383 pg/ml (normal 35-836), Osteocalcin 181 ng/ml (normal 9-42), Bone Specific Alkaline Phosphatase 622 mcg/L (normal 0-20)]. 24 hou urinary calcium and phosphate were inappropriately low. Bone surveys show progressive diffuse, uniform bone formation with loss of medullary space in the long bones. A transiliac crest bone biopsy after tetracycline double labeling performed in 08/2009 exhibited increased bone and osteoid formation with extremely thick cortices and difficulty delineating trabecular bone. Further testing ruled out vitamin A excess, hepatitis C, mastocytosis, and fluorosis. He has gradually developed arthralgias and pancytopenia with mild splenomegaly, consistent with extramedullary hematopoiesis.

Conclusion:

There are many causes of osteosclerosis, including genetic, iatrogenic, and acquired causes. It is unclear why this patient has such high bone mass and continues to build bone at an astounding rate. Evaluation of biomarkers involved in bone remodeling and potential genetic polymorphisms may clarify the cause of extreme bone formation and lead to a therapeutic target to stabilize or reverse the process and preserve marrow function. Understanding the pathophysiology behind excessive bone formation may identify new therapeutic targets for osteoporosis.

Nothing to Disclose: EMA, WAM, JS, CJ, BL, MIH

Pub #	P2-152
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Autosomal Dominant Osteopetrosis Type II (ADO-II), with Possible New Mutation Recognition
Author String	Z Maani, D Abuelo, J Hyland, N Beck, G Gopalakrishnan Alpert Medical School of Brown University- Hallett Center for Diabetes and Endocrinology, East Providence RI; Rhode Island Hospital, Providence, RI; Connective Tissue Gene Tests, LLC, Allentown, PA
Body	Background Osteopetrosis is a rare disorder of increased bone density due to deficits in bone resorption by osteoclasts. There are three known types: infantile or malignant autosomal recessive osteopetrosis (ARO), intermediate autosomal recessive osteopetrosis (IARO), and autosomal dominant osteopetrosis (ADO). The severity and clinical features of the different types vary. Several gene mutations have been identified so far, but not all cases have those mutations, which suggests that there are still more gene alterations to be identified. Clinical case: A 47 year old female was incidentally found to have sclerotic bands along the end plates of her spine on X-ray. She was asymptomatic, and had no history of bone pain or fractures. Laboratory tests showed corrected Ca 8.9 mg/dL (n 8.5- 10.5), PTH 63 pg/mL (n 10-65), Cr 0.5 mg/dL, 25-hydroxyvitamin D 23 ng/mL and 52 ng/mL after replacement (n >30). A bone scan showed increased uptake mainly at the metaphyses, and a bone density revealed a T score of 7.4 at the hip, and 10.6 at the spine. Acid phosphatase was 18.9 U/L (n 3.1- 7). These findings were consistent with osteopetrosis. We suspected that she has ADO-II, and molecular testing showed two discrete changes in the CLCN7 gene. One of them was in exon 10 and the other in exon 23. Neither had been previously reported as a mutation or polymorphism. However, the exon 10 transition was detected in two other patients, one of whom had a definite clinical diagnosis of osteopetrosis. It is not clear whether these changes are in cis or in trans; we plan further family studies on her daughters to enable interpretation of her findings and offer genetic counseling to the family. The patient is being followed clinically, and continues to have no symptoms. It is unlikely that she will need treatment. Conclusion: We present a case of clinically diagnosed osteopetrosis, with two ADOII related gene changes. One of the changes has been found in another patient with clinical osteopetrosis. The significance of these changes is still not clear, but either one or both may be pathogenic. Nothing to Disclose: ZM, DA, JH, NB, GG

Pub #	P2-153
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Treatment of Monostotic Fibrous Dysplasia with Zolendronic Acid -- A Case Report
Author String	D Bulchandani, B Lukert University of Kansas School of Medicine, Kansas City, KS
Body	Fibrous dysplasia is a rare benign skeletal disease associated with poorly mineralized bone matrix. The bisphosphonate , pamidronate, has shown clinical and radiological improvement but little is known about the efficacy of once a year zolendronic acid in this condition. We report a case of fibrous dysplasia and associated bone pain that showed significant improvement on treatment with zolendronic acid. Case summary: A 45 y/o female presented to the endocrine clinic with longstanding history of arm pain and working diagnosis of Paget's disease. Her previous MRIs and bone scans done at an outside facility were suggestive of changes consistent of Paget's disease. She failed a trial of etidronate. Review of her records indicated a consistently normal alkaline phosphatase (100 IU/l) and calcium levels as well as a mildly low Vitamin D level (23ng/ml) which was appropriately replaced. Other endocrinopathies were ruled out. During her clinic visit alkaline phosphatase, calcium, and phosphorus were within normal limits. Bone biopsy was negative for malignancy but inconclusive for any particular disease. CT scan was compatible with a diagnosis of monostotic fibrous dysplasia. She was treated with IV zolendronic acid with remarkable improvement in her bone pain. Her X-ray improvement was consistent with filling of the lytic lesions as well as thickening of the cortices. Conclusion: Our case highlights the use of zolendronic acid as an alternative agent to pamidronate which had been used in the treatment of fibrous dysplasia. Zolendronic acid resulted in clinical and radiological improvement. There is no published study/guideline recommending the use of zolendronic acid for monostotic fibrous dyplasia. Previous studies of the use of zolendronic acid to treat fibrous dysplasia have shown equivocal results. The effect of this bisphosphonate in our patient was rapid and dramatic. Nothing to Disclose: DB, BL

Pub #	P2-154
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Hypophosphatemia in Metastatic Prostate Carcinoma: Case Report
Author String	J Tloczkowski, SA Weinerman North Shore-LIJ Health System, Manhasset, NY
Body	**Background**: Tumor induced osteomalacia (TIO) is a well-known paraneoplastic syndrome in which a phosphatonin, fibroblast growth factor 23 (FGF-23), is overexpressed by tumor cells leading to hypophosphatemia (1). FGF-23 causes urinary phosphate wasting and suppresses 1,25 vitamin D. The majority of reported cases are in mesenchymal tumors, while few case reports exist of TIO with prostate cancer. Typical lab findings include renal phosphate wasting, hypophosphatemia, low 1,25 OH vitamin D and elevated FGF-23. Serum calcium, 25-OH vitamin D and PTH levels are normal. We present a case of presumed TIO in the setting of metastatic prostate cancer with atypical PTH and vitamin D findings. **Case**: A 73 y. o. AA male presented to NSUH-LIJ with sepsis. He had known prostate cancer metastatic to bone and was receiving monthly zoledronate elsewhere. On admission, the serum phosphorus was 0.9 mg/dL (2.5-4.5) which persisted despite aggressive PO and IV phosphate repletion. The patient was asymptomatic, and no prior phosphorous levels were measured. His magnesium, potassium and renal function were normal. The serum calcium 7.9 mg/dl (8.4-10.5) PTH 330 pg/mL (15-65), 25 OH vitamin D 25.5 ng/mL (30-100), 1,25 OH vitamin D 29, (repeat 52) pg/ml, (18-64), alkaline phosphatase 256 u/L (30-120), 24-hr urine phosphorus 2611 mg/dL (900-1300) and FGF-23 404 RU/mL ($\leq$180). He was started on supplementation with calcitriol, cholecalciferol, potassium phosphate and Posture D (contains calcium, phosphorus, magnesium, vitamin D). The serum phosphorus was 2.4 mg/dL on discharge. **Discussion**: This case adds to the rare reports of TIO due to FGF- 23 in prostate cancer. The low calcium, high PTH, and normal 1,25 vitamin D are atypical for TIO. We believe the pretreatment with bisphosphonate caused hypocalcemia, and secondary hyperparathyroidism. Both the elevated FGF-23 and PTH will cause hyperphosphaturia. The [ldquo]normal[rdquo] 1,25 vitamin D is inappropriately low for the PTH elevation, possibly from suppression by FGF-23. Routine measurement of serum phosphorous should be considered in patients with metastatic diseases, especially if receiving anti-resorptive therapy. (1) Jan de Beur, SM. [ldquo]Tumor Induced Osteomalacia.[rdquo] JAMA. 2005; 294; 1260-67 Nothing to Disclose: JT, SAW

Pub #	P2-155
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Tumor-Induced Osteomalacia (TIO): Treatment and Course of Recovery
Author String	T Thompson, WH Chong, MH Kelly, P Andeopolis, MT Collins, MA Banerji SUNY Downstate Medical Center, Brooklyn, NY; CSDB, NIDCR, National Institute of Health, Bethesda, MD
Body	Introduction TIO is a rare paraneoplastic syndrome caused by fibroblast growth factor 23 (FGF23) secreting mesenchymal tumors. FGF23 regulates phosphate & vitamin D metabolism. TIO causes phosphaturia, low serum phosphorus,1,25-OH vitamin D & generalized pain, weakness and fractures. Our patient, undiagnosed for years before successful treatment, had a varying time course for recovery of different features. Clinical Case A 46 yo woman was admitted with chest pain, generalized body pain, muscle weakness & inability to walk unassisted. Evaluation showed vertebral & rib fractures, normal serum calcium 9.9 mg/dl (8.4-10.2), low phosphorus, 0.9 mg/dl (2.5-4.5), 25 OH-vitamin D, 6 ng/ml (20-100), 1,25 OH-vitamin D 2 pg/ml (19-67) & elevated alkaline phosphatase. An elevated FGF23, 1050 pg/ml (nl <50)confirmed the diagnosis of TIO. The ratio of FGF23 in the venous drainage to general circulation was 1.6 and a 4x7x7 cm popliteal, phosphaturic mesenchymal tumor was removed totally. Following treatment with phosphorus & calcitriol, immediately pre op, she had normal levels of 1,25 OH-vitamin D (43 pg/ml), PTH (54 pg/ml), calcium and low phosphorus (1.9). Abnormalities normalized at different times.There was an unexplained increase in 1,25 OH -vitamin D levels from 40 pg/ml on day 2 to 225 pg/ml on day 4 post-op for at least 6 weeks, despite normal serum levels of FGF23, PTH , calcium and phosphorus. The lumbar bone mineral density (BMD) rose from a T score of -3.2 to -2.3 & ulna BMD from -1.5 to -0.2 by 12 months while alkaline phosphatase, a marker of osteomalacia, normalized in 24 months. There was full recovery of strength & ambulation. However, at 30 months, she has persistent anterior thigh and lumbar pain on prolonged walking requiring chronic pain management, presumably due to residual vertebral compression. Conclusions Diagnosing TIO is challenging. Clinical & biochemical features of TIO recover at different times after tumor resection & require follow-up. This patient's sudden post-op decrease in FGF23 was associated with a 4-fold, supra-normal, transient rise in 1,25 OH-vitamin D suggesting an unknown regulation of vitamin D metabolism. Occasionally this is associated with high serum calcium and low PTH levels. Clinically, despite recovery of function, our patient requires chronic pain management despite early normalization of FGF23 and late normalization of osteomalacia. Finally, bisphosphanates should probably be avoided in treating low BMD in osteomalacia. Nothing to Disclose: TT, WHC, MHK, PA, MTC, MAB

Pub #	P2-156
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Pycnodysostosis and Syringomyelia: An Association Not yet Reported
Author String	R Quezado, AG Costa, MN Hissa Hospital Universitario Walter Cantidio/Federal University of Ceara, Fortaleza, Brazil
Body	Pycnodysostosis (PYCD) is a rare skeletal abnormality of autosomal recessive inheritance due to a defect on the gene encoding the enzyme cathepsin K, located in the chromosome 1q21. It is characterized by important short stature (SS), osteosclerosis, acroosteolysis, craniofacial deformities and bone fragility. Although SS is a constant characteristic in this disease, growth hormone (GH) deficiency has been reported in some cases. We are presenting a case with typical clinical and radiological profile of pycnodysostosis associated with syringomyelia. A female,11 years old, in prepurbety stage, came to consult reporting respiratory distress, reduction of auditory acuity and bone fragility. Presented important SS, bilateral proptosis, blue sclerae, frontal bossing, brachycephaly, prominent nose, micrognathia, abnormal dental implantation, brachydactyly and dystrophic nails. Diffuse osteosclerosis, wide cranial sutures, open fontanels, platybasia, omega-shaped sella turcica, tibial model alteration (saber type), and acroosteolysis of the distal phalanges were evidenced. A the age of 15, she presented tonic-clonic seizure without relevant electroencephalographic alterations. The cranial magnetic resonance (NMR) evidenced medullary cavity from C2 to T2 (syringomyelia). No hormonal deficiency was diagnosed in this case. Conclusion: Syringomyelia is related to compressive processes of the medulla, tumors, traumas, arachnoid ossification, arachnoiditis and to compression of the craniocervical neuroaxis, as occurs in the basilar impression, in the Chiari malformation and also in the Hajdu-Cheney syndrome. There is no report in literature regarding the association between syringomyelia and pycnodysostosis so far. Nothing to Disclose: RQ, AGC, MNH

Pub #	P2-157
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	A Variation of a Classic Disorder or a New Genetic Syndrome? A Case of Recurrent Fractures in a Woman with Congenital Hip Dislocation, Sensorineural Hearing Loss, Blue Sclera and Globe Rupture
Author String	Z Maani, N Shur, L Ala-Kokko, G Gopalakrishnan Alpert Medical School of Brown University- Hallett Center for Diabetes and Endocrinology, East Providence RI; Rhode Island Hospital, Providence, RI; Connective Tissue Gene Tests, LLC, Allentown, PA
Body	Background: Osteogenesis imperfecta (OI) is a genetically heterogeneous disorder characterized by recurrent fractures, sensorineural hearing loss, blue sclera and dentinogenesis imperfecta. Globe rupture is a rare finding in OI. Other conditions associated with globe rupture include Ehlers Danlos syndrome type VI and Brittle Cornea Syndrome. We report a case of recurrent fractures in a woman with globe rupture, congenital hip dislocation, skin hyperelasticity and sensorineural hearing loss. Clinical Case: A 57 year old postmenopausal woman presented with history of multiple minimal trauma fractures including a pelvic fracture. Her past medical history was significant for congenital hip dislocation, hyperextensible joints and ruptured globe after light touch. Family history was not significant for connective tissue abnormalities. Physical examination revealed blue sclera, and doughy skin with extensive scars. A beighton scale was 0 due to arthritis. Initial lab screening included calcium of 9.2 mg/dL (n 8.5-10.5), PTH 137 pg/mL (n 10-65), 25OH-vit D 13.2 ng/mL (n >30), and urine NTX to creatinine ratio 73 (n 4-64). DEXA scan showed a T-score of -0.1 at the spine, -2.5 at the femur neck and -2.1 at the hip. In our patient, radiographs did not reveal Wormian bones or other characteristic signs of OI. Evaluation of urine lysyl hydroxylase and deoxypridinoline: pyridinoline ratio were normal. Sequencing of ZINF469, COL1A1 and COL1A2 genes did not reveal a disease causing mutation. A skin biopsy for biochemical analysis of collagen is pending. Conclusion: Our patient's clinical findings and negative work-up to date suggest that she may have either an atypical presentation of a known disorder or a novel connective tissue disorder. In cases of recurrent fracture and unusual additional findings, such as globe rupture, broadening the differential to include disorders beyond OI offers the best chance of solving the diagnostic puzzle. Nothing to Disclose: ZM, NS, LA-K, GG

Pub #	P2-158
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Serum 1,25 (OH)2-Vitamin D_3 as a Surrogate Marker for Clinical Improvement in Neurocysticercosis
Author String	AS Sacerdote, O Salamon, G Bahtiyar Woodhull Medical Center, Brooklyn, NY; SUNY Downstate Medical Center, Brooklyn, NY; NYU School of Medicine, New York, NY; NYU School of Medicine, New York, NY; St George's University School of Medicine, St George's, Grenada
Body	We've previously reported that the serum calcitriol level was elevated in the presence of normal 25-OH-Vitamin D level in a 34 year old female patient from Mexico with widespread neurocysticercosis. Subsequently we've made the same observation in a male patient with Mexico with disseminated neurocysticercosis. We suggested that either the encysted parasite or the surrounding macrophages (similar to the pulmonary sarcoidosis mechanism) might be a source of extrarenal 1-α-hydroxylase in these patients. After treatment with raloxifene (2010) our first patient demonstrated a significant reduction in parasite burden on MRI; following a second standard course of treatment with albendazole and dexamethasone.(2010) she showed further reduction in neuroparasite burden. 25-OH-Vitamin D3 and calcitriol were measured by immunoextraction. After the two treatments serum 1,25 (OH)-Vitamin D3 fell from 81 to 41 pg/ml (30-67) [49%], while 25-OH-vitamin D fell from 34 to 30 ng/dl (30-100) [13%]. We suggest that 1,25-(OH)-Vitamin D3 may prove to be a useful biomarker for parasite burden in neurocysticercosis, which, if further corroborated, may replace a portion of the expensive imaging required fo therapeutic follow-up. Nothing to Disclose: ASS, OS, GB

Pub #	P2-159
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Decreased Responsiveness to Epinephrine in Patients with Acrodysostosis and Multiple Hormonal Resistance
Author String	A Linglart, P Chanson, E Clauser, P Bougneres, C Silve APHP, St-Vincent de Paul Hospital, Paris, France; APHP, Bicetre Hospital, le Kremlin-Bic[ecirc]tre, France; APHP, H[ocirc]pital Européen Georges Pompidou, Paris, France; St-Vincent de Paul Hospital, Paris, France
Body	Acrodysostosis is a rare disease characterized by developmental abnormalities resembling Albright osteodystrophy that characterizes pseudohypoparathyroidism type Ia (PHP1a). As in PHP1a, obesity is frequently observed in acrodysostosis. Gsa mutations have not been identified in acrodysostosis. Recently we identified a germline recurrent de novo heterozygous mutation in the PRKARIA gene, coding for the major regulatory subunit of the cAMP-dependent protein kinase (PKA) in three patients who presented with acrodysostosis and multiple hormonal resistance. The mutation causes PRKAR1A gain-of-function, which therefore represses constitutively the catalytic subunit and results in PKA insensitivity to cAMP, thereby explaining the phenotype and the similarities with PHP1a. We documented in these patients resistance to PTH, TSH, GHRH and gonadotropins. Epinephrine also signals through Gsa and PKA. Resistance to this hormone, previously demonstrated in PHP1a, could contribute to the obesity seen in acrodysostosis, but responsiveness to epinephrine has not been evaluated in this latter disease. To evaluate this question, we investigated the effect of exogenous epinephrine infusion (35 microgr/1h) on heart rate (HR), plasma glycerol and free fatty acids (FFA) in two patients and six lean controls. Results are presented as a mean ± SD of 3 repeated measures. Basal HR was higher in patients with acrodysostosis (74 ± 4) than in controls (60 ± 3). However, HR in patients did not increase in response to epinephrine infusion (98 ± 2% of basal rate), whereas in controls it increased significantly (114 ± 3 % of basal HR, p=0.001 compared with patients). Patient basal glycerol (80.00 ± 14.14 pM) and free fatty acids (0.33 ± 0.03 [mu]M) were comparable to those of controls (91.83 ± 10.08 and 0.52 ± 0.07, p=0.52 and 0.06, respectively for glycerol and FFA). However, epinephrine infusion induced a lower production of both glycerol and FFA in patients with acrodysostosis (91.25 ± 4.79 and 0.55 ± 0.08, respectively) when compared to that in controls (146.2 ± 8.62 and 1.00 ± 0.07, respectively). These results are consistent with reduced epinephrine responsiveness in the two patients affected with acrodysostosis and PRKAR1A mutation compared to that in controls, as observed in patients with PHP-Ia. The data indicate that the deficient cAMP signaling and end-organ resistance to adrenergic stimulation might contribute to the obesity seen in both diseases. Nothing to Disclose: AL, PC, EC, PB, CS

Pub #	P2-160
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Anti-Hepatitis B Viral Agents and Osteomalacia: A 2-Year Longitudinal Observatonal Study in Severance Hospital
Author String	KJ Kim, KM Kim, S Hwang, KH Park, Y Rhee, S-K Lim, JY Park Severance Hospital, Yonsei University College of Medicine, Seoul, Korea; Severance Hospital, Yonsei University College of Medicine, Seoul, Korea
Body	Backround: Adefovir dipivoxil (ADV) is commonly used as an antiviral agent in the treatment of chronic hepatitis B or human immunodeficiency virus infection. Large clinical trials advocated the safety of ADV at a daily dose of 10 mg, the standard dose given to patients with hepatitis B. However, many recent reports showed that hypophosphatemic osteomalacia could be induced by ADV. Obejective: To elucidate the long term effect of ADV and Entercavir on renal function and serum phosphate level Method: we retrospectively reviewed medical records of 1321 patients who were prescribed ADV 10mg daily Furthermore, we analyzed medical chart of 2012 patients who have taken entecavir 3mg daily to compare the renal safety and prevalence of hypophosphatemia of two drugs. Patients with liver cirrhosis were excluded. Result: Mean follow up duration of patients who have taken ADV is 29 months and entecavir is 23 months. Among 1321 patients who were prescribed the ADV, 64 patients have taken ADV for more than 2 yrs. Seven of 64 (11%) had hypophosphatemia, and three had eGFR less than 30 ml/min per 1.73 m^2. Among 2012 patients who was prescribed entecavir, 97 patients have taken Entecavir for more than 2 yrs. Ten of 97 (10%) cases had hypophosphatemia, and four had eGFR less than 30 ml/min per 1.73 m^2. During follow up, mean serum phophonate level decreased by 0.4mg/dL in ADV group and 0.3mg/dL in Entecavir group. Mean GFR decreased by 9ml/min per 1.73m in ADV group, 3ml/min per 1.73m in Entecavir. We did not found the patient with osteomalacia with hypophosphatemia in Entecavir group but we found 2 cases in ADV group. Conclusion: Despite large clinical trials advocating the safety of adefovir dipivoxil at 10-mg daily and Entecavir 0.5mg daily, long-term use of this agent may be nephrotoxic and in rare cases, cause Fanconi syndrome and severe hypophosphatemic osteomalacia. Clinicians prescribing this drug should be aware of this potential complication. Nothing to Disclose: KJK, KMK, SH, KHP, YR, S-KL, JYP

Pub #	P2-161
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	A Patient with Renal Cell Cancer and Osteoblastic-Appearing Bone Lesions
Author String	H Gharwan, E Krug Johns-Hopkins-University/Sinai Hospital of Baltimore, Baltimore, MD; Johns-Hopkins-University/Sinai Hospital of Baltimore, Baltimore, MD
Body	***Background:*** Osteopoikilosis (OPK) is a rare benign sclerosing bone dysplasia characterized by the development of multiple bone islands clustered near articular ends of a bone. It is usually detected incidentally on imaging studies. Most cases are transmitted in an autosomal dominant pattern with high penetrance. The disorder can be associated with skin manifestations, such as juvenile elastomas or dermatofibrosis lenticularis disseminata. The combination of bone abnormality and connective tissue nevi is referred to as Buschke-Ollendorf syndrome. ***Clinical Case:*** A 35-year-old man was referred to endocrinology clinic for evaluation of stable osteoblastic-appearing bone lesions throughout his skeleton discovered several years ago. Four years prior he had been involved in a motor vehicle accident. At that time CT scan of abdomen/pelvis revealed a right renal mass. The patient underwent a right nephrectomy. Histopathology revealed solid variant of papillary renal cell cancer with Fuhrman nuclear grade 2. Staging CT scan detected lesions suspicious for blastic skeletal metastases. A total body scan and PET/CT confirmed multiple blastic-appearing lesions throughout the spine and pelvis, but failed to demonstrate increased Tc99m uptake or increased metabolic activity typical for active metastatic disease. Baseline alkaline phosphatase was 56 Units/L (normal up to 117). The patient received chemotherapy and was started on a regimen with zolendronic acid, initially monthly and thenekly over the following four years. All repeat PET/CT and whole body scans remained negative for skeletal activity. The appearance of skeletal lesions has remained stable. Open bone biopsy revealed a subtle mosaic pattern with packets of osseous lamellae often at odd angles to the direction of adjacent lamellar bone with no evidence of malignancy. DXA images revealed patchy bone appearance, but normal bone density based on Z-scores. Upon endocrine evaluation the diagnosis of OPK has been suggested and treatment with zolendronic acid was stopped. ***Conclusion:*** The radiographic presentation of OPK can mimic osteoblastic bone metastases. Normal markers of bone turnover and normal results of radionuclide bone scan are usually key differenciating features. Timely recognition may prevent unnecessary and potentially dangerous treatment. In the presented case the diagnosis of renal cell cancer confounded and significantly delayed the recognition of the patient's bone lesions as osteopoikilosis. (1) Serdaroglu M et al.,Rheumatol Int 2007; 27:683-686 (2) Baasanjav et al.,BMC Medical Genetica 2010.11:110 Nothing to Disclose: HG, EK

Pub #	P2-162
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	A Novel Syndrome of Neurocutaneous Melanosis, FGF23-Mediated Hypophosphatemic Rickets, and a Mosaic Skeletal Dysplasia
Author String	AM Boyce, WH Chong, MH Kelly, RI Gafni, EW Cowen, PS Thornton, MT Collins National Institutes of Health, Bethesda, MD; National Institutes of Health, Bethesda, MD; National Institutes of Health, Bethesda, MD; Cook Children's Medical Center, Fort Worth, TX
Body	**Background** Hypophosphatemic rickets due to FGF23 overproduction has been rarely reported in association with congenital cutaneous lesions, including epidermal nevus syndrome and linear sebaceous nevus syndrome. **Case** We report a novel syndrome of neurocutaneous melanosis associated with FGF23-mediated hypophosphatemic rickets and a mosaic skeletal dysplasia. The patient is a 3 year-old AA girl with an unremarkable family history who at birth was noted to have a bathing trunk-type congenital pigmented nevus covering her entire posterior torso, flank, chest, and scalp. Her extremities were covered with large, well-defined, raised plaques. Coarse terminal hair covered a significant portion of the nevi, leading to striking hypertrichosis. Two linear plaques were present on the left arm and leg. Development was significant for poor linear growth and severe motor delay, with inability to crawl, stand or bear weight on her upper or lower extremities by age 3 years. Cognitive development was notable for speech delay, but otherwise unremarkable. CNS imaging revealed foci of increased signal consistent with melanin deposition and a large enhancing intraventricular mass in the left temporal horn. Radiographs revealed unilateral brachydactyly and dysplasia o the long bones on the left, in addition to severe rickets. Karyotype was normal. Blood chemistries showed marked hypophosphatemia with phosphorus 1.1 mg/dL (n 3.1-5.5), calcium 8.5 mg/dL (n 8.2-10), alkaline phosphatase 586 U/L (n 108-317), intact PTH 61 pg/mL (n 16-87), 25-D 60 ng/mL, and 1,25-D 37 pg/mL (n 24-86). FGF23 was elevated at 795 RU/mL (n <108). In an effort to determine if the cutaneous lesions were the source of FGF23, as has been described in related cases, an octreotide scan was performed, but was negative. Phosphate and calcitriol treatment were initiated, however in part due to poor compliance, she remained profoundly hypophosphatemic with active rickets, poor linear growth and severely limited mobility. Over the course of a two-week inpatient admission with directly observed medication administration, she had rapid normalization of her serum phosphorus. With continued medication compliance, functional status has markedly improved. At 4.5 years there has been no progression of the CNS lesions and the patient is ambulating. **Conclusion** To our knowledge, this is the first report of FGF23-mediated hypophosphatemia and a mosaic pattern skeletal dysplasia in association with neurocutaneous melanosis. Disclosures: PST: Consultant, Endo Pharmacuticals; Speaker Bureau Member, Endo Pharmacuticals. Nothing to Disclose: AMB, WHC, MHK, RIG, EWC, MTC

Pub #	P2-163
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Tumor-Induced Osteomalacia (TIO): Report and Long-Term Follow-Up of Four Cases after Surgical Resection
Author String	MP Ferraz, LZ Bussmann, FG Graciolli, CRGCM Oliveira, V Jorgetti, PHS Correa, RM Martin HC-FMUSP, São Paulo, Brazil; HC-FMUSP, São Paulo, Brazil; HC-FMUSP, São Paulo, Brazil; HC-FMUSP São Paulo, Brazil
Body	**Background:** TIO is frequently small, slow-growing which has mesenchymal origin and may situate in any anatomical site. It causes an acquired, paraneoplastic syndrome of renal phosphate wasting. FGF23 is the main phosphaturic factor secreted by these tumors implicated in the low serum phosphate and abnormal bone mineralization. **Clinical cases**: We followed 4 patients (2 men and 2 women) with progressive weakness, skeletal pain, multiply fragility fractures starting at age ranging from 38 to 51 yo. Laboratorial tests showed: high alkaline phosphatase (124-307 U/L, N 25-104), hypophosphatemia (1.3-2.3 mg/dL, N 2.7-4.5) and low TmP/GFR. Renal tubular acidosis was ruled out. These data were consistent with hypophosphatemic osteomalacia, which was confirmed by iliac crest bone biopsy and therapy with phosphate and calcitriol was started. An oncogenic origin was suspected due to late onset and the absence of familial cases but the detection of tumors was only possible with octreotide scan since most of TIO expresses somatostatin receptors. Different anatomical sites (parotid region, thigh, first rib and ethmoid) were revealed by this technique and the tumoral localization was confirmed by a topographic image which helped in surgical resection. In all cases, histologic analysis was compatible with mesenchymal tumor and the immunohistochemistry was positive for FGF23. Moreover, the patients had high FGF23 presurgical levels (233-8,000 pg/mL; N 10-50), which rapidly normalized after tumors removal, except in one case, where incomplete resection occurred because vessels-nerve involvement by the tumor. In the other 3 cases, from 1 week to 1 month after tumor removals were enough to restore and remain normal phosphate metabolism without further phosphate therapy. Noteworthy, 2 patients who received long-term phosphate supplementatio (around 15 yrs) developed tertiary hyperparathyroidism (tHPT) probable because chronic parathyroid stimulation. **Clinical lessons:** Despite the fact that TIO is a rare disorder, the precocious diagnosis is essential to prevent bone deformities. Because the tumor removal is curative, its localization is mandatory and the use of octreotide scan should be considered as the initial imaging study. When the tumor is not found, tHPT is a potential complication of the long term of phosphate therapy. Furthermore, high serum FGF23 reinforces the TIO diagnosis and it is an excellent test to detect cure, persistence or recurrence of tumor. Nothing to Disclose: MPF, LZB, FGG, CRGCMO, VJ, PHSC, RMM

Pub #	P2-164
Session Information	POSTER SESSION: CLINICAL - Metabolic Bone Diseases, Case Reports & Rare Disorders (1:30 PM-3:30 PM)
Title	Tandem Spinal Stenosis in a Child with Albright Hereditary Osteodystrophy
Author String	TQ Cheng, MA Mittler, PM Kreitzer, GR Frank Cohen Children's Medical Center of NY, New Hyde Park, NY; Cohen Children's Medical Center of NY, New Hyde Park, NY
Body	Background: Albright Hereditary Osteodystrophy (AHO) is caused by the loss of function of one allele of the gene encoding the stimulatory G protein alpha subunit (GNAS1). Affected individuals have a typical phenotypic appearance (short stature, round facies, short 4th metacarpals, developmental delay, dental hypoplasia, and subcutaneous calcifications) as well as hormone resistance states. These patients may also develop spinal cord compression as a result of vertebral anomalies and/or soft tissue calcification. Case: An 8-year-old female with known AHO (typical phenotype plus hypothyroidism and pseudohypoparathyroidism) presented to an outlying hospital with a one month history of progressive difficulty walking and frequent falls to the extent that she could no longer walk more than a few steps independently. She complained of intermittent urinary incontinence and episodes of numbness and tingling in both lower extremities. Initial imaging studies of the lumbar spine showed significant lumbar spinal stenosis for which surgical intervention was recommended. The family requested a second opinion and she was transferred to our facility for evaluation. On account of hyperreflexia in the lower extremities, an MRI of the entire spinal axis was performed which revealed significant spinal stenosis with cord compression of the cervical spine in addition to the lumbar spine. She underwent both cervical (C2-C4) and lumbar (L2-L5) laminectomy and spinal fusion. Surgery was uneventful and she was referred for rehabilitation/physical therapy. She is now walking independently once more and expected to make a full recovery. Clinical lessons: Although uncommon, spinal stenosis has been reported in pseudohypoparathyroidism and should be a consideration in these patients in the appropriate clinical settings (weakness, loss of balance, etc.). When symptoms of lumbar spinal stenosis (eg urine incontinence) are present, careful neurologic examination of the deep tendon reflexes should be performed. It is critical to appreciate that hyperreflexia cannot be explained by lumbar spinal stenosis and if elicited, the possibility of tandem spinal stenosis involving the cervical spine, in addition to the lumbar spine, should be considered. Had the initially planned lumbar spinal surgery taken place, not only would her symptoms not be cured, but in addition, positioning for surgery may have caused further compression of her cervical spine and possibly rendered her quadraplegic. van Lindert EJ, Bartels RHMA, Noordam K. Spinal Stenosis with Paraparesis in Albright Hereditary Osteodystrophy. Pediatric Neurosurgery 2008; 44:337-340. Nothing to Disclose: TQC, MAM, PMK, GRF

Pub #	P2-165
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	B-Type Natriuretic Peptide and Aortic Dilation in Turner Syndrome
Author String	LS Gutin, VK Bakalov, CA Bondy National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD
Body	**Background:** Turner syndrome is a relatively common chromosomal disorder that is caused by the complete or partial loss of a second sex chromosome. The risk for aortic dilation and dissection is increased in young women with TS, therefore close monitoring of aortic size is important. B-type natriuretic peptide (BNP) is a cardiac hormone that is elevated in patients with ventricular hypertrophy and congestive heart failure. It is unknown whether the concentration of BNP is increased in individuals with aortic dilation. **Methods:** Study subjects included 119 consecutive participants [ge]18 years of age (range 18-67, average age 38) enrolled in the NICHD Institute Review Board-approved TS genotype-phenotype protocol since 2008. The diagnosis of Turner syndrome was confirmed in all study subjects by 50-cell peripheral karyotype in which 70% or more of cells demonstrated loss of all or part of the second X-chromosome. Participants that had recent cardiac or aortic surgery or a left ventricular ejection fraction of less than 50% were excluded. Plasma N-terminal-pro-BNP concentration was measured by immunoassay at the NIH Clinical Center using a Siemens Vista Analyzer. Ascending and descending aortic diameters were measured on T1-weighted images obtained in the axial plane at the level of the right pulmonary artery using a 1.5-Tesla magnetic resonance scanner. Left ventricular ejection fraction was also measured by cardiac MRI. **Results:** We used linear regression to analyze the correlation between aortic diameters normalized to body surface area (aortic size index; ASI) and BNP levels. There was a strong, positive correlation between ascending ASI and BNP (R^2=0.15, p<.0001) and descending ASI and BNP (R^2=0.17, p<.0001). We compared BNP values of a group with a non-dilated ascending aorta (ASI<1.9, n=79) to those of a group with a dilated ascending aorta (ASI[ge]1.9 cm/m^2, n=40), using ANCOVA with age as the covariate. Mean BNP concentration was 79.27 ± 6.32 pg/ml in the non-dilated group vs. 119.33 ± 13.06 pg/ml in the dilated group (p=.0018). BNP concentrations were not correlated to estrogen use or to left ventricular ejection fraction. **Conclusions:** This novel data suggests that circulating BNP levels reflect aortic dilation in women with TS. Further studies are necessary to confirm these observations and determine if BNP levels could be used in clinical practice for the detection and monitoring of aortic dilation. Nothing to Disclose: LSG, VKB, CAB

Pub #	P2-166
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	Education, Employment, and Marriage for U.S. Adults with Turner Syndrome
Author String	HN Gould, CJ Tankersley, VK Bakalov, CA Bondy NIH, Bethesda, MD
Body	Turner Syndrome (TS) affects ~ 1/2500 live-born females. The diagnosis requires the complete or partial absence of the second sex chromosome, associated with short stature and early ovarian failure. There is little information on adjustment to adult life for women with TS. We surveyed the educational attainment, employment, and marital status of 240 women with TS, aged 25-67 (mean 40 years), participating in an NIH natural history study in 2001-2010. All subjects had a 50-cell karyotype demonstrating < 30% normal cells. Normative data for the US female population was obtained from the US Census Bureau (www.factfinder.census.gov). Education: 70% of our TS cohort had a baccelaureate degree or higher, compared to 26% of US women. In addition, 6% of our TS cohort had a professional degree in medicine, law, or research vs. 3% of the general population. Employment: 80% of our TS group was employed outside the home, compared to 70% of the US female population. The most common job categories reported by the TS population were Healthcare Practitioners and Technical Occupations (18%); Education, Training, and Library Occupations (15%); and Healthcare Support Services (12%) according to the Bureau of Labor and Statistics classification system, accounting for 9, 10, and 4.5% of employment for the general female population, respectively. Marriage: 48% of our TS group had married, vs. 78% of the general female population. Thirty-three women had children; 24 had adopted and 9 women had spontaneous or donor egg with IVF pregnancies. A multivariate analysis was used to determine contributions of current age, age at diagnosis, and adult height on education, employment, and marriage. For education level and employment status, none of these factors proved significant. However, we found that age was positively correlated ($p=0.01$), and height negatively correlated ($p=0.028$), with marriage rate. Women with TS participating in the NIH study achieved higher than average education levels and were employed at comparable rates, but were less likely to marry than the general female population. Nothing to Disclose: HNG, CJT, VKB, CAB

Pub #	P2-167
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	A Novel Mutation in the *CBX2* Gene in a Brazilian Patient with 46,XY Disorders of Sex Development (DSD) Due to Gonadal Dysgenesis
Author String	CR Gomes, FC Soardi, MP Brandao, RB Silva, MP Mello, S Domenice, IJP Arnhold, BB Mendonca, EMF Costa Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Universidade Estadual de Campinas Campinas, Brazil
Body	**Introduction**: CBX2 mouse homologue, *M33,* was found to play role in the gonadal development. One compound heterozygous mutation, the P98L/R443P, in CBX2 gene has been described in a 46,XY patient with ovaries, uterus and female phenotype. **Objective:** To analyze *CBX2* gene in patients with 46,XY and 46,XX Disorders of Gonadal Development (DGD). **Patients/Methods**: We evaluated 60 patients. Forty 46,XY DGD patients from 36 families, eighteen 46,XX DGD patients from 13 families and a family with one sibling with 46,XX DGD and other with 46,XY DGD. Mutations in *SRY*, *SF1* and *FSHR* gene were excluded as well as autoimmunity. The entire coding region and the splicing sites flanking regions of *CBX2* was amplified and sequenced. Molecular modeling of mutant protein was performed by MODELLER[reg] 8v2. The evaluation method Procheck[reg] and the Ramachandran[reg] plot of each model was compared with the template structure. The models images were examined and edited using Millennium STING (CNPTIA-Embrapa, Brasil) and PyMOL[reg] program. **Results:** We identified 12 allelic variants in our patients: 7 known polymorphisms and five new ones. Moreover, the novel homozygous mutation, C132R, was found in a 46,XY DGD patient with partial gonadal dysgenesis and was absent in 206 normal control alleles. In the model of human CBX2 protein is observed the discrepancy between the residues. Comparing the internal contacts, C132 interacts with Arg119 by hydrophobic interaction and with Gly128, Val136 by hydrogen bond main chain. R132 establishes new interactions with Trp129 by hydrophobic interaction and Glu120 by charge attractive interaction. The mutant protein new contacts suggest relevant α-helix mobility reduction whereas the cysteine loss causes drastic structural alterations. **Conclusion:** This is the first homozygous mutation in CBX2 gene causing 46,XY DSD due to partial gonadal dysgenesis. The molecular modeling of mutant protein strongly suggests that the new mutation causes drastic damages on CBX2 protein. Functional studies will be performed to confirm these interesting findings. In addition we expanded the repertoire of CBX2 allelic variants. Nothing to Disclose: CRG, FCS, MPB, RBS, MPM, SD, IJPA, BBM, EMFC

Pub #	P2-168
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	A Novel Heterozygous Missense Variant of the *FGFR2* Gene in Two 46,XY Sisters with Non-Syndromic Partial Gonadal Dysgenesis
Author String	AZ Machado, MG Santos, MY Nishi, MP Brandao, EMF Costa, BB Mendonca, S Domenice Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil
Body	Fgf9/Fgfr2 signaling seems to be essential to maintain *Sox9* expression after sex determination, a critical effect for normal male gonadal development. *Fgfr2* knockout mice present severe abnormalities in male gonadal development that cause male-to-female sex reversal. Differently, in humans no association between *FGFR2* and testes development could be confirmed until now. Deletions of 10q chromosome are classically associated with dysmorphic facial features, heart defects, neurodevelopmental deficits (1, 2) and urogenital anomalies including 46,XY gonadal dysgenesis (3). *FGFR2* is one of the genes located at 10q26 region, suggesting its potential role in the abnormal sex development phenotype of 10q deletion syndrome. Our aim is to analyze if *FGFR2* inactivating mutations or deletions would be involved in the etiology of non syndromic 46,XY gonadal dysgenesis. **Patients and methods:** We studied 39 46,XY patients with gonadal dysgenesis (GD), 13 with the complete and 26 with the partial form. The entire coding region of *FGFR2* was PCR amplified and directly sequenced using a BigDye Terminator in ABI PRISM 3100 DNA sequencer. *FGFR2* copy number variation was determined by multiplex ligation probe amplification (MLPA) using the commercial SALSA MLPA P231 Kit. The PCR products were analyzed by GeneScan. **Results:** A novel heterozygous nonsynonymous *FGFR2* variant c.1361 C>T (p.S453L) located at exon 11 was identified in two sisters with partial GD. Their mother is a carrier of this variant which was absent in 100 control males. This variant was tested in two prediction sites (PolyPhen and SIFT) and both confirmed that this protein is possibly damaged. No *FGFR2*deletions were identified in MLPA analysis. **Discussion:** In the present study, in a large group of patients with gonadal dysgenesis the novel heterozygous variant (p.S453L) was identified in two 46,XY DSD sisters and their mother suggesting that gonadal dysgenesis in 10q syndrome is probably caused by FGFR2 damage. **Conclusion** A novel *FGFR2* variant c.1361 C>T (p.S453L) was identified in two sisters with 46,XY DSD due to partial GD. If the prediction sites results were confirmed in further functional studies a real role of *FGFR2* in human testis embryogenesis will be defined. (1) McCandless, S.E. et al., 2000. (2)Mulcahy, M.T. et al., 1982. (3)Wilkie, A.O. et al., 1993. Sources of Research Support: Fapesp #08/55952-8; CNPq #483416/2009-6. Nothing to Disclose: AZM, MGS, MYN, MPB, EMFC, BBM, SD

Pub #	P2-169
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	Quality of Life in a Large Cohort of Adult Patients with 46,XX and 46,XY Disorders of Sex Development (DSD)
Author String	M Inacio, RC Amaral, VN Brito, TAAS Bachega, S Domenice, MH Sircili, IJP Arnhold, G Madureira, EMF Costa, BB Mendonca Hospital das Clinicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil
Body	Background: Disorders of sex development (DSD) refer to a group of congenital conditions in which atypical development of sex occurs. Few studies have focused the quality of life (QoL) of affected individuals and none of them used a questionnaire to measure physical, mental, psychological and social well-being objectively to evaluate the impact of the disease on quality of life in patients with 46, XY and 46, XX DSD patients in adulthood. Our aim is to evaluate the QoL using the WHOQOL-BREF questionnaire in 46,XX and 46,XY DSD patients followed until adulthood in a single center. Patients and Methods: 112 adult DSD patients (43 patients with 46,XX DSD - 37 with female social sex and 6 with male social sex) and (69 patients with 46, XY DSD - 44 with female social sex and 25 with male social sex) answered the 26 questions related to general QoL and health and to its four domains: physical, psychological, social and environment. Results: The general QoL of 46, XX DSD patients was similar in 46, XY DSD patients considering the whole group. However, both 46,XX and 46,XY DSD patients with male social sex (n=28) had better general quality of life as well as in physical, psychological and social domains than those patients with female social sex (n = 84) ($p<0.05$). The aspect that most influenced the outcome of the physical domain was the discomfort which indicates the impact of physical pain preventing the patient from exercising their day-to-day activities. Regarding psychological domain, the facet that most influenced was the body image and appearance, indicating greater acceptance of body image in patients with male social sex. Interestingly, the quality of sexual activity did not influence quality of life suggesting that this is not the cause of better quality of life in patients with male social sex. In patients with 46,XY DSD, general QoL and at physical, psychological and social domains were better in patients with male social sex than the ones with the female social sex ($p<0.05$). Finally, among the patients with female social sex, the 46,XX patients (n=32) are more satisfied with their health than 46,XY patients (n=46) ($p = 0.05$). Conclusion: In general, adult DSD patients with male social sex have a better quality of life than patients with female social sex, independently of the quality of sexual life, probably reflecting the greater acceptance of body image in male patients. Nothing to Disclose: MI, RCA, VNB, TAASB, SD, MHS, IJPA, GM, EMFC, BBM

Pub # P2-170

Session Information POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)

Title *GATA4* Variants (p.L269F and V380M) Identified in 46,XY Patients with Disorders of Sex Development (DSD) without Congenital Heart Defects

Author String TE Silva, EMF Costa, MY Nishi, RB Silva, BB Mendonca, S Domenice
Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

Body

GATA4, a member of the GATA family of transcription factors, is a crucial regulator of cell-specific gene expression in many tissues, including gonads and heart. In mouse, *Gata4* is prominently expressed in somatic cells of testis and ovary, where it has been demonstrated to regulate the transcription of genes involved in sex development, as *Sry*, *Amh* and *Sox9*. In humans, *GATA4* mutations have been related to congenital heart defects (CHD) and rarely to 46,XY DSD. Our goal is to search for *GATA4* mutations in patients with 46,XY DSD due to Gonadal Dysgenesis (GD). **Subjects and Method** We studied 41 patients with 46,XY GD: 26 with partial (P) and 15 with complete (C) form of the disease. The *GATA4* (ENST00000335135) coding region (exons 2-7) was amplified by PCR. The amplicons were sequenced and analyzed by the ABI Prism 3100 Genetic Analyzer. The screening of the novel variant found was performed in 150 health individuals. **Result** A novel heterozygous nonsynonymous allele variant c.805C>T (p.L269F), located at exon 4, was identified in a patient with PGD and it was not found in the control group. In exon 6 were identified the heterozygous variant c.1138G>A (p.V380M) in a patient with CGD and the polymorphisms rs3729856 and rs56206007, all previously described. **Discussion** The majority of *GATA4* mutations are associated with CHD The p.V380M variant was formerly reported in a Chinese patient with sporadic CHD by Tang *et al.* We identified the same variant in a CGD patient without heart defects. The valine 380 is conserved in a few species and *Polyphen* (http://genetics.bwh.harvard.edu/pph/) predicted this alteration to be benign. The second *GATA4* variant (p.L269F) identified in a PGD patient is located next to the zinc finger C-terminal and can probably disrupt on GATA4 ability to interact with its transcriptional partner WT1 consequently, modifying the level of expression of *SRY* and *AMH*. The leucina 269 is highly conserved in several species and this variant is predicted to be possibly damaging. The large phenotypic spectrum of *GATA4* mutation was reported by Lourenco *et al.* in a French family with 46,XY DSD and CHD, suggesting that genotyping of *GATA4* mutant is influenced by the strain background. **Conclusion** The functional studies of these GATA4 protein variants will allow evaluating their role in gonadal disease. Although to date *GATA4* mutations have been related especially with CHD and rarely with gonadal anomalies they represent one more factor to cause human DSD.

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Sources of Research Support: FAPESP 2009/03872-3; CNPq 301339/2008-9.

Nothing to Disclose: TES, EMFC, MYN, RBS, BBM, SD

Pub #	P2-171
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	Comprehensive Genetic Analyses of *AR, SF1,* and *SRD5A2* in 95 Japanese Patients with 46,XY Disorders of Sex Development
Author String	M Hayashi, S Narumi, F Kato, T Ishii, T Hasegawa Keio University School of Medicine, Tokyo, Japan
Body	**Background:** *AR, SF1,* and *SRD5A2* have been assumed to be the top three monogenic causes of 46,XY disorders of sex development (DSD). Expansion or contraction of trinucleotide CAG repeat polymorphism in *AR*, G146A polymorphism in *SF1*, and V89L polymorphism in *SRD5A2* have been reported to reduce the activity of the protein *in vitro*. **Objective:**The aims of this study were **1)** to estimate frequency of *AR, SF1,* and *SRD5A2* mutations and **2)** to analyze whether those three polymorphisms in the three genes constitute susceptibility factor(s), in Japanese patients with 46, XY DSD. **Subjects:** **1)** We enrolled 95 Japanese index patients with 46,XY DSD. The Quigley stage(S) of the patients were as follows; S1=0, S2=0, S3 or S4=88, S5=0, S6/7=7, respectively. **2)** We studied 79 male-assigned Japanese patients with 46,XY DSD enrolled in the subjects1) and 171 fertile male people as control. The patients proven to have monogenic causes in 1) were excluded. **Methods:** **1)** All exons and exon-intron boundaries of the three genes were amplified by polymerase chain reaction and directly sequenced in the subjects 1). **2)** We determined alleles of the three polymorphisms in the subjects 2) and subsequently compared the range or frequency of alleles in the three polymorphisms between patients and control statistically. **Results:** **1)** Monogenic mutations were found in 13 patients (14% of index patients); *AR* mutations in 9 (10%), *SF1* mutation in 1 (1%), *SRD5A2* mutations in 3 (3%), respectively. **2)** (Range of lengths of CAG repeat)18-32 (Control 15-32) p=0.67 (Genotype of G146A)GG:GA:AA(%) =41:50:9 (Control 65:27:8) p=0.0002, AA+GA/GG OR=2.7 (95%CI:1.5-4.7) p=0.0003, GG+GA/AA OR=1.1 (95%CI:0.4-3.1) p=0.86 (Genotype of V89L)VV:VL:LL(%)=32:45:23 (Control 31:49:20) p=0.78 **Discussion and Conclusions:** We estimated the prevalence of *AR, SF1,* and *SRD5A2* mutations in Japanese patients with 46,XY DSD to be 14%. G146A in *SF1* polymorphism is likely to be a susceptibility factor in Japanese male-assigned patients with 46, XY DSD. Nothing to Disclose: MH, SN, FK, TI, TH

Pub #	P2-172
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	Novel Heterozygous Mutations of the *SF1* Gene in Patents with Severe Penoscrotal Hypospadias without Adrenal Insufficiency
Author String	J-H Choi, B-W Jeon, H-Y Jin, S-H Choi, G-H Kim, H-W Yoo Asan Medical Center, Seoul, Korea; Asan Medical Center, Seoul, Korea; Asan Medical Center, Seoul, Korea
Body	**Purpose:** Hypospadias is a frequent congenital anomaly. However, the exact etiology of hypospadias remains unknown in most cases. One candidate gene known to be involved in testis development and steroidogenesis is *SF1*. SF1 is a nuclear receptor that regulates multiple genes involved in adrenal and gonadal development, steroidogenesis, and the reproductive axis. Recent studies have suggested that heterozygous mutations in SF1 may be found in patients with 46,XY disorders of sex development (DSD) with normal adrenal function. This study was aimed to identify the *SF1* mutations in patients with severe hypospadias and micropenis without adrenal insufficiency. **Methods:** The cohort studied consisted of 16 46,XY DSD patients who presented at birth with variable degrees of hypospadias. Of these, 12 patients had both descended testes and 4 patients had at least one undescended testis. Adrenal insufficiency and dysmorphic features have not occurred in any of the patients. Genomic DNA was extracted from peripheral blood leukocytes and direct sequencing of the 6 coding exons o *SF1* was performed. **Results:** All patients manifested micropenis and hypospadias in the neonatal period, and urethroplasty was performed in infancy. Heterozygous novel missense mutations of the *SF1* gene were found in 6 cases (p.Gln352His, p.Gln457Pro, p.Gln215Lys, p.Ser195Cys, p.Ala280Thr, p.Gln294Pro). *In silico* analysis using Polyphen tool suggested all missense substitutions were classified as [ldquo]probably or possibly damaging [rdquo]. These 6 individuals represented the most severe end of the spectrum studied as they presented with penoscrotal hypospadias, and variable androgenization of the phallus. Endocrine evaluation at diagnosis showed low basal testosterone levels and significantly elevated testosterone levels after hCG stimulation. All patients did not have uterus and had a male gender assignment. **Conclusion:** This study demonstrates that *SF1* mutations should be considered in 46,XY individuals with severe penoscrotal hypospadias, especially if low testosterone levels are present. *SF1* mutations in milder forms of idiopathic hypospadias are unlikely to be common. Although the functional studies of these mutations of the *SF1* gene were not assayed, the fact that the sequence of these missense mutations of the *SF1* gene is highly conserved cross various species makes us speculate that it might abolish the function of the protein. Nothing to Disclose: J-HC, B-WJ, H-YJ, S-HC, G-HK, H-WY

Pub #	P2-173
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	Postnatal Development of the Gonad Specific SF-1 KO Mouse Testis
Author String	Y Ikeda, T Kato School of Medicine Yokohama City University, Yokohama, Japan
Body	The orphan nuclear receptor steroidogenic factor 1 (SF-1, Nr5a1), is expressed in steroidogenic and non-steroidogenic cells of limited tissues at multiple levels of the reproductive axis, and plays essential roles in their development and function. SF-1 is expressed in the two cell types, Leydig and Sertoli cells, in the testis during development and in adulthood. To study SF-1's roles in specific gonadal cell types, gonad-specific SF-1 KO mice were generated using the Cre-loxP system to inactivate SF-1 mediated by anti-Müllerian hormone type 2 receptor (Amhr2)-Cre. The previous analysis of the gonad-specific SF-1 KO revealed that expression of markers for testosterone biosynthesis was markedly reduced at embryonic day 14 (E14) and E16, indicating impaired differentiation of Leydig cells. The adult testis of the gonad-specific SF-1 KO mice was markedly hypoplastic and abnormal in morphology but had abundant Leydig cells. In the present study, we investigated the postnatal development of the gonad-specific SF-1 KO mouse testis. Noticeably, we found a population of cells that were morphologically mature Leydig cells and were immunoreactive for the P450 cholesterol side-chain cleavage enzyme (P450scc), a marker for steroidogenesis, and SF-1, in the interstitium of both KO and WT mouse testes at all developmental stages examined. During postnatal development, fetal-type Leydig cells (FLCs), which contribute to steroidogenesis in the embryonic testis, reduce, while adult-type Leydig cells (ALCs), which contribute to adult testicular steroidogenesis, differentiate from the precursor cells that are distinct from FLCs. It has been reported in rats that Amhr2 is expressed in FLCs during embryonic period, and in progenitor and immature ALCs, which appear at around postnatal day 21 (P21) and P35, respectively, as well as in mature ALCs in adulthood. Taken together, our results suggest that the Leydig cell population detected in the postnatal testis emerges probably during late embryonic period, produces testosterone but does not express Amhr2. Furthermore, the number of Sertoli cells that were identified by the immunoreactivities for AMH, Sox9, and SF-1, was decreased in the SF-1KO mice at P14 and P21, indicating that not all but some Sertoli cells in the postnatal testis are affected. The impaired Sertoli cell function may be associated with the abnormal morphology of the seminiferous tubules observed at P14 and P21. Nothing to Disclose: YI, TK

Pub #	P2-174
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	Testosterone Synthesis in Patients with 17β-Hydroxysteroid Dehydrogenase 3 Deficiency
Author String	SO Hiort, R Werner, H Merz, F Riepe, P-M Holterhus University of Luebeck, Luebeck, Germany; University of Luebeck, Luebeck, Germany; Christian-Albrechts University, Kiel, Germany
Body	17β-hydroxysteroid dehydrogenase 3 (17β-HSD 3) deficiency is a rare cause of 46,XY disorders of sex development (DSD). At the time of puberty, these patients experience a surge of androstenedione (A) and also testosterone (T), leading to substantial virilization. The origin of T synthesis in these patients remains elusive. **Objective:** To determine the expression of AKR1C3 (17b-HSD 5) in the testis as well as the ability of patients' genital skin fibroblasts (GSF) to synthesize testosterone. **Methods:** Four unrelated patients with the same disruptive mutation in HSD17B3 were investigated. Expression of AKR1C3 under varying incubations with androstenedione was studied in three patient derived GSF cultures. Serum samples of one patient before and after gonadectormy as well as the supernatants of the cultures were analyzed by LCMSMS and GCMS. The androgenic potential of supernatants was assessed by AR-mediated transactivation of an androgen responsive luciferase reporter gene in transiently transfected CHO cells. **Results:** AKR1C3 is expressed both in the testes and in GSF of the patients. However, in 17b-HSD 3 negative GSF, A is rapidly metabolized and is not synthesized to T. The transactivation potential towards the AR of the GSF supernatant is declining within 48 hours. Only under testis-equivalent A concentration, T can be synthesized in GSF. After gonadectomy, both A and T decline rapidly in vivo. **Conclusion:** In 17b-HSD 3 deficiency, relevant amounts of T are synthesized most probably through AKR1C3 in the testis and not peripherally in GSF. This explains the pubertal virilisation of the patients. Sources of Research Support: BMBF grant no. 01GM0625; FP7 grant 201444. Nothing to Disclose: SOH, RW, HM, FR, P-MH

Pub #	P2-175
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	Developmental Changes in Androgen Receptor (AR), Estrogen Receptor (ER) (Alpha and Beta) and Aromatase (ARO) Immuno-Expression in the Human Testis: Comparison of Mini-Puberty and Definitive Puberty
Author String	A Della Gaspera, E Berensztein, R Ponzio, MA Rivarola, A Belgorosky Hospital de Pediatria Garrahan, Buenos Aires, Argentina; Research Reproduction Institute, UBA, Buenos Aires, Argentina
Body	Androgen receptor (AR), estrogen receptors (ERα, ERβ) and aromatase (ARO) immunoexpression was studied in testicular cells of 32 testes as follows: 1) mini-puberty (MINI) vs puberty (PUB). Autopsy tissue of 0- to 7-month-old subjects who did not have endocrine or metabolic diseases (MINI Group, n=16) was compared with biopsy tissue of 14-15-year-old varicocele patients with normal histology for pubertal testis (PUB Group, n=5); 2). Post MINI, prepuberty (n=11)-puberty correlation with age, in 1- to 15-year-old subjects. Interstitial (IC), Leydig (LC), peritubular (PC), Sertoli (SC) and germ cells (GC) immunohistochemistry was estimated as % of positive cells. Statistics: ANOVA and Bonferroni, Kruskal-Wallis (ERα) tests, linear regression analysis. In LC, AR in MINI was lower than in PUB (8.8±13.1 vs 52.4±5.1, $p<0.05$); in IC and LC, ARO during MINI was significantly higher than in PUB (LC, 76.3±17.7 vs < 1%, $p<0.05$), while ERβ in MINI and PUB was similar (around 10-30%). In IC and LC, ERα in MINI was lower than in PUB (LC range 0-25 vs 89.4-100, $p=0.002$). In PC, ERβ in MINI was significantly higher than in PUB (24.7±10.8 vs <1%, $p<0.05$) while AR was high in MINI and PUB. In SC, AR and ERβ were lower ir MINI than in PUB (4±6.39 vs 92.3±11.8%, $p<0.05$ and 27.2±25.7 vs 69.2±6.75%, $p<0.05$, respectively). In GC (spermatogonia), ARO (45±25 vs 95.7±8.56%, $p<0.05$) and ERβ (30.6±16.9 vs 92±16%, $p<0.05$, respectively) in MINI were lower than in PUB. In PUB, ARO was also found in primary spematocytes and in spermatids. In the study of age correlation, % ERβ in PC decreased ($p=0.007$, $r=0.819$) while % AR increased ($p=0.007$, $r=0.73$). In SC, ERβ ($p=0.0007$, $r=0.82$) and AR ($p=0.000$, $r=0.97$) increased with the age. In GC, ERβ ($p=0.002$, $r=0.88$) and ARO ($p=0.000$, $r=0.94$) expression increased with the age. These results suggest that in MINI estrogens, locally synthesized by IC and LC might preserve the LC pool acting through ERβ, while in PUB they act through ERα on LC. SC expressed no AR in MINI; AR expression starts in late prepuberty to increase at PUB, when ERβ is also high. In spermatogonia, ARO and ERβ are expressed in MINI, and even more in PUB, suggesting a role for local estrogens through ERβ on spermatogenesis. In conclusion, LC ARO, ER and AR expression in MINI and PUB are very different, indicating changing local roles for sex hormones. The gradual changes preceding PUB confirm that the human testis is locally active during the so-called quiescent prepubertal period. Nothing to Disclose: ADG, EB, RP, MAR, AB

Pub #	P2-176
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	The Effects of Prenatal and Peripubertal Androgen Exposure on Female Puberty and Reproduction
Author String	EA Witham, JD Meadows, AS Kauffman, PL Mellon University of California, San Diego, La Jolla, CA
Body	Sex steroid hormone production and feedback mechanisms comprise critical components of the hypothalamic pituitary-gonadal (HPG) axis, and are important in fetal development, puberty, fertility, and menopause. In females, exposure to androgens can alter HPG axis organization, resulting in pathophysiological effects on reproduction. We have characterized a murine model of prenatal androgenization (PNA) in which females receive a low dose of dihydrotestosterone (DHT) during the late prenatal critical period of sexual differentiation in the brain, embryonic days 16-18. This dose of DHT does not significantly alter the external genitalia. The timing of pubertal onset (as determined by the age of vaginal opening) was significantly advanced by 4 days in PNA females compared to controls. Fertility assessments showed that PNA females have significantly delayed times to their first litter (12 days) compared to controls. Interestingly adult PNA females had increased serum testosterone levels compared to controls. However, PNA females did not display differences relative to controls in *Kiss1* gene expression in the hypothalamic arcuate nucleus. Since *Kiss1* levels in the arcuate are normally decreased by high circulating sex steroids, our results in PNA females suggest a disruption in sex steroid negative feedback on *Kiss1* neurons. We also observed disruptions in estrous cyclicity as the PNA mice aged so that by 8 months of age, 40% of PNA females had entered into persistent vaginal estrus (a marker of reproductive aging) compared to only 5% of controls. This difference in estrous cyclicity was maintained at 10 months. These results suggest an overall effect of prenatal DHT that accelerates reproductive aging throughout the life cycle. To assess the importance of the window of androgen exposure we administered DHT to peripubertal female mice on 21-23 days of age. Peripubertal DHT also significantly advanced the timing of pubertal onset compared to vehicle (by approximately 4 days), yet had no lasting effects on estrous cycling or testosterone levels, suggesting different mechanisms of DHT action between the prenatal and peripubertal periods. It remains to be determined whether the prenatal and peripubertal androgen effects on pubertal timing are via the same mechanism and if they occur in the brain. These studies will lead to further insight into the mechanisms underlying female reproductive disorders, including precocious puberty and polycystic ovarian syndrome. Sources of Research Support: U54 HD012303, R01 HD020377, R01 DK044838, R01 HD065856, T32 GM008666. Nothing to Disclose: EAW, JDM, ASK, PLM

Pub #	P2-177
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	Expression of a New 3[prime] End Variant of Human P450 Aromatase (ARO) Transcript (Intron 9, IN9) in the Human Term Placenta (Pl)
Author String	R Sainz, N Saraco, G Iniguez, MA Rivarola, V Mericq, A Belgorosky Hospital de Pediatria Garrahan, Buenos Aires, Argentina; Hospital San Borja Arriaran, Santiago, Chile
Body	Aromatase is the key enzyme for estrogen biosynthesis and is encoded by Cyp19 gene. In human placenta, Aro is expressed exclusively in syncytiotrophoblast. Alternative splicing of the coding region of Aro was previously described in other species but not in human. In male rat germ cells two unusual isoforms of Aro mRNAs lacking the last coding exon are expressed. In ovarian follicle of the fish Medaka, one of the two cDNAs described also lacks the sequence coding for the heme-binding domain. In rabbit preovulatory granulosa cells, a truncated Aro mRNAs is expressed. These findings suggest that alternative splicing events could be involved in the control of Aro expression. The aim o our study was to evaluate the expression of the IN9 variant (truncated mRNA lacking exon 10) in Pl. Total RNA was isolated from 15 Pl tissues from 6 males (m) and 9 females (f) newborn deliveries. Aro mRNA variant expression was analyzed by Real-time RT-PCR. Aro protein was analyzed by Western blot. We found expression of the IN9 variant in Pl. IN9 mRNA related to total Aro mRNA (CYP19) or active Aro mRNA (Arom) in m was significantly higher than in f (IN9/CYP19: 6.99±5.24 and IN9/Arom: 9.36±7.15 AU, mean±SD, n=6) (IN9/CYP19: 2.67±2.89 and IN9/Arom: 2.33±2.56 AU, mean±SD, n=11), $p<0.05$. We are describing for the first time a new Aro mRNA variant in human placenta that has a gender-specific expression. As the IN9 variant is a truncated Aro mRNA translating a non active protein, we propose that the expression of this variant would be involved in the regulation of Aro activity in human term placenta. Nothing to Disclose: RS, NS, GI, MAR, VM, AB

Pub #	P2-178
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	LIN28 in Human Ovary Development and Function
Author String	R El-Khairi, R Parnaik, L Lin, MT Dattani, GS Conway, JC Achermann UCL Institute of Child Health, University College London, London, UK; University College London Hospitals, London, UK
Body	The Lin28 family of proteins are emerging as important regulators of microRNAs in endocrine systems and key modulators of the hypothalamic-pituitary-gonadal axis in females. *Lin28* (also known as *Lin28a*) influences primordial germ cell development in mice, and overexpression of *Lin28a* in transgenic mice has recently been shown to influence body size, timing of puberty and litter size. Similarly, several different genome-wide association studies in humans have shown that polymorphic variability in the related factor *LIN28B* can be associated with age at menarche and stature. Despite these recent advances, relatively little is known about the role of this family of proteins in the developing female reproductive system in humans, or in human reproductive disorders. To address this, a study was undertaken to investigate expression of *LIN28A* and *LIN28B* in early human ovary development and as a potential cause of primary ovarian insufficiency (POI) in women. Expression studies showed that *LIN28A* was upregulated during a critical stage of early human ovary development (6-9 weeks post-conception, wpc), whereas levels were lower in the developing testis and did not shown any variation over this time period. In contrast, *LIN28B* was expressed at a lower level than *LIN28A*, and did not show differential expression in the gonads. These findings suggest that LIN28A is the major regulator of early ovary development. In keeping with these results, immunohistochemistry revealed strong expression of LIN28A together with OCT4 in a population of peripheral germ cells in the developing human ovary at 7 wpc. LIN28A seemed to be upregulated in germ cells located cortically in the developing gland, possibly representing those cells undergoing rapid mitotic division or ready to enter meiosis. These results led us to hypothesize that LIN28A may regulate primordial germ cell formation and expansion of the germ cell pool, and that disruption of *LIN28A* might lead to germ cell depletion and primary ovarian insufficiency (POI) in humans. Mutational analysis of *LIN28A* was undertaken in a cohort of women with POI but did not reveal any significant non-synonymous changes in this gene. Taken together, these findings support a role primarily for LIN28A rather than LIN28B in early human germ cell development, but suggest that changes in *LIN28A* are not a common cause of POI. Sources of Research Support: The Wellcome Trust (079666). Nothing to Disclose: RE-K, RP, LL, MTD, GSC, JCA

Pub #	P2-179
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	Porcine Granulocyte-Macrophage Colony-Stimulating Factor (pGM-CSF) Improves Development of Porcine Preimplantation Embryos Produced *In Vitro*
Author String	S-S Kwak, D Biswas, S-H Hyun College of Veterinary Medicine, Chungbuk National University, Cheongju, Republic of Korea
Body	This study investigated the effects of porcine granulocyte-macrophage colony-stimulating factor (pGM-CSF) on the developmental potential of porcine *in vitro*-fertilized (IVF) embryos in chemically defined medium. In experiment 1, zygotes were treated with different concentrations of pGM-CSF (0, 2, 10, 100 ng/ml). The results indicated that 10 ng/ml pGM-CSF significantly ($p < 0.05$) increased blastocyst development and total cell number (15.1% and 53.5, respectively) compared with the control (6.1%, 38.8, respectively). Comparing blastocyst formation, early and expanded blastocyst formation was significantly higher in the 10 ng/ml-pGM-CSF group than in the control on days 6 and 7 of the culture period. However, there was no significant difference in cleavage rate. Experiment 2 demonstrated that pGM-CSF influenced the percentage of blastocys formation when pGM-CSF was added during days 4-7 (14.6%) or days 0-7 (15.2%) compared with the control (7.8%) and compared with days 0-3 (8.7%). Similarly, early blastocyst formation rates were significantly higher at days 4-7 than in the control, and expanded blastocyst formation was significantly higher at days 4-7 or days 0-7. No significant difference in cleavage rates appeared among the groups. In experiment 3, in the presence of BSA, pGM-CSF also increased the percentage of embryos that developed to the blastocyst stage and the total cell number (20.3% and 59.8, respectively) compared with the control (14.9% and 51.4, respectively), whereas there was no significant difference in cleavage rate. Experiment 4 found that the total cell number and the number of cells in the inner cell mass (ICM) were significantly increased compared with the control when zygotes were cultured in either PZM-3 or PZM-4 medium supplemented with 10 ng/ml pGM-CSF. The number of trophectoderm (TE) cells was significantly higher in PZM-3 medium supplemented with pGM-CSF than in the control, and the number tended to increase ($p = 0.058$) in PZM-4 medium supplemented with pGM-CSF. The ratio of ICM to TE cells was significantly higher in PZM-4 medium supplemented with 10 ng/ml pGM-CSF, but not in PZM-3 medium. Together, these results suggest that pGM-CSF may have a physiological role in promoting the development of porcine pre-implantation embryos and regulating cell viability and that addition of pGM-CSF to IVC medium at days 4-7 or 0-7 improves the developmental potential of porcine IVF embryos.

Sources of Research Support: Grant (# 20070301034040) from BioGreen 21 program, Rural Development Administration, Republic of Korea.

Nothing to Disclose: S-SK, DB, S-HH

Pub #	P2-180
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	Cleavage Pattern and Survivin Expression in Porcine Embryos by Somatic Cell Nuclear Transfer
Author String	Y Jeon, E-B Jeung, S-H Hyun Chungbuk National University, Cheongju, Republic of Korea
Body	Numerous, somatic cell nuclear transfer(SCNT) embryos undergo arrest and show abnormal gene expression in the early developmental stages. The present study, we were performed to analyze porcine SCNT embryo development and investigate the cause of porcine SCNT embryo arrest. The temporal cleavage pattern of porcine SCNT embryos was analyzed first, and the origin of the blastocyst was found on day 7. To investigate markers of arrest in the cleavage patterns of preimplantation SCNT embryos, the expression of survivin - the smallest member of the inhibitor of apoptosis gene family, which suppresses apoptosis and regulates cell division - was compared between embryos showing normal cleavage and arrested embryos. A total of 511 SCNT embryos were used for cleavage pattern analysis. 24 hours post activation (hpa), embryos were classified into five groups based on the cleavage stage as follows; 1-cell, 2-cell, 4-cell, 8-cell and fragmentation (frag). In addition, 48 hpa, embryos were more strictly classified into 15 groups based on the cleavage stage of 24 hpa; 1-1 cell (24 hpa-48 hpa), 1-2 cell, 1-4 cell, 1-8 cell, 1 cell-frag, 2-2 cell, 2-4 cell, 2-8 cell, 2 cell-frag, 4-4 cell, 4-8 cell, 4 cell-frag, 8-8 cell, 8 cell-frag, and frag-frag. These groups were cultured until 7 days post activation, and were evaluated for blastocyst formation. At 24 hpa, the proportion of 2-cell stage was significantly higher (44.5%) than those in the other cleavage stages. At 48 hpa, the proportion of embryos in the 2-4cell stage was significantly higher (32.4%) than those in the other cleavage stages. Blastocyst formation rates were significantly higher in 2-4 cell cleavage group (52.5%) than in other groups ($p<0.05$). Therefore SCNT embryos in 2-4 cell stage showed the greatest stability and excellent developmental competence. In addition, we investigated survivin expression in porcine SCNT embryos during the early developmental stages. The levels of survivin mRNA in 2-cell, 4-cell stage SCNT embryos were significantly higher than those of arrested embryos. Survivin protein expression showed a similar pattern to that of survivin mRNA. Normally cleaving embryos showed higher survivin protein expression levels than arrested embryos. These observations suggested that 2-4 cell cleaving embryos at 48 hpa have high developmental competence, and that apoptosis is a factor in the induction of embryonic arrest, which may be influenced by survivin expression in porcine SCNT embryos. Sources of Research Support: Grant (# 20070301034040) from BioGreen 21 program, Rural Development Administration, Republic of Korea. Nothing to Disclose: YJ, E-BJ, S-HH

Pub #	P2-181
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	Barriers to Gender Reassignment Surgery (GRS) in Transsexuals in the U.S.: Influence of Gender and Psychiatric Disorders
Author String	M Fiore-Urizar, N Patel, S Bettadahalli, M Luo, N Galambos, M Leinung Albany Medical College, Albany, NY; Statistical Power Inc, Stony Brook, NY
Body	Objectives: GRS is considered standard therapy for transsexualism by the World Professional Association for Transgender Health (WPATH). We looked at rates of GRS in a population of transsexual patients in upstate NY. Methods: We reviewed the charts of patients seen in our institution from 1986 to 2010 for hormonal therapy of transsexualism. Results: A total of 242 patients have been seen, 192 male to female (MTF) and 50 female to male (FTM). Forty six (24%) MTF underwent vaginoplasty, and 8 underwent orchiectomy only. Prolonged follow up changed the percentage little. Of those on hormonal therapy for 10 or more years, only 31% (14 of 45) have had vaginoplasty (an additional 4 have had orchiectomy). HIV status did not have a significant effect: of 9 patients HIV positive, 4 have had vaginoplasty. Thirty two of 50 (64%) FTM had some form of GRS: 27 breast reductions and 21 hysterectomies. Prolonged follow up produced little change: Of 35 FTM patients with >2 years of therapy, 17 (49%) have had hysterectomy, and an additional 8 (23%) have had mastectomy (hence 25 (71%) with surgery). Note that 4 patients had hysterectomy prior to initiation of hormone therapy. Kaplan Meier survival analysis was used to study the difference in time to surgery for MTF and FTM with and without mood and adjustment disorders (depression, mania, adjustment disorder). Time to surgery was shorter for FTM and was not affected by the presence of mood and adjustment disorders (mean 4.84 years). The average time to surgery among MTF was 19.4 years and was effected by presence of mood disorders (11.5 years without, 23.2 with). Conclusions: Few transsexual patients are undergoing GRS, especially FTM. One major barrier is lack of coverage by Medicaid and most insurance carriers, thus forcing patient self pay. We also found that the presence of mood and adjustment disorders added to the delay in surgery for MTF. This is likely due to the impact of mood and adjustment disorders, wealth accumulation, and overall functionality. FTM patients were more likely to undergo surgery, probably due to lower cost and additional medical reasons for hysterectomy. Nothing to Disclose: MF-U, NP, SB, ML, NG, ML

Pub #	P2-182
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	Efficiency and Safety of Low Doses of Estrogens Associated with Antiandrogens in Hormonal Treatment of Male-to-Female Transsexual Patients
Author String	CA Hohl, VL Camara, UV Zanardi, M Inacio, EU Verduguez, MHP Sircili, S Domenice, BB Mendonca, EMF Costa Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil
Body	**Background:** The cross-sex hormone treatment of transsexual persons is a challenging task for the endocrinologist. The treatment goals are suppress endogenous hormone secretion and maintain the cross-sex hormone levels within the adult normal range. Typical transsexual estrogen therapy were two to three times higher than recommended for hormone replacement therapy in postmenopausal women (1). However, side effects as venous thrombosis, hyperprolactinemia and breast cancer are reported and estrogens dose dependen **Objective:** To demonstrate our long-term experience with low-dose hormone treatment in MTF transsexual patients. **Patients and Methods:** A retrospective study of forty-three cross-sex hormone treated MTF transsexual patients. **Results:** The mean age of treatment onset with low doses of cross-sex hormones was 21 years and the average treatment time was 10.8 years. Most patients reported previous irregular use of estrogen preparations. The patients were treated with oral conjugated equine estrogen (CEE) 0.625mg in 52.7% of them and 1.25mg in 47.3%, associated with cyproterone acetate100mg in 57.5% and 50mg in 32%. Physical examination after at least 1 year of treatment revealed breast development T3 stage in 12.1% of the patients, T4 in 36.4% and T5 in 51.5% of them, unrelated to the dose of estrogen used. The facial and body were scarc in the majority of the patients. The penis and testicular size were smaller than the average for normal adult males. Decreased spontaneous erections occurred in 99% of the patients. Laboratory results revealed that the mean LH levels before treatment was 7.78 UI/L and after treatment was 1.54. The mean pretreatment FSH levels was 11.1 UI/L and after treatment 3.42 UI/L. Estradiol levels before and after treatment was 36 and 42.6 pg/mL respectively, and testosterone levels decreased from an average of 733 before treatment to 41.3 ng/dL pos treatment. No cases of venous thromboembolism or breast cancer were found. The bone mineral density after treatment was in normal average for male in 90% of the patients. Interestingly, hyperprolactinemia was detected in 55.8% of the patients (11.3 to 39.5 ng/mL) and all of them were taking cyproterone acetate. **Conclusion:** Low-dose hormone treatment in MTF transsexual patients was effective to achieve normal androgen levels for female sex and normal female secondary sex development with no relevant side effects.

(1) Hembree, WC et al., J Clin Endocrinol Metab 2009; 94:3132

Nothing to Disclose: CAH, VLC, UVZ, MI, EUV, MHPS, SD, BBM, EMFC

Pub #	P2-183
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	Demographic, Clinical, and Psychiatric Characteristics of Transsexual Patients: Possible Influence of Age at Treatment on Mood and Adjustment Disorders
Author String	M Fiore-Urizar, N Patel, S Bettadahalli, M Luo, N Galambos, M Leinung Albany Medical College, Albany, NY; Statistical Power, Inc, Stony Brook, NY
Body	Objectives: We sought to describe the characteristics of transsexual patients presenting to our institution in upstate New York over the past 24 years. Methods: Retrospective chart review of transsexual patients seen and followed from 1986 to 2010 for hormonal therapy. Results: A total of 242 patients were seen: 80% (192) male to female (MTF) and 20% female to male (FTM). A psychiatric disorder at presentation existed in 137 (56.6%) with 106 (77.4%) having mood and adjustment disorder (depression, mania, adjustment disorder). MTF transsexuals were older than FTM at initiation of hormonal therapy (38.0 vs. 29.6 years). Transsexual patients frequently had low employment status with 41 % of those of working age being unemployed or on disability. Most of the disabilities were related to mental health issues. At presentation, there was a higher percentage of MTF subjects on disability (22.0 vs. 8.7%), and a higher percentage of FTM subjects who were students (26.1 vs. 6.1%: both p values <0.001). The most frequent psychiatric diagnoses were mood and adjustment disorder. Alcohol and substance-related disorders are more prevalent in the MTF group. There has been a notable increase over time in the number of patients presenting to the clinic, with a commensurate decline in age. The incidence of mood and adjustment disorder was lower in those patients that began hormonal treatment at age <= 32. Overall 36.3% of the age <=32 group had a mood and adjustment disorder, while 51.7% of the age 32+ group had a mood and adjustment disorder. (Fisher's Exact 1-side test, p=0.011). The age at presentation correlated inversely with the presence of psychiatric problems. Conclusion: Psychiatric problems and low employment status are common in our transsexual population. Patients beginning hormonal treatment at <=32 (median age of therapy) have a significant lower incidence of mood and adjustment disorders. It is possible that initiation of therapy at younger age may help prevent such disorders in transsexual patients. The recent increase in the number of patients presenting and the younger age may be a result of increasing social acceptance and general awareness of transsexualism. Nothing to Disclose: MF-U, NP, SB, ML, NG, ML

Pub #	P2-184
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	Management of Disorders of Sex Differentiation: An International Survey
Author String	N Josso, L Audi INSERM, Clamart, France; Hospital Vall d'Hebron - Institut de Recerca, Barcelona, Spain
Body	A questionnaire on management of disorders of sex differentiation (DSD), CAH excluded, was e-mailed to th 650 international members of the European Society for Paediatric Endocrinology (ESPE). 62 answers were received from 21 different countries. **The response rate** from Northern and Central Europe (NCE: Germany/Austria, Benelux, Switzerland, Sweden and UK), Latin Europe (LE: France, Spain/Portugal, Italy) was approximately 8%, lower than that of Eastern Europe (EE) 15% or Turkey 18,5%. Three out of the seven South-American (SA) members responded. Responses from other parts of the world were not representative. **Nomenclature** 86% of respondents have adopted the "Chicago" 2005 nomenclature with colleagues, 68% use it at least partially with parents. The rate of **prenatal diagnosis** is highest in LE: a 10% incidence was reported by 42% of respondents compared to 13% in NCE and EE and none in other regions. Standards of prenatal care probably explain this discrepancy. Most respondents have access to **hormonal and genetic tests** they require but the cost and delay (up to one month for a karyotype) are much higher in EE and Turkey, explaining a significant delay in sex assignment**. AMH assay** is available to 83% of NCE, 67% of LE and SA members and 40% elsewhere. In the West, 80% DSDs are **sex-assigned** within 2 days, 49% in EE, only 10% in Turkey and SA. **Initial interviews** are always carried out by the pediatric endocrinologist, often assisted by a surgeon/urologist and a psychologist but rarely by a gynecologist, except in EE. Physicians from NCE were the most **open with family and patient,** elsewhere karyotype and prospects for fertility were often revealed late or only after specific questioning. Regarding **parameters implicated in sex assignment**, fertility was highly rated by 80% Turkish respondents, compared to 30-40% elsewhere. Parental views were considered crucial by 60-70% of LE and SA respondents versus 27% in NCE, 17% in EE and none in Turkey. Globally, ease of male reconstruction, fertility and size of penis topped the list, karyotype was last. In LE, EE and Turkey, [ldquo]male factors[rdquo], ie ease of male genitoplasty and penile size, were given significantly more weight than [ldquo]female factors[rdquo] such as ease of female genitoplasty and presence of an uterus, maybe because the results of male reconstruction are immediately apparent, while problems with female plasty are detectable only later. No sex bias was seen in SA or NCE where surgical considerations were not paramount. Nothing to Disclose: NJ, LA

Pub #	P2-185
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL/CLINICAL - Sex Determination, Differentiation & Reproductive Development (1:30 PM-3:30 PM)
Title	The Effect of Transdermic Dihydrotestosterone Gel Treatment on Penile Size: Experience with Fifteen Patients
Author String	RB Silva, CR Gomes, MP Brandao, S Domenice, AAL Jorge, EM Costa, BB Mendonca Hospital das Clinicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil
Body	Dihydrotestosterone (DHT) is the most powerful naturally occurring androgen and, unlike testosterone (T), cannot be aromatized to estradiol. Treatment with DHT gel represents a more physiological way of promoting virilization of the external genitalia. The advantages of DHT gel are the transdermal route of administration and the absence of estrogen-related effects, such as gynecomastia and acceleration of growth rate and bone age progression. Objective: To evaluate the results of treatment with DHT gel 2.5% in patients with isolated micropenis or ambiguous genitalia with microphalus. Patients: Data from 15 patients attending the Endocrinology Center from 1987 to 2010 were retrospectively evaluated. The majority (10/15) had Disorders of Sex Development (DSD) of various etiologies: four patients with disorders associated to defects in synthesis, action or metabolization of T and six with defects in gonadal development. Five patients had isolated micropenis. Mean age at the beginning of treatment was 6.43+/-3.95 yr. The mean daily dose of DHT gel 2.5% was 3.55+/-1.42 g and the duration of treatment was 2.75+/-1.88 mo. The stretched penile length was expressed as mean +/-1 SD in centimeter (cm) and Z score and treatment response was evaluated by the difference between the Z score at the beginning and after completing treatment. Data from penile anthropometry of Brazilian patients were used as reference (1). Results: The penile size before treatment was 3.34+/-0.86 cm (Z -3.35+/-0.68) and after DHT was 4.76+/-0.74 cm (Z -1.86+/-0.88). Z score of treatment response was 1.49+/-0.29 ($p<0.0001$). There was no statistically significant difference in treatment response between the groups: DSD associated to defects in synthesis, action or metabolization of T; DSD with defects in gonadal development and patients with isolated micropenis. Only early age at the beginning of treatment correlated significantly with a better response on final penile size ($p = 0,024$). Tanner Stage of pubic hair development was applied to 12/15 patients: 6 remained unchanged; 5 progressed one stage and 1 progressed two stages. Conclusions: Treatment with DHT gel was an effective treatment on penile growth induction, particularly when initiated in earlier ages. Moreover, the only side effect was a mild progression of pubic hair (1) Gabrich PN, Vasconcelos JS, Damiao R, Silva EA. Penile anthropometry in Brazilian children and adolescents. J Pediatr (Rio J). 2007; 83(5): 441-6. Nothing to Disclose: RBS, CRG, MPB, SD, AALJ, EMC, BBM

Pub #	P2-186
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Male Reproductive Physiology (1:30 PM-3:30 PM)
Title	The Tyrosine Phosphatase SHP2 Regulates Sertoli Cell Junction Complexes
Author String	P Puri, WH Walker University of Pittsburgh, Pittsburgh, PA
Body	Within the seminiferous tubules of the testes, Sertoli cells provide the support required for germ cell maturation. Sertoli cells create a unique environment for meiotic and post-meiotic germ cells due to the formation of a cluster of tight, adherens and desmosomal junctions, known as the blood testis barrier (BTB). The absence or dysfunction of BTB leads to male infertility. BTB functions are regulated by the phosphorylation of BTB protein components. We have found that a phosphatase, SHP2, is localized to Sertoli Sertoli cell junctions. Constitutive activation of SHP2 causes Noonan syndrome in humans, which results in male infertility. Furthermore, there is evidence that Noonan syndrome patients have Sertoli cell dysfunction. Our studies of cultured rat Sertoli cells indicated that the activity of known BTB regulatory kinases was altered after infection with an adenovirus expressing a constitutively active SHP2 mutant (SHP2 Q79R). Specifically, ERK was activated and FAK activity was inhibited. The SHP2 Q79R-mediated increase in phosphorylation of ERK was partially inhibited by the Src kinase inhibitor PP2, suggesting that SHP2 acts partially through Src kinase to regulate MAP kinase pathway in Sertoli cells. Over-expression of SHP2 Q79R also resulted in fewer polymerized actin filaments within the cell body and disrupted the actin cytoskeleton. In addition, Sertoli cells over-expressing AdSHP2 Q79R had reduced staining of the tight junction marker ZO-1 and adherens junction marker β-catenin at cell junction sites. Thus, our preliminary studies indicate that the expression of SHP2 Q79R in Sertoli cells causes mis-localization of BTB component proteins and disruption of actin structure in Sertoli cells, two mechanisms by which BTB integrity can be compromised. Further studies will be performed to determine whether SHP2 Q79R disrupts BTB integrity and spermatogenesis in vivo and to identify the mechanisms by which SHP2 Q79R disturbs the structure of the BTB. The expected outcome of the proposed investigations will enhance our understanding of the regulation of BTB integrity by SHP2 and may define the etiology of male infertility in Noonan syndrome patients. This information is needed to develop novel strategies for the diagnosis and treatment of male infertility. Sources of Research Support: 1RO1 HD043143 awarded to WHW. Nothing to Disclose: PP, WHW

Pub #	P2-187
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Male Reproductive Physiology (1:30 PM-3:30 PM)
Title	The LKB1/CRTC Pathway Regulates Aromatase in the Human Testis
Author String	S Ham, SJ Meachem, ER Simpson, KA Brown Prince Henry's Institute, Clayton, Australia; Monash University, Clayton, Australia; Monash University, Clayton, Australia; Monash University, Clayton, Australia; Monash University, Clayton, Australia
Body	Estrogens play an important role in spermatogenesis and their biosynthesis is dependent on the expression of aromatase, which catalyzes their conversion from androgens. The proteins involved in the regulation of aromatase expression in the testis are largely uncharacterized. Our laboratory has implicated LKB1 as a negative regulator of aromatase expression in the breast by inhibiting the nuclear translocation of the CREB co-regulator CRTC2, a potent inducer of aromatase expression. As aromatase expression in breast cancer and the testis is primarily dependent on the activation of aromatase promoter II (PII), we hypothesized that the LKB1-CRTC pathway may be involved in regulating aromatase in the testis. Luciferase assays were performed to determine the effect of CRTC1-3 on aromatase PII activity using the Sertoli-like cell line NT2/D1. Immunofluorescence was performed on archived Bouin's fixed samples from qualitatively fertile men (n=3) to assess the localization of CRTC1, CRTC2, and CRTC3, LKB1, and aromatase within the testis. Our results showed that all three CRTCs significantly increased the activity of aromatase PII, with CRTC3, having the greatest effect. Furthermore, the CRTC-mediated effect was suppressed significantly in the presence of LKB1. Using confocal microscopy, we demonstrated that aromatase is expressed in the seminiferous epithelium, consistent with published results in rodent models. Conversely, LKB1 was mainly localized in interstitial cells. CRTC1 and CRTC3 were predominantly cytoplasmic and localized in the interstitial cells and seminiferous epithelium. CRTC3 was highly expressed in pachytene spermatocytes and round spermatids. Notably, CRTC2 was localized in the nucleus of the seminiferous epithelium and in the cytoplasm of interstitial cells. Interestingly, CRTC2 nuclear localization was inversely correlated with LKB1 protein expression. As CRTC activity is positively correlated with its nuclear localization, our data suggests that it is CRTC2 that is involved in regulating aromatase within the testis, and that its localization is tightly coupled to the expression of LKB1. Future studies will involve identifying the functional role of this signaling pathway in the healthy and diseased testis, including Peutz-Jegher's syndrome where the LKB1 gene is mutated and aromatase expression is supra-physiological. Sources of Research Support: NHMRC program grant 494802 to ERS. Nothing to Disclose: SH, SJM, ERS, KAB

Pub #	P2-188
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Male Reproductive Physiology (1:30 PM-3:30 PM)
Title	Role of FSH Prior to LH + FSH on Testes Development in Humans: Effects on Histologic, Biochemical, and Fertility Parameters in Men with Isolated Gonadotropin-Releasing Hormone (GnRH) Deficiency and Prepubertal Testes
Author String	AA Dwyer, G Sykiotis, FJ Hayes, PA Boepple, KR Loughlin, M Dym, WF Crowley, Jr, N Pitteloud Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland; University of Patras Medical School, Patras, Greece; St Vincent's University Hospital, Dublin, Ireland; Massachusetts General Hospital, Boston, MA; Brigham & Women's Hospital, Boston, MA; Georgetown University Medical Center, Washington, DC
Body	**BACKGROUND:** Men with isolated GnRH-deficiency and prepubertal testes provide a rare and unique human disease model to explore the role of FSH in testes maturation. This study examined the impact of FSH pre-treatment on testes development, inhibin B (I_B) secretion, testicular volume (TV), and sperm production. We hypothesized that outcomes could be improved by inducing growth with FSH alone before providing FSH + LH to induce growth & maturation simultaneously. **METHODS:** 18 men with isolated GnRH deficiency, prepubertal testes ($\leq$ 3 mL), and no cryptorchidism or prior gonadotropin Rx were enrolled. Following baseline (BL) neuroendocrine profiling, TV by ultrasound, and testicular biopsy, subjects were randomized to either (i) 24 mos of pulsatile GnRH therapy (LH + FSH stimulation), or (ii) 4 mos rFSH (Gonal-F 75-150 IU sc daily) followed by 24 mos pulsatile GnRH. Repeat biopsies were performed at + 4 mos. Serial FSH, I_B, LH, testosterone (T), TV & seminal fluid analyses were performed throughout. **RESULTS:** 5 men were excluded for compliance issues. At BL, the rFSH pre-treatment group (n=7) and the LH + FSH group (n=6) were similar in all parameters, with undetectable LH & FSH (< 1.6 IU/L), low T (18$\pm$2 ng/dL), & low I_B (22$\pm$6 pg/mL). TV was uniformly small (1$\pm$0.1 mL) with prepubertal histology: absent Leydig cells (LC), small seminiferous cords lacking lumen, containing only immature Sertoli cells [SC] & spermatogonia (SGA). On rFSH (8$\pm$0.4 IU/L), I_B [uarr] to normal (24$\pm$11 to 107$\pm$31, p<.05) while LH & T were unchanged. TV doubled in 4 months (1$\pm$0.2 to 2$\pm$0.3, p<.005) and biopsies revealed SC/SGA proliferation with [uarr] SC: germ cell ratio yet no change in cord diameter. Marked maturational changes were observed: cytoskeletal rearrangement, appearance of tight junctions, SC polarization, & SGA migration. On pulsatile GnRH (LH + FSH) both groups normalized T (> 280 ng/dL) and though not statistically significant, the rFSH group exhibited: i) [darr] LH & FSH (13$\pm$0.5 vs.17$\pm$0.7 IU/L , 10$\pm$0.4 vs. 16$\pm$1.1 IU/L), [uarr] I_B (110$\pm$5 vs. 74$\pm$4 pg/mL), iii) [uarr] TV (9$\pm$2 vs. 7$\pm$1 mL), iv) [uarr] rates of developing sperm in the ejaculate (7/7 vs. 4/6), and [uarr] counts (range: 1-18 vs. 0-7 X 10^6/mL, 6$\pm$2 vs. 2$\pm$1 X 10^6/mL). **CONCLUSIONS:** FSH induces SC/SGA proliferation & testicular growth in immature testes and triggers cytoskeletal rearrangements in the seminiferous tubules. Pre-treatment with rFSH prior to pulsatile GnRH appears to have beneficial effects on maximizing potential for fertility in these men. Sources of Research Support: R01 HD15788 Eunice Kennedy Shriver National Institute of Child Health and Human Development/NIH Cooperative Agreement U54 HD028138. Nothing to Disclose: AAD, GS, FJH, PAB, KRL, MD, WFC, NP

Pub #	P2-189
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Male Reproductive Physiology (1:30 PM-3:30 PM)
Title	Human Prepubertal Testis in Organotypic Culture: A Useful Tool for Basic and Clinical Research
Author String	E Berensztein, A Della Gaspera, MA Rivarola, A Belgorosky Hospital de Pediatria Garrahan, Buenos Aires, Argentina
Body	Organotypic culture of testis is a good system for studying the development of somatic and germ cells. Some reports studied this model animals or human fetal and adult testis. Little experience has been reported in human prepubertal testis (HPPT). We have previously described a method to obtain and develop primary cell cultures from HPPT collected at necropsies. However, biopsies of dysgenetic testes frequently provide insufficient material for primary cell cultures. The aim of this work was to develop a culture micro-method to study the function of Sertoli (SC), Leydig (LC), and germ cells (GC), as well as macrophages (MO), in HPPT biopsies to study *in vitro* normal and pathological testicular tissues inside a preserved histo-architecture. Four human testis samples were studied: 3 prepubertal (PP, ages: 2 months, 2 months, and 2.5 years) and 1 puberta (Pu, age: 13 years) subjects. The study was approved by the Institutional Review Board of the Garrahan Pediatric Hospital. Testicular tissues were carefully dissected into 3 mm^3 fragments. In each well of a six-wel plate, four-six fragments were placed on Millipore filters (pore size, 0.45 mm). The filter bearing the pieces o testis was placed on 1.5 ml DMEM/F12 medium and cultured at 37[deg]C, in a humidified atmosphere containing 95% air:5% CO2, for 5 days. The medium was changed every 24 h. At the end of the culture period, 100 ng/ml hCG for the last 3 h of culture was added. Conditionned media (CM) were collected to determine testosterone (LC), estradiol (LC) and AMH secretion (SC). For immunohistochemical analyses, the explants were fixed in formalin. Several markers were used to identify different cell subpopulations in the testicular parenchyma: P450scc for LC, AMH for SC, c-kit and MAGE for GC, and TNFα / CD68 for MO. The histoarchitecture of the explants during culture was conserved Testosterone secretion was detected in CM either increasing with culture days (two PP testis) or decreasing (one PP and Pu). T secretion increases significantly (275% and 250%) under hCG stimulation in two cultures (one PP and Pu respectively). Estradiol and AMH secretion were also detected in all cultures.. Positive immunoexpression of all somatic and germ cell markers was observed. In conclusion, organotypic culture of human PP testis proved to be an efficient tool for studying the early development of the testicular functions and interactions, particularly when the sample is too small for primary cultures. Nothing to Disclose: EB, ADG, MAR, AB

Pub #	P2-190
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Male Reproductive Physiology (1:30 PM-3:30 PM)
Title	Identification of Ubiquitin Proteasome System Genes and Proteins in Rat Testicular Gonocytes
Author String	G Manku, O Sarkar, S Wing, M Culty McGill University Health Center, Montreal, Canada; McGill University, Montreal, Canada; McGill University, Montreal, Canada
Body	The ubiquitination of proteins represents one of the main mechanisms for protein turnover and is involved in the regulation of various cellular functions and signaling pathways, both in physiological and pathological conditions. Alterations of components of the Ub proteasome system (UPS) have been associated with subfertility/infertility. We have shown that ubiquitination is activated during spermatogenesis and identified ubiquitin (Ub) system genes in mice from postnatal day (PND) 10 to 65 (1). Spermatogenesis relies on the formation of a pool of spermatogonial stem cells (SSCs) from their precursor cells, the gonocytes. Moreover, failure of gonocytes to differentiate is believed to lead to the formation of testicular germ cell tumors, the mos common cancer in young men, stressing the importance of studying this germ cell stage. We have studied rat gonocyte development, identifying factors and pathways regulating proliferation and differentiation, as well a the impact of endocrine disruptors on this process (2). Based on the hypothesis that UPS proteins might be involved in the turnover of key proteins during gonocyte development, we determined which UPS genes/proteins are expressed in gonocytes and compared their levels to those found in spermatogonia, using both gene-targeted and gene expression array approaches. Among the genes expressed in gonocytes were genes previously shown to be involved in spermatogenesis, including Ub, the E2 Ub conjugases UBC2 and UBC4, the Ub ligase Huwe1/LASU1 and the deubiquitination enzyme USP2, as well as genes with no known function in male reproduction such as the Ub conjugase Ube2l6 and the deubiquitination enzyme USP25. Interestingly, LASU1 was localized in the cytosol of gonocytes whereas it was found to be nuclear in spermatogonia, suggesting a different function between the two germ cell stages. These studies identified several UPS-related genes that may be involved in gonocyte development. Deciphering the role of these molecules in gonocytes should provide clues on the mechanisms involved in the transition from gonocyte to SSC and lead to a better understanding of male reproductive pathologies such as testicular cancer and infertility. 1. Wing SS 2003 Int J Biochem & Cell Biol 35:590-605 2. Culty M 2009 Birth Defect Research (Part C), 876:1-26 Sources of Research Support: In part by an award from the Center for the Study of Reproduction at McGill University and by the Research Institute of the McGill University Health Center. Nothing to Disclose: GM, OS, SW, MC

Pub #	P2-191
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Male Reproductive Physiology (1:30 PM-3:30 PM)
Title	A Novel Mouse Model for Severe Congenital Hypospadias
Author String	PE Gradie, P Overbeek, R Behringer, AJ Pask The University of Connecticut, Storrs-Mansfield, CT; Baylor College of Medicine, Houston, TX; University of Texas MD Anderson Cancer Center, Houston, TX; The University of Melbourne, Victoria, Australia
Body	Hypospadias (the abnormal placement of the urethral opening on the penis) is one of the most common birth defects in the USA, affecting approximately 1 in every 140 live male births. Despite its high incidence, relatively little is known about the causes of this common disease. A few genes have been isolated that predispose the penis to hypospadias. Here we report a novel mutant mouse model isolated from a mutagenesis screen that presents with severe hypospadias. Adult homozygous males displayed bilateral cryptorchidism with an unfused scrotum. Interestingly, the urethral epithelium is present along the entire ventral surface of the phallus but urethra fails to close. Defects in the developing phallus are apparent as early as E16.5, and mutants could be visually distinguished on the day of birth. In addition, homozygous females carrying the mutation also displayed urogenital abnormalities, with reduced anogenital distance or persistent cloaca. Mutant mice are deleted for approximately 200kb of chromosome 8 in a region over 230kb downstream of the EphrinB2 gene. While the region is devoid of protein coding genes, it does contain several noncoding RNAs and estrogen-receptor-related-receptor-beta (ESRRB1) binding sites. This region is essential for initiating urethral closure in males and may be susceptible to endocrine disruption. This mouse presents a novel model of severe hypospadias. With the current lack of animal models to study this condition, this mouse will provide an important model to define the molecular causes of this disease. Nothing to Disclose: PEG, PO, RB, AJP

Pub #	P2-192
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Male Reproductive Physiology (1:30 PM-3:30 PM)
Title	Identification by LTQ-MS/MS Proteomic Approach and EmPAI Index Determination of Proteins Involved in Male Fertility
Author String	D Milardi, G Grande, F Vincenzoni, A Astorri, A Giampietro, A Bianchi, M Mormando, M Castagnola, L De Marinis, A Pontecorvi, R Marana Catholic University, School of Medicine, Rome, Italy; Catholic University, School of Medicine, Rome, Italy; Catholic University, School of Medicine, Rome, Italy
Body	Seminal plasma contains a large array of proteins required for normal physiology of spermatozoa and fertilization. To provide informations about the physiology of male fertility and to identify new fertility markers we performed proteomic studies of human seminal plasma. Semen samples were collected by 5 fertile normospermic men with normal levels of serum testosterone, estradiol, FSH, LH. An aliquot of seminal plasma was mixed with aqueous trifluoroacetic acid and centrifuged. The upper acidic supernatant was analyzed by an Ultimate 3000 Nano/Micro-HPLC apparatus equipped with an FLM-3000-Flow manager module coupled to an LTQ Orbitrap XL apparatus. The LTQ-Orbitrap mass spectrometer was operated in data dependent mode in which each full MS scan was followed by three MS/MS scans. The most abundant molecular ions were dynamically selected and fragmented by collision-induced dissociation. Tandem mass spectra were searched against the Swiss-Human.fasta database. Filtering criteria were XCorr versus charge 1.8 for 2+, 2.5 for 3+ ions; mass accuracy 3 ppm; high value peptide confidence. Peptide informations were then analysed by using ProteinCenter bioinformatic software. Statistical evaluations were performed to exclude proteins with False Discovery Rate>0.5%. Non-exclusively annotation of each protein using Gene Ontology System was performed. To estimate protein abundance we calculated the EmPAI index. A total of 1504 unique proteins were present in all samples. The most abundant GO Molecular Function grou was protein binding (n:943, 63%). The most frequent annotations for cellular distribution were membrane proteins (n:930, 62%) and cytoplasmatic proteins (n:899, 60%). 903 proteins (60%) resulted involved in metabolic processes and 689 (46%) in the regulation of biological processes. Among most abundant proteins, we observed Romo1 (Reactive oxygen species modulator 1), identified for the first time in seminal plasma, which might be involved in the control of sperm capacitation; semenogelin, the main protein of semen coagulum; podocin, whose gene mutation is associated with sperm abnormalities; CRISP1 (cysteine-rich secretory protein 1), a protein involved in sperm-egg interaction. By identification of seminal plasma proteome we provided better insight into the physiology of male fertility and identified abundant proteins, which could have main role in male fertility. Nothing to Disclose: DM, GG, FV, AA, AG, AB, MM, MC, LDM, AP, RM

Pub #	P2-193
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Male Reproductive Physiology (1:30 PM-3:30 PM)
Title	Regulation of Adult Leydig Cell Development from Stem Cells in Adult Mouse Testis
Author String	Z Su, Y Huang, R-S Ge Population Council, New York, NY; Jinan University, Guangzhou, China
Body	Stem cells have the capacity to self-renew and to develop into differentiated cells. Adult stem cells have been found in many adult tissues to replenish the loss of the differentiated cells. We hypothesize that adult stem Leydig cells are present in mature testis interstitium to restore adult Leydig cells. In the present study, we developed an organ culture system in vitro to induce stem Leydig cells to differentiate into Leydig cell lineage. Seminiferous tubules from 90-day-old mouse testes were separated from each other and washed 9 times in buffer (PBS or Medium 199), and then were incubated with different concentrations (75 to 1500 [micro]g/ml) of ethane dimethanesulfonate (EDS) for 24 hrs to kill the residual adult Leydig cells. After EDS treatment, seminiferous tubules were cultured for additional 2-5 days and media were collected for testosterone assay. EDS treatment killed all residual Leydig cells (histochemical staining for 3β-hydroxysteroid dehydrogenase, 3βHSD, a Leydig cell marker) and led to undetectable levels of testosterone. At day 5, seminiferous tubules were cultured in vitro with/without luteinizing hormone (LH) for additional five weeks. In the first two weeks culture after LH treatment, testosterone levels were undetectable. However, after 15 days in culture with LH, testosterone was detectable in the medium of the tubule fraction (7.7 pg/ml). Testosterone production by the tubules increased gradually at least through four weeks with 19.0, 59.5 and 159.9 pg/ml testosterone 21, 28 and 35 days after LH treatment. The seminiferous tubules without LH did not produce testosterone. Immunohistochemical staining showed that 3βHSD positive cells were located around the tubules. The present study indicates that LH is a critical factor of inducing stem Leydig cells to develop into adult Leydig cells in mouse testis. Sources of Research Support: Grants RO1 HD050570 and RO1 AG030598. Nothing to Disclose: ZS, YH, R-SG

Pub #	P2-194
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Male Reproductive Physiology (1:30 PM-3:30 PM)
Title	A Novel Treatable Form of Male Infertility Linked to Mutation of Follicle Stimulating Hormone (FSH) Beta Subunit Promoter
Author String	A Ferlin, C Vinanzi, R Selice, A Garolla, AC Frigo, C Foresta University of Padova, Padova, Italy; University of Padova, Padova, Italy
Body	**Background** High follicle stimulating hormone (FSH) plasma levels indicates severe male factor infertility due to a primary testicular disorder. However, about half of oligozoospermic men have inappropriately low-normal FSH levels. A single nucleotide polymorphism in the *FSHB* gene promoter modulates the transcriptio of the gene for the beta subunit of FSH. We tested whether this variant is associated with male infertility, sperm count and FSH plasma levels, and whether it could be a pharmacogenetic tool for the treatment of male infertility with FSH. **Methods** 248 subjects with normozoospermia, 79 with azoospermia (absence of sperm in the ejaculate even after semen centrifugation) and 435 with oligozoospermia (total sperm count <40 million/ejaculate).were evaluated for semen parameters, reproductive hormone levels and *FSHB* -211 G/T polymorphism (rs10835638). **Results** *FSHB* -211 TT genotype was associated with significantly lower FSH levels (mean ± SD, 3.3±2.5 IU/L *vs* 9.1±8.9 IU/L in GG homozygotes, P=0.0002). TT homozygotes were 25% (5/20) of subjects with azoo-oligozoospermia and low FSH levels ([le]1.5 IU/L). We did not observe this genotype in men with high FSH levels (>8 IU/L). TT homozygous men (13 subjects) had a primary testicular disorder but low or inappropriately normal FSH levels. Treatment with FSH in these subjects induced a dramatic improvement in sperm count and quality, significantly higher with respect to carriers of the G allele. **Conclusions** *FSHB* -211 TT genotype represents a novel treatable form of male infertility characterized by severe spermatogenic impairment and low or inappropriately normal FSH plasma levels. This genetic marker can be used as a molecular diagnostic tool for male infertility and might represent a valid pharmacogenetic approach for identification of potential responders to FSH treatment. Sources of Research Support: Italian Ministry of University and Research Grant 2007TKYYJR. Nothing to Disclose: AF, CV, RS, AG, ACF, CF

Pub #	P2-195
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Male Reproductive Physiology (1:30 PM-3:30 PM)
Title	Humanin Attenuates the Effect of Insulin-Like Growth Factor Binding Protein-3 on Male Germ Cell Apoptosis
Author String	N Ilani, Y-H Lue, J Ma, RS Swerdloff, P Cohen, K-W Lee, LJ Cobb, C Wang Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, Torrance, CA; David Geffen School of Medicine, UCLA, Los Angeles, CA
Body	*Background:* Humanin (HN), an endogenous 24-amino acid peptide, is a pro-survival factor in neuronal cells and many other cell types. Modification of HN with substitution of Glycine for Serine14 (HNG) enhances the neuroprotective activity of HN. We have previously shown that intratesticular administration of HN inhibits germ cell apoptosis induced by gonadotropin releasing hormone antagonist in rats. In this study, we investigated if HNG could neutralize the effect of insulin-like growth factor binding protein-3 (IGFBP-3) on germ cell apoptosis in an *ex vivo* culture system. *Study design:* Immediately after removal of one testis of an adult male rat under anesthesia, the seminiferous tubules (ST) were dissected to one mm length light (late or early stages) segments. Twelve to fifteen pieces of ST were cultured in each well with serum free culture medium as four treatment groups: (1) Scrambled peptide at 3 mcg/ml for the control group; (2) HNG at 3 mcg/ml; (3) IGFBP-3 at 30 mcg/ml (equimolar to HNG dose) and (4) combination of HNG (3 mcg/ml) and IGFBP-3 (30 mcg/ml). The culture plates were incubated at 34[deg] C for 15 hours. The ST were then dispersed as single cell suspension with collagenase (0.2%) and filtered through cell strainer to remove Sertoli cells. Then the samples, containing mainly germ cells, were labeled with AnnexinV-APC (allophycocyanin conjugate) to detect early stages of apoptosis by flow cytometric analysis. *Results*: In this *ex vivo* ST culture system, spontaneous germ cell apoptosis occurred in serum free culture medium. The apoptotic rate was suppressed by 24% at 15h after HNG treatment and increased by 60% by IGFBP-3 treatment in both early and late stages of spermatogenesis in comparison with controls. HNG treatment attenuated the effect of IGFBP-3 induced germ cell apoptosis by 76%. *Conclusion*: We demonstrated a significant protective effect of HNG in preventing spontaneous germ cell death in cultured seminiferous tubules and showed that HNG can attenuate the apoptotic effect of IGFBP-3 on germ cell apoptosis. This *ex vivo* model can facilitate future studies to investigate the mechanisms of action of HN which may be a new target in the development of a male contraceptive or infertility treatment. Nothing to Disclose: NI, Y-HL, JM, RSS, PC, K-WL, LJC, CW

Pub #	P2-196
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Male Reproductive Physiology (1:30 PM-3:30 PM)
Title	Engineering Transgenic Mice to Identify the Classical and Non-Classical Actions of Testosterone That Regulate Spermatogenesis
Author String	EJ Easton, WH Walker, P Jeyasuria University of Pittsburgh, School of Medicine, Pittsburgh, PA
Body	Testosterone targets Sertoli cells in the mammalian testis through the androgen receptor (AR) to produce the factors and environment required for germ cell development and survival. We have characterized a rapid (<1 min) and sustained mechanism of testosterone action (non-classical pathway) that causes the phosphorylation and activation of the Src and ERK kinases, the epidermal growth factor receptor and the CREB transcription factor. Our overall long-term goal is to establish the relative functions of the classical (DNA binding and direct gene regulation) and non-classical (kinase activation) pathways of androgen receptor (AR) actions in maintaining spermatogenesis. To accomplish this goal, we will engineer mouse models where the wildtype androgen receptor is replaced during fetal development with mutant forms of the receptor that selectively activate either the classical or the non-classical pathway. First, we are establishing the feasibility of restoring AR activity in an AR-defective mouse by incorporating a wildtype AR transgene. Specifically, we have incorporated a bacterial artificial chromosome (BAC) that contains an AR transgene into the mouse genome. To produce this AR trangene, we used recombineering techniques to combine the relevant regions of two BACs together to make a larger BAC that contained the entire AR gene plus 40 Kb each of 5' and 3' regulatory sequence. We then recombinereed an internal ribosome entry site (IRES) and EGFP gene into the 3' UTR of the AR transgene. The final product contains the entire genomic AR gene linked to the IRES and the EGFP gene allowing for bicistronic expression of both AR and EGFP using AR promoter and enhancer regulatory sequences. Pronuclear injection of the BAC resulted in 9 founder transgenic mice of which 5 have transmitted the AR-IRES-EGFP gene to the germline. To complete the goals of this pilot study, we will cross mice expressing the AR-IRES-EGFP transgene with an AR-defective tfm mouse strain to determine whether the transgene is able to restore functional AR activity and restore fertility. These initial studies will determine the feasibility of expressing pathway-specific AR mutants to identify the processes and factors regulated by the classical or non-classical action of testosterone in vivo. Our long-term objectives will fill a long-standing gap in the understanding of the molecular and cellular mechanisms by which testosterone acts in Sertoli cells to maintain spermatogenesis and fertility. Sources of Research Support: NIH HD008610. Nothing to Disclose: EJE, WHW, PJ

Pub #	P2-197
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproduction & Mammary Gland (1:30 PM-3:30 PM)
Title	Mouse Embryonic Long-Label Retaining Cells in the Adult Mammary Gland
Author String	K Boras-Granic, P Dann, JJ Wysolmerski Yale School of Medicine, New Haven, CT
Body	Studies in several tissues have confirmed the presence of organ-specific stem cells that contain the capacity to proliferate and differentiate into all the lineages of a given tissue. Mouse embryonic mammary rudiments have the capacity to generate an entire gland when transplanted into the cleared fat pad of adult mice. However, the relationship of embryonic mammary cells to adult mammary stem cells remains unknown. We were interested in determining whether embryonic cells only contribute to the formation of the ducts or whether they also contribute to the stem cells that are responsible for tissue homeostasis that support the cyclical development of the mammary gland during reproductive cycles. Long label-retaining cells (LLRCs) have been the basis for identifying adult stem cell distribution in situ in many tissues, since stem cells are thought to cycle slowly and retain DNA labels for prolonged periods of time. We hypothesized that if embryonic cells contributed to the adult stem cell pool, then DNA labeling during the first phase of rapid ductal development (e15 to birth) might generate LLRCs that would be found in the developing ducts during puberty. We pulsed embryos with EDU during this phase,and then chased these cells for up to 7-1/2 weeks after birth. We identified LLRCs, as well as epithelial cells expressing the lineage markers, K14, Gata3, p63. We found that 1% of the total cells retained EDU and the majority expressed the myoepithelial markers. Interestingly, LLRCs were located only near the origins of the duct system, far from the terminal end buds, the active growth fronts of the ducts. We next performed sequential labeling with EDU (e15 to birth) and BrDU at the onset of puberty for five days to label two different phases of active growth. These experiments demonstrated that LLRCs are found only at the site of active cell division at the time of DNA labeling. Furthermore, some of these LLRCs were mitotically active during puberty as demonstrated by BrDU labeling. Thus, our data suggest that stem/progenitor cells in the mammary gland do not come from a subset of the embryonic cells that are carried forward with the growing ducts, but rather may be set aside at the site of active cell division and growth. Using FACS cell sorting, we found that a subset of these LLRCs express cell surface markers previously described for mammary stem cells. We are currently investigating whether these LLRCs have functional characteristics of stem cells. Sources of Research Support: NIH R01 DK055501. Nothing to Disclose: KB-G, PD, JJW

Pub #	P2-198
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproduction & Mammary Gland (1:30 PM-3:30 PM)
Title	Ephrin Signaling Alters Granulosa Cell Morphology and Adhesion in the Mouse Ovary and Is Regulated by Follicle-Stimulating Hormone and Estrogen Receptor β
Author String	AV Buensuceso, BJ Deroo The University of Western Ontario, London, Canada
Body	Granulosa cell differentiation leading to the formation of a preovulatory follicle requires the synergistic actions of follicle-stimulating hormone (FSH) and estrogens acting through Estrogen Receptor β (ERβ). ERβ is essential for estrogen signaling in granulosa cells, and ERβ-null females are subfertile and possess ovaries with impaired folliculogenesis. It is also well-established that FSH-mediated changes in granulosa cell adhesion and morphology are essential for preovulatory follicle development, given the dramatic changes in follicle size and granulosa cell number that occur during this transition. Based on microarray studies comparing the gene expression of granulosa cells isolated from FSH-treated wildtype and ERβ-null mice, we have found that several members of the ephrin family of cell-positioning and adhesion molecules regulate granulosa cell morphology and adhesion, and that expression of these genes is disrupted in ERβ-null mice. Members of the ephrin family, which consists of ephrin ligands and their receptors, regulate cell location, adhesion, and migration during embryonic development, and while ephrin signaling has been best-characterized in the developing brain, very little is known about ephrin signaling during folliculogenesis. We find that FSH induces the expression of the ephrin ligand, Efna5, and its high-affinity receptors, Epha3, Epha5, and Epha8, in wildtype granulosa cells, suggesting the coordinated regulation of ephrin ligand and receptors in response to FSH. However, induction of these genes by FSH was greatly reduced in the absence of ERβ. Immunofluorescence studies suggest that Efna5 and Epha5 are located in the membrane of granulosa cells of developing follicles. We also show for the first time that ephrin signaling directly affects granulosa cell morphology and adhesion. Recombinant Efna5 ligand reduced cell spreading and increased cell rounding in mouse primary granulosa cells and a rat granulosa cell line. In addition, recombinant Epha5 receptor reduced granulosa cell adhesion in both model systems. We also investigated the regulation of Efna5 and Epha5 expression by FSH, and found that both FSH and forskolin increased expression in rat and human granulosa cell lines, indicating that FSH may regulate these genes via the cAMP/PKA pathway and that this regulation is conserved across different species. These studies identify ephrin signaling as a novel FSH-mediated pathway regulating granulosa cell morphology and adhesion. Sources of Research Support: The Canadian Institutes of Health Research; The University of Western Ontario; The Children's Health Research Institute; The London Regional Cancer Program. Nothing to Disclose: AVB, BJD

Pub #	P2-199
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproduction & Mammary Gland (1:30 PM-3:30 PM)
Title	Decreased Luteinizing Hormone Pulse Frequency Is Associated with Elevated 24-Hour Ghrelin after Calorie Restriction and Exercise Training in Non-Obese Women
Author String	JL Scheid, MJ De Souza, HJ Leidy, NI Williams Pennsylvania State University, University Park, PA; University of Missouri, Columbia, MO
Body	Exercise associated energy deficiency is accompanied by suppressed reproductive function but the underlying mechanism remains unclear. Ghrelin has been shown in men, Rhesus monkeys and rats to be associated with reduced luteinizing hormone (LH) pulse frequency. We previously reported that 24 hour ghrelin concentrations are chronically elevated in women following a 3 month exercise and diet program leading to weight loss and menstrual disturbances. To further elucidate the mechanism of exercise related menstrual disturbances, the primary purpose of this study was to investigate if the elevations in circulating ghrelin following a ~3 month exercise and diet program leading to weight loss are associated with a decrease in LH pulsatility in non-obese premenopausal women. Twelve non-obese (BMI =18 to 25 kg/m^2), non-exercising women (age, 18 to 24 years) were randomly assigned to a non-exercising control group (Control, n=4) or a diet and exercise group (Energy Deficit, n=8). The Control group was provided a controlled diet that matched baseline energy needs, while the Energy Deficit group was provided a diet in which energy intake was reduced from baseline energy requirements with the goal of weight loss. Supervised exercise training occurred five times a week for up to 90min at 70-80% of maximum heart rate in the energy deficit group. Repeated blood sampling over 24 hours to measure LH and total ghrelin occurred before and after the intervention. Significant decreases in body weight (- 2.53 ± 0.90 kg) and body fat (- 2.82 ± 0.77 kg) were observed in only the Energy Deficit group ($p < 0.05$). Significant increases in mean 24 hour ghrelin were found in only the deficit group ($p<0.05$). A negative correlation between the changes in LH pulse frequency and the changes in the mean 24 hour ghrelin (R=-0.591, p=0.043) and a positive correlation between changes in LH pulse frequency and changes in body weight (R=0.788, p=0.002) were found. However, when both change in ghrelin and change in body weight were entered into a multiple stepwise regression to predict change in LH pulse frequency, the change in body weight alone explained the greatest variance of change in pulse frequency (R^2=0.622, F=16.434, p=0.002). While the change in ghrelin is associated with the change in LH frequency, the effect of ghrelin appears to be mediated by the change in body weight during a weight loss program in non-obese premenopausal women. Sources of Research Support: NIH Grants 1R01HD39245-01A1 and M01 RR 10732. Nothing to Disclose: JLS, MJDS, HJL, NIW

Pub #	P2-200
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproduction & Mammary Gland (1:30 PM-3:30 PM)
Title	*In Vitro* and *In Vivo* Positive Modulation of Gonadotropin Activity with Enhancing Monoclonal Antibodies
Author String	E Kara, J Decourtye, V Wehbi, G Durand, E Reiter, M-C Maurel INRA, Nouzilly, France; Institut National de la Recherche Agronomique (INRA), Nouzilly, France
Body	Gonadotropins, namely follicle stimulating hormone (FSH), Luteinizing hormone (LH) and chorionic gonadotropins (CG), are key hormones for reproduction. We have previously described anti-equine CG antibodies (Ab) enhancing FSH and LH bioactivities of eCG (1). These eCG/anti-eCG Ab-enhancing complexes can also differently modulate signaling pathways at the FSH receptor, hence acting as biased ligands (2). To further analyze enhancing effect, we developed monoclonal antibodies (mAb) directed against ovine LH. They were selected on their ability to enhance LH activity using LH *in vitro* bioassay based on cAMP and progesterone secretion in MLTC cells. We obtained six mAbs enhancing both LH and hCG activities. mAb/LH complexes enhanced LH activity by five to seven fold. Since they cross-reacted with FSH, we analyzed their effect on FSH bioactivity using *in vitro* assay based on cAMP secretion in LTK cells. When complexed with the hormone, two mAbs enhanced FSH bioactivity by two-fold. These *in vitro* effects were confirmed *in vivo*, by standardized bioassays in rat. To assess the effect of mAbs on LH bioactivity, Parlow bioassay was performed in females, and growth of seminal vesicles was measured in males. Steelman and Pohley bioassay was used to determine the effects on FSH bioactivity in females. Highly significant results were obtained in both genders, exhibiting a two to three fold enhancing effect of the complexed mAb on LH and FSH bioactivity. The correlation between *in vitro* and *in vivo* data demonstrates that the enhancing effect of the mAb was not due to an increased half-life of the hormone. To complete *in vivo* investigations, mAb effects were studied in ewes treated for 14 days with progestagen to synchronize oestrus. At the end of treatment, females were injected either with LH alone, mAb/LH complexes or mAb alone. Ovulation time was dated by endoscopy and progesterone secretion was measured by ELISA. Ewes injected with mAb alone or in complex with LH ovulated 24 hours before the control group, that correlated with a 24 hour-shift of progesterone secretion. Importantly, these results suggest that mAb can target the endogenous LH and enhance its activity as it does for complexed LH. In conclusion, we describe mAbs able to enhance gonadotropin activity *in vitro* as well as *in vivo*, providing new pharmacological tools to modulate hormone activity in breeding animals. (1) Herve et al., Endocrinology 2004, 145(1): 294-303 (2)Wehbi et al, Endocrinology 2010, 151(6):2788-2799 Nothing to Disclose: EK, JD, VW, GD, ER, M-CM

Pub #	P2-201
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproduction & Mammary Gland (1:30 PM-3:30 PM)
Title	Mitochondrial DNA Content in Peripheral Blood Cells and Premature Ovarian Aging
Author String	M Bonomi, E Somigliana, M Busnelli, C Cacciatore, R Rossetti, A Paffoni, D Mari, G Ragni, L Persani Fondazione IRCCS Istituto Auxologico Italiano, Milano, Italy; Fondazione IRCCS Istituto Auxologico Italiano, Milano, Italy; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy; Universit[agrave] degli Studi di Milano, Milano, Italy
Body	Primary ovarian insufficiency (POI) is a critical fertility defect characterized by a progressive impairment of the follicular reserve. This process is generally silent without evident menstrual irregularity so that women are diagnosed with POI due to the premature cessation of menses (secondary amenorrhea, SA) before 40 years of age in the stage of complete follicular depletion (premature ovarian failure, POF or overt POI). POI pathogenesis is largely unexplained but a frequent maternal inheritance is present in idiopathic forms. Therefore, we hypothesized a possible involvement of a mitochondrial defect since mitochondrial biogenesis and bioenergetics play an essential role in oocyte maturation and embryo development. We therefore aimed to verify whether the content of mitochondrial DNA (mtDNA) is significantly reduced in the peripheral blood cells of POI women. We recruited 101 women with an impaired ovarian reserve: 59 women with premature ovarian failure (POF) and 42 poor responders (PR) to ovarian hyperstimulation (OH). A Taqman copy number assay revealed a significant mtDNA depletion (P<0.001) in both POF and PR women in comparison with 43 women of similar age and intact ovarian reserve, or 53 very old women with a previous physiological menopause. Consistently, a lower percentage of mothers was seen among POF and PR women with the mitochondrial defect. No variations in a mitochondrial DNA polymerase gene (POLG) were detected in POF or PR women with mtDNA depletion. In conclusion, blood cell mtDNA depletion is a frequent finding among women with premature ovarian aging, suggesting that a generalized mitochondrial defect appears frequently involved in the predisposition to POI. Since overlaps with control values were limited to about half of POF or PR cases, blood cell mtDNA determination may become an useful tool for POI risk prediction. Nothing to Disclose: MB, ES, MB, CC, RR, AP, DM, GR, LP

Pub #	P2-202
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproduction & Mammary Gland (1:30 PM-3:30 PM)
Title	Changes in Corpus Luteal Expression of Prolactin Receptors during Luteal Phase of Rats: Implication of Local Gonadotropin-Releasing Hormone (GnRH) Expression
Author String	T Yonezawa, A Shioya, S Kurusu, M Kawaminami Kitasato University, Towada, Japan
Body	We have already reported that luteal expression of annexin A5 mRNA, which is known to be stimulated by GnRH, is decreased during pseudopregnancy [(1)]. It was increased after systemic treatment with a dopamine agonist, CB-154, at mid-pseudopregnancy. Furthermore, when GnRH antagonist, Cetrorelix, was administered into the ovarian bursa with the systemic CB-154 treatment, both the expression of annexin A5 and the apoptotic reaction were inhibited. These findings suggest that local GnRH would induce apoptosis to functional luteal cells once prolactin secretion is ceased [(1)]. Recently, we have reported that GnRH mRNA was expressed in the luteolytic corpus luteum during estrous cycle and found that GnRH affected prolactin receptor expression. As these findings suggest a functional relationship between local GnRH and prolactin receptors for regulation of luteal function, we examined, in this study, changes in transcriptional levels of GnRH and prolactin receptor isoforms in the corpus luteum throughout luteal phases, pregnancy and pseudopregnancy. All mRNA levels in this study were measured using real-time RT-PCR. At early- to mid-pseudopregnancy, GnRH and annexin A5 mRNA levels in the corpus luteum remained to be low, and significantly augmented toward the end of pseudopregnancy. In the pregnant corpus luteum, these alterations were also similarly observed. In contrast, the long form and short form of prolactin receptor mRNA levels in corpus luteum were increased at mid-pseudopregnancy and mid-pregnancy, and reduced to the terminal of both luteal phases. After systemic treatment with CB-154 at mid-pseudopregnancy, the local GnRH and annexin A5 mRNA levels were dramatically increased and both of the prolactin receptor isoform mRNA levels were suppressed. When GnRH agonist (Des-Gly(Pro9)-GnRH Ethylamide) was locally administered into the ovarian bursa using osmotic mini-pumps at mid-pseudopregnancy, the transcriptional levels of prolactin receptors tended to be decreased. These results demonstrate that luteal GnRH is increased along witl the regression of functional corpus luteum. The inverse changes of GnRH and prolactin receptors at luteal regression together with our earlier observation suggest that GnRH is involved in regulation of luteal responsiveness to prolactin. (1) Kawaminami M et al., Endocrinology 2003; 144:3625 Sources of Research Support: Japan Society for the Promotion of Science, Grant-in-Aid for Scientific Research B (22780263); Kitasato University Research Grant for Young Researchers. Nothing to Disclose: TY, AS, SK, MK

Pub #	P2-203
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproduction & Mammary Gland (1:30 PM-3:30 PM)
Title	Mast Cells May Be a Source of Gonadotropin-Releasing Hormone (GnRH) in the Ovary
Author String	T Laoharatchatathanin, D Rieanrakwong, S Kurusu, Y Hasegawa, M Kawaminami Kitasato University, Towada, Japan; Kitasato University, Towada, Japan; Mahanakorn University of Technology, Bangkok, Thailand
Body	It has been reported that gonadotoropin releasing hormone receptor (GnRHR) is expressed in ovarian cells, especially in granulose cells. GnRH is believed to be involved in follicular atresia in the ovary. We also found that ovarian GnRH facilitates the apoptosis of luteal cells after disruption of prolactin surges at mid-pseudopregnancy of rats (1). Although GnRH receptor and its function have been repeatedly described, how the ligand is delivered in the ovary is still obscure. We recently observed that mast cells in the mammary tissues contain GnRH immunoreactivity and GnRH facilitates the mammary involution after lactation (Rieanrakwong et al., ENDO2009). These results suggest that mast cells would be a common producer of GnRH in various tissues. So, we examined in the present study whether mast cells could be a source of GnRH in the ovary. It was clearly demonstrated by the immunohistochemistry that only mast cells were GnRH positive in the ovary. Mast cells distribute to the interstitial tissues, especially to ovarian hilus and medulla. The number of toluidine blue stained mast cells in the ovary varied during the estrous cycle with two peaks at 2000 h of diestrus 2 and at 1700 h of proestrus. Furthermore, it was demonstrated by reverse transcription-PCR that mast cells prepared from peritoneal lavage fluid express GnRH mRNA. We previously observed that GnRH stimulates luteal apoptosis in the afternoon of diestrus 2 (Yonezawa et al, ENDO2007). So, the first peak of mast cell number is thought to coincide with luteal regression during estrous cycle. The second peak in proestus suggests a relation to follicular atresia. Both peaks of ovarian mast cell number were suppressed in pregnant rats, suggesting hormonal milieu would affect the distribution of mast cells. Prolactin (10 IU/0.2 ml ip) given at 1000 h of diestrus 2 decreased the number of ovarian mast cell at 2000 h. Present results suggest that GnRH is a paracrine hormone synthesized and secreted by mast cells. It is postulated that the migration of mast cells into the ovary would be a hormonally regulated mechanism to deliver GnRH in the ovary. (1) Kawaminami M et al., Endocrinology 2003; 144:3625 Nothing to Disclose: TL, DR, SK, YH, MK

Pub #	P2-204
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproduction & Mammary Gland (1:30 PM-3:30 PM)
Title	Inactivation of the Circadian Clock Prevents Lactotroph Plasticity in Cryptochrome-Deficient Female Mice
Author String	AO Martin, N Romano, A Guillou, IM Bur, P Mollard, X Bonnefont CNRS, Montpellier, France

Body

The misalignment between our internal timing system and the external world is usually associated with major health problems, such as an increased risk of cancer, or metabolic syndrome. Another important issue lies in the higher occurrence of subfecundity concerns in women working at night. However, the causal link between the biological clock ticking and the fine regulation of non-24 hour hormonal rhythms involved in reproduction remains puzzling.

A number of studies have shown that the hypothalamic-pituitary-gonadal axis is under circadian control. Indeed, both surgical ablation of the central clock located in the suprachiasmatic nuclei of the hypothalamus, or mutation of the molecular circadian clockwork, provoke irregular estrous cyclicity and absence of coordinated LH surge. However, the circadian system may also interfere with the female reproductive function through prolactin secretion, as suggested by shortened pseudo-pregnancy in homozygous Clock/Clock circadian mutants. We thus decided to address further the potential link between circadian timekeeping and the lactotroph axis.

We first observed that pups nourished by circadian clock-deficient Cry1-/- Cry2-/- dams have a severely reduced body growth, although they manage to reach weaning. This prompted us to investigate the functionality of the lactotroph axis of lactating Cry1-/- Cry2-/- mice:

- At the peripheral level, we noticed a dramatic atrophy of mammary glands from lactating Cry1-/- Cry2-/- females, as compare to controls. The histology of these glands revealed a nearly total absence of alveolar differentiation.
- At the pituitary level, we identified figures of parapoptosis in PRL-containing cells within the gland of lactating Cry1-/- Cry2-/- mice, which might be related to an overproduction of dopamine.
- At the hypothalamus level, we noticed that tubero-infundibular neurons (TIDA) that regulate prolactin secretion undergo a dramatic plasticity in control females from the virgin to lactating states, whereas this plasticity does not occur in Cry1-/- Cry2-/- mice, neither at the functional nor at the morphological levels.

Altogether, these results provide the first evidence for impaired lactation in circadian clock mutant mice, and reveal that a functional circadian clockwork is required for undergoing the plasticity of the lactotroph properly.

Sources of Research Support: INSERM (PNRRE to XB and AM).

Nothing to Disclose: AOM, NR, AG, IMB, PM, XB

Pub #	P2-205
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproduction & Mammary Gland (1:30 PM-3:30 PM)
Title	Influence of FSH Receptor Genotype on Ovarian Response to FSH in Brazilian Women Undergoing Assisted Reproduction
Author String	C Tusset, M Badalotti, E Trarbach, MY Nishi, BB Mendonca, AC Latronico, MB Kohek Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Fertilitat, Porto Alegre, Brazil; Universidade Federal de Ciencias da Saude de Porto Alegre, Porto Alegre, Brazil
Body	In assisted reproduction programs, the response of ovulating women to exogenous FSH therapy is quite variable. Studies in diverse populations showed that polymorphisms at the promoter region and at the positions 307 and 680 of the FSH receptor gene play a fundamental role in determining the physiological responsiveness of the target organ to FSH stimulation. **Aim:** To evaluate the genotypic frequencies of FSH receptor gene polymorphisms, -29G>A, Ala307Thr, and Ser680Asn in infertile Brazilian women undergoing controlled ovarian stimulation for assisted reproduction, and investigate the role of the FSH receptor genotype on ovarian response to FSH therapy. **Patients and Methods:** Forty-two ovulatory women with infertility caused by male factor and/or tubal factor were included in this study. In all cases, controlled ovarian stimulation was performed before genetic analysis according to standard protocols. Basal FSH levels were obtained in one of the previous cycles before ovarian stimulation. A group of one hundred fertile Brazilian women was used as controls. Genomic DNA was extracted from peripheral blood and the FSH receptor gene polymorphisms were analyzed by Real Time PCR. Stattiscal analyses were performed by chi-square test and simple linear regression. **Results:** The genotypic frequency distribution of the polymorphism at position 680 was 38.1% for the Asn/Asn, 52.38% for the Asn/Ser, and 9.52% for the Ser/Ser variant. HapMap analysis of the polymorphic site at position 307 displayed a linkage of Thr307 to Asn680 and of Ala307 to Ser680 variants in all patients investigated. The genotype frequency distribution of the polymorphism locate on the promoter region was 47.62% for the G/G, 40.48% for the G/A, and 11.90% for the A/A genotype. The comparison of allelic and genotypic frequencies between patients and controls showed no statistically significant differences. The association between FSH receptor gene polymorphisms and ovarian response to exogenous FSH was analyzed. No statistically significant differences were found between different genotypes of FSH receptor gene at position -29 and 680 and the basal FSH level, as well with the number of FSH ampoules required for successful stimulation. **Conclusion:** In this Brazilian cohort of patients, the ovarian response to exogenous FSH does not depend on the FSH receptor genotype. Sources of Research Support: FAPESP # 2005/04726-0. Nothing to Disclose: CT, MB, ET, MYN, BBM, ACL, MBK

Pub #	P2-206
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproduction & Mammary Gland (1:30 PM-3:30 PM)
Title	Secretion of LH and PRL Induced by Leptin May Be Mediated by Nitric Oxide in the MPOA under Modulation of Estrogen
Author String	BDB Borges, CR Franci FMRP, Ribeirão Preto, Brazil
Body	Leptin (Lep) is a possible mediator for interaction between energy homeostasis and reproduction. Studies showed that leptin action on reproduction depends on estrogen. However, the gonadotropin-releasing hormon (GnRH) neurons do not express leptin receptor and nitric oxide is a possible candidate to mediate the action o leptin on these neurons. The aim of this work was verify if the expression of neural nitric oxide synthase (nNOS) in the medial preoptic area (MPOA) depends on leptin action and modulation by estrogen. ***Methods*:**Wistar female rats with regular cycles estrous receive a cannula in the lateral ventricle. After convalescence, the animals were treated with estrogen antagonist, Tamoxifen s.c. (TMX, 3mg/rat) or Vehicle (veh), in metestrous and diestrous. Only animals that showed vaginal smear for proestrous received leptin i.c.v. (0,3; 1; 3 and 10ug/ml) or saline (C) at 11:00 am. These animals were killed by decapitation at 5:00 pm, to collect blood and brain. Ovariectomized (OVX) animals were included as additional control. Plasma luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin (PRL) were measured by radioimmunoassay. MPOA was microdissected to analyze the expression of gene (RT-PCR) and protein (western blot) for nNOS. ***Results:*** Lep-3[mu]g amplified plasma LH (C 28.7 $\pm$ 7.1 x Lep 78.9 $\pm$ 24.7ng/ml) and Lep-10[mu]g amplified plasma PRL (C 234.8 $\pm$ 35 x Lep 377.2 $\pm$ 71.9 ng/ml). On the other hand, TMX reduced the secretions of LH (veh 28.7 $\pm$ 7.1 x TMX 4.7 $\pm$ 0.5 ng/ml), FSH (veh 3.1 $\pm$ 0.5 x TMX 1.1 $\pm$ 0.1 ng/ml) and PRL (veh 234.8 $\pm$ 35 x TMX 15.1 $\pm$ 1.7 ng/ml) and prevented the effects of LEP i.c.v. on secretio of LH and PRL. TMX and Lep-10mg decreased the gene expression for nNOS in the MPOA, 53% and 56%, respectively. Lep-10ug decreased (veh 0.75 $\pm$ 0.02 / Lep 0.66 $\pm$ 0.01) the nNOS protein in the MPOA, while TMX did not change. OVX rats decreased both gene for nNOS (64%) and protein expression (Veh 0.75 $\pm$ 0.02 x OVX 0.58 $\pm$ 0.02) in the MPOA. ***Conclusion:*** Our results suggest that: amplification of the secretion o LH and PRL by leptin may be mediated by nitric oxide in the MPOA and modulated by estrogen; LH secretion is more responsive to the leptin action than PRL secretion; some others ovarian factor may be involved in the mechanism of leptin action in the MPOA since TMX decreased gene expression for nNOS bu not protein expression while the ovariectomy decreased also nNOS expression. Sources of Research Support: FAPESP and CnPQ in Brazil. Nothing to Disclose: BDBB, CRF

Pub #	P2-207
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproduction & Mammary Gland (1:30 PM-3:30 PM)
Title	Polymorphisms in the Element Response Estrogen (ERE) in the *GnRH1* Promoter Region Are Not the Cause of Suppressed Gonadotropin Levels in Patients with Turner Syndrome under Sex Steroid Replacement
Author String	EB Trarbach, R Paranhos, AF Braz, AC Almeida, EMF Costa, BB Mendonca HC-FMUSP, São Paulo, Brazil
Body	Hypergonadotropic hypogonadism is characterized by absent pubertal development associated with low levels of sex steroids and high gonadotrophin levels. **Turner syndrome** (TS) is the major cause of hypergonadotropic hypogonadism in girls. During estrogen replacement, around 10% of these patients present suppressed gonadotropin levels whereas most of them maintain elevated gonadotropin levels. Estrogen (E2) is the major hormonal regulator of GnRH release and act as a classic homeostatic (negative and positive) feedback molecule between gonad and brain. The presence of an ERE (element response estrogen) in human GnRH gene suggests that estrogen directly controls gonadotropin secretion from GnRH neurons in the hypothalamus. We hypothesized that allelic variant in ERE elements GnRH promoter region could be involved in the suppression of gonadotropin levels in females with TS. To investigate this possibility, a genetic screening of selected segments (-985 to -472) located in the middle of *GnRH1* promoter region containing an ERE domain was performed. Eight females with TS who had suppressed LH during estrogen replacement (LH <0.6 UL) were evaluated and compared with a group of 17 TS patients who kept elevated gonadotropin levels (LH: 16.8-29.9 UL; FSH: 50.4-104.4 UL) during estrogen replacement. No allelic variants in the *GnRH1* promoter region were identified. In conclusion, polymorphisms in the element response estrogen (ERE) in the *GnRH1* promoter region are not the cause of suppressed gonadotropin levels in patients with TS under estrogen replacement. Sources of Research Support: FAPESP 2005/04726-0. Nothing to Disclose: EBT, RP, AFB, ACA, EMFC, BBM

Pub #	P2-208
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproduction & Mammary Gland (1:30 PM-3:30 PM)
Title	Sequence Analysis of the Ovarian Kiss1 cDNA in the Mongolian Gerbil and Syrian Hamster
Author String	O Suzuki, M Koura, Y Noguchi, K Uchio-Yamada, J Matsuda National Institute of Biomedical Innovation, Ibaraki, Japan
Body	Kisspeptins are peptide products of the Kiss1 gene, expressed in the ovary in addition to the hypothalamus and brain. We have been determining the cDNA sequences of gonadotropins and their receptors in laboratory animals in order to examine the molecular basis for an efficient superovulation technique in laboratory animals (1-5). In the present study, we determined the full cDNA sequences of ovarian Kiss1 in the Mongolian gerbil and Syrian hamster to examine a role of kisspeptin signalling in the ovarian function in the animals. Total RNA was isolated from the gerbil and hamster ovaries. Rapid amplification of cDNA ends (RACE) was conducted using Smarter RACE cDNA Amplification Kit (Clontech). Primers were designed using NCBI Primer-BLAST with mouse kiss1 sequence (accession #NM_178260.3). The PCR products were gel-purified and directly sequenced. The complete sequences of the Kiss1 cDNA in gerbil (AB571125) and hamster (AB571126) were obtained by combining the overlapping 5'- and 3'-RACE sequences. The inferred amino acid sequences of the gerbil and hamster Kiss1 were 134 amino acids in length and shared highly homologous sequences near the N-terminus with mouse and rat Kiss1, especially the highly conserved active 10-amino-acide peptide (YNWNSFGLRY). The sequence information would be a useful tool for analyzing kisspeptin expression in gerbil and hamster ovaries. (1) Suzuki et al. (2007) Gen Comp Endocrinol. 151:231-235. (2) Noguchi et al. (2006) Gen Comp Endocrinol. 147:231-235. (3) Koura et al. (2004) Gen Comp Endocrinol 136:406-410. (4) Suzuki et al. (2003) Mol Reprod Dev 64:219-225. (5) Suzuki et al. (2002) Mol Reprod Dev 62:335-342. Sources of Research Support: Grant from the Ministry of Health, Labour, and Welfare of Japan. Nothing to Disclose: OS, MK, YN, KU-Y, JM

Pub #	P2-209
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproduction & Mammary Gland (1:30 PM-3:30 PM)
Title	Adrenal Androstenediol Contributes to Estrogenicity during the Menopausal Transition
Author String	BL Lasley, FZ Stancyzk, DS McConnell University of California at Davis, Davis, CA; University of Southern California, Los Angeles, CA; University of Michigan, Ann Arbor, MI
Body	The increase in circulating dehydroepiandrosterone sulfate (DHEAS) during the menopausal transition (MT) is accompanied by increases in other adrenal steroids. These increases range from non-detectable in some women to several orders of magnitude in others. Such a large dynamic range in circulating steroid levels has the potential to explain the wide inter-woman differences in steroid hormone-related menopausal symptoms. The objective of this study was to examine the circulating adrenal steroid hormone dynamics to better understand their relationship to the wide range of estrogen-related changes that are observed during the MT. Annual serum samples from the Study of Women's Health Across the Nation (SWAN) (n=144) were selected from women identified to be in early- and late-perimenopause stages of the MT. Sera were analyzed for bioactive estrogens and androgens using in vitro bioassays, as well as immunoreactive androstenedione (Adione), testosterone (T), dehydroepiandrosterone (DHEA), DHEAS, androstenediol (Adiol) and estradiol (E_2) by immunoassay methods. A modest decline in circulating E_2 levels was observed two years before and two years following menopause. In contrast, a five-fold increase in serum Adiol (which has both androgenic and estrogen bioactivity) from approximately 700 pM to over 3,500 pM was found to parallel the rise in DHEAS four years before and two years following menopause. During the six year time interval studied herein, circulating Adione and T increased only two- and three-fold, respectively, and were not related to total estrogenicity. Circulating Adiol, however, was correlated ($p<0.02$) to circulating estrogen bioactivity during the MT when circulating E_2 concentrations were low and circulating Adiol concentrations were high. The wide range of circulating Adiol and its potential contribution to total estrogenicity during the MT is consistent with the observed inter-woman differences in estrogen-related symptoms at this time. We conclude that the higher circulating levels of Adiol may contribute to estrogenicity, when E_2 production falls during the MT, and to the endocrine changes experienced by midlife women. The Study of Women's Health Across the Nation (SWAN) has grant support from the National Institutes of Health (NIH), DHHS, through the National Institute on Aging (NIA), the National Institute of Nursing Research (NINR) and the NIH Office of Research on Women's Health (ORWH) (Grants NR004061; AG012505, AG012535, AG012531, AG012539, AG012546, AG012553, AG012554, AG012495). The content of this abstract is solely the responsibility of the authors and does not necessarily represent the official views of the NIA, NINR, ORWH or the NIH. Nothing to Disclose: BLL, FZS, DSM

Pub #	P2-210
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Ovarian & Endometrial Cancer (1:30 PM-3:30 PM)
Title	The Gonadotropins Induce Expression of Oncogenic Pathways in a 3D Model of Normal Ovarian Surface Epithelium
Author String	TS Hilliard, S Quartuccio, JE Burdette University of Illinois at Chicago, Chicago, IL
Body	Epithelial ovarian carcinomas (EOC) account for 90% of all ovarian cancers and are believed to originate from the ovarian surface epithelium (OSE), a single layer of epithelial cells surrounding the ovary. Understanding the events leading to the initiation and progression of ovarian cancer is critical for detecting early stages of ovarian cancer. Ovarian surface proliferation, associated with ovulation, and the gonadotropins that regulate ovulation are factors in ovarian surface transformation and cancer progression. Based on the gonadotropin theory, we hypothesized that follicle-stimulating hormone (FSH) and luteinizing hormone (LH) play a role in the pathogenesis of EOC. A three-dimensional (3D) alginate-hydrogel organ culture system, developed in our lab, supported the growth of normal OSE and responded to the gonadotropins. Cultures treated with FSH, LH, or both FSH and LH as well as 1mM H_2O_2, proliferated more on day 8 when compared to basal conditions. From the proliferation data, experiments were developed to verify that FSH and LH play a role in the progression rather than the initiation of ovarian cancer. To confirm this, a traditional soft agar transformation assay was performed. Our lab previously demonstrated that OSE treated with H_2O_2 undergoes transformation. Ovaries were cultured in H_2O_2 to induce transformation and the OSE was removed with collagenase, added to soft agar with media supplemented with FSH, LH or both to investigate progression and FSH increased colony formation suggesting it helps progress initiated OSE cells. To evaluate if FSH or LH initiate ovarian cancer, ovaries were cultured in 3D and stimulated with FSH, LH or both and added to soft agar with basal media. LH and FSH alone did not initiate OSE transformation. FSH and LH pathways were studied downstream using transcriptional array analyses of OSE cells grown as a 3D organ culture. FSH transcriptionally up-regulated Cdkn2a (cyclin dependent kinase inhibitor 2A), and EGFR (epidermal growth factor) mRNA. LH up-regulated AKT1, cdkn2a, and EGFR mRNA, which all increase proliferation. FSH and LH both up-regulated AKT2, which might contribute to increased proliferation, and down-regulated caspase 8, reducing apoptosis. This 3D ovarian organ culture identified key cancer pathways regulated by gonadotropins and revealed how these hormones contribute to OSE tumorigenesis by increasing proliferation and leading to the progression of colony formation stimulated by oxidative stress. Sources of Research Support: UIC Cancer Center Pilot Grant, K12HD055892, and CO6RR15482. Nothing to Disclose: TSH, SQ, JEB

Pub #	P2-211
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Ovarian & Endometrial Cancer (1:30 PM-3:30 PM)
Title	Kallikrein-Related Peptidase 4 Promotes Mesenchymal-Epithelial Cell Plasticity and Paclitaxel Resistance in Serous Epithelial Ovarian Carcinoma
Author String	Y Dong, C Stephens, C Walpole, J Swedberg, J Harris, G Boyle, P Parsons, M McGuckin, J Clements Queensland University of Technology, Kelvin Grove, Australia; Queensland Institute of Medical Research, Herston, Australia; Mater Medical Research Institute, South Brisbane, Australia
Body	High levels of many kallikrein-related peptidases (KLKs) are associated with poor outcome in women with serous epithelial ovarian carcinoma (EOC). We have previously shown that KLK4 is highly expressed in EOC but its role in the progression of this disease is unknown. To determine the function of KLK4 in EOC peritoneal dissemination, we used KLK4-transfected SKOV-3 EOC cells in 3-dimensional (3D)-suspension culture to mimic the ascites microenvironment. KLK4-SKOV-3 cells formed multicellular aggregates (MCAs indicative of that seen clinically in ascites, as did native SKOV-3 cells treated with active KLK4 enzyme, which were blocked with a KLK4 blocking antibody or the sunflower trypsin derived selective KLK4 inhibitor (SFTI-FCQR). Increased E-cadherin and decreased N-cadherin levels were observed in the phenotypically mesenchymal SKOV3 native and vector cells day 1 in 3D suspension suggesting a mesenchymal-epithelial transition was occurring. Notably, this effect was delayed until days 7 and 14 in the KLK4-SKOV3 cells. Activation of the EGF receptor (a pathway known to be activated during epithelial-mesenchymal plasticity) was induced by day 1 and sustained for 14 days in both KLK4-transfected and native/vector control SKOV-3 cells. However, Src phosphorylation and lipocalin 2 (another early indicator of epithelial-mesenchymal plasticity) induction was more pronounced in the KLK4-SKOV-3 over this period. Importantly, many of these changes were not seen in 2D-culture emphasizing that 3D-models more faithfully recapitulated the *in vivo* microenvironment. The KLK4-MCAs formed invasive cancer cell foci in a mesothelial cell monolayer. They had unchanged sensitivity to cisplatin but were paclitaxel resistant which could be reversed by the selective SFTI-FCQR inhibitor. High tumor *KLK4* levels were also associated with poor prognosis and chemoresistance in patients. Our findings show that KLK4 induces MCA formation and delays the mesenchymal-epithelial phenotypic change which may be an early driver of the paclitaxel chemoresistance observed in these SKOV-3-derived MCAs. Combining KLK4 inhibition with paclitaxel may improve the outcome for women with high KLK4 levels in serous EOC.

Sources of Research Support: National Health & Medical Research Council of Australia.

Nothing to Disclose: YD, CS, CW, JS, JH, GB, PP, MM, JC

Pub #	P2-212
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Ovarian & Endometrial Cancer (1:30 PM-3:30 PM)
Title	Epidermal Growth Factor Regulation of Granulosa Cell Tumor Proliferation Via Multiple Signaling Pathways
Author String	C Wang, JS Davis University of Nebraska Medical Center, Omaha, NE; VA Medical Center, Omaha, NE
Body	Granulosa cell tumors (GCTs) represent ~5% of all ovarian cancers. These tumors often produce estrogen leading to symptoms and signs of estrogen excess such as endometrial hyperplasia. GCTs may also produce androgens leading to virilization. Moreover, cases of recurrence or metastases have been described following initial tumor debulking. In contrast to the extensive studies on the development and progression of epithelial ovarian cancer, the mechanisms promoting GCT development are unclear. Epidermal growth factor (EGF) is a potent mitogen for many cells, including ovarian granulosa cells. However, little is known on the action of EGF in GCTs. The aim of this study was to determine whether EGF plays a role in the proliferation of GCT cells. KGN cells were used as a cellular model to detect the possible function and cellular signaling mechanisms of EGF. Immunohistochemistry, Western blot and RT-PCR techniques were used to determine the expression of EGF receptors (EGFR). Flow cytometry (FCM) was used to detect the effect of EGF on KGN cell cycle progression. Western blot results indicated that EGFR, Her2, Her3 and Her4 were all expressed in KGN cells. This result was confirmed by fluorescent immunohistochemistry and RT-PCR. EGF treatment enhanced the transition of KGN cells from G1 into S phase, and significantly increased the number of cells in G2 phase. The increase in cell number was associated with increased cyclin D2 expression. Treatment with TGFα had similar effects. Both EGF and TGFα rapidly stimulated the phosphorylation of ERK1/2 and Akt in KGN cells. The effect of EGF on the phosphorylation of Akt returned to base line within 60 minutes, while the levels of phosphorylated ERK1/2 remained elevated for 72 hours. Pretreatment with AG1478 significantly reduced EGF and TGF-α induced phosphorylation of ERK1/2 and abrogated EGF and TGF-α stimulated phosphorylation of Akt. Pretreatment with wortmannin blocked EGF and TGFα stimulated phosphorylation of Akt, but had no effect on the phosphorylation of ERK1/2. Pretreatment with U0126 significantly reduced EGF and TGFα stimulated phosphorylation of ERK1/2, but had little on the phosphorylation of Akt. Treatment with U0126 significantly reduced EGF-stimulated cell proliferation. In comparison, treatment with LY294002 caused a modest reduction in EGF-stimulated cell proliferation. In conclusion, EGF contributes to the proliferation of granulosa tumor cells via activation of multiple signaling pathways. Sources of Research Support: NIH (1K99HD059985) and Olson Center for Women's Health. Nothing to Disclose: CW, JSD

Pub #	P2-213
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Ovarian & Endometrial Cancer (1:30 PM-3:30 PM)
Title	p53 Directs Role of Smads in Ovarian Cancer Growth and Metastasis
Author String	RA Ahmed, JE Burdette University of Illinois at Chicago, Chicago, IL

Body

Ovarian cancer is the most lethal gynecological malignancy due to the lack of early detection measures and ar incomplete understanding of the cell of origin. Ninety-six percent of high-grade serous ovarian cancers have p53 mutations. Additionally it is known that interplay between the transforming growth factor beta (TGFβ) pathway and p53 occurs such that TGFβ differentially affects cell proliferation, apoptosis and metastasis. The role of TGFβ in cancer changes as the disease progresses; in the early stages TGFβ suppresses tumor growth, however after tumor formation TGFβ promotes metastasis. If p53 mutation is the essential early event in ovarian cancer that changes the role of TGFβ, TGFβ signaling should prevent tumor progression in ovarian cancer p53 wildtype cell lines, while p53 nulls exhibit TGFβ-dependent migration, proliferation and tumorigenesis.

To determine the role of p53 in the TGFβ pathway, a panel of four human ovarian cancer cell lines was chosen based on p53 status (OVCA420, HEYC2 - p53WT; SKOV3, OVCAR5 - p53 null). Proliferation, apoptosis, migration, and protein expression were measured in these cell lines after stimulation with TGFβ (10ng/mL) and activin (20ng/mL). Luciferase assays were performed to verify the ability of the cells to elicit a Smad dependent transcriptional response after TGFβ and activin ligand stimulation. In response to TGFβ, p53 wildtype ovarian cancer cells, unlike p53 nulls, are arrested in G1 suggesting interplay between p53 and p21 expression. Known tumor suppressor Maspin, shown to mediate BRAt in breast cancer, is expressed more in p53 wildtype cell lines than null cell lines, which could account for increased metastatic properties. Re-expression of p53 in the null SKOV 3 cell line reinstated that both p21 and Maspin are somehow regulated by p53. We have identified p53 as a modifier of Smad signaling that encourages cell cycle arrest in its wildtype form as opposed to its null form. Null p53 cell lines have shown a gain of function in the reduced expression of tumor suppressor Maspin. Further studies will help determine whether the reduction of Maspin accounts fo increased metastasis.

Sources of Research Support: R03CA139492.

Nothing to Disclose: RAA, JEB

Pub #	P2-214
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Ovarian & Endometrial Cancer (1:30 PM-3:30 PM)
Title	Small-Molecule Tyrosine Kinase Inhibitors as Potential Therapy for Granulosa Cell Tumors of the Ovary
Author String	S Jamieson, PJ Fuller Prince Henry's Institute of Medical Research, Clayton, Australia; Monash University, Clayton, Australia

Body

Granulosa cell tumors of the ovary (GCT) represent a specific subset of malignant ovarian tumors of which there are two distinct subtypes, the juvenile and the adult form. Aside from surgery no reliable therapeutic options exist for patients with GCT. Our finding that the tyrosine kinase inhibitor (TKI) imatinib, and its more potent analog, nolitinib, inhibited two human GCT-derived cell lines, COV434 and KGN, in an off-target manner (1) prompted us to investigate a potential role for TKI in the clinical management of GCT. Utilizing TKI with distinct but overlapping multi-targeted specificities, which are either in clinical use or in advanced development, cellular proliferation, viability and apoptosis were evaluated in the COV434 and KGN cells. Sunitinib, which targets the imatinib-inhibited tyrosine kinases: VEGFR, KIT, PDGFR and FLT-3, was without effect. Sorofenib elicited dose dependent inhibition of cellular proliferation and viability in both cell lines at concentrations equivalent to that seen in other systems. Sorafenib has a broad spectrum inhibitory activity with a high affinity for RAF1 and BRAF, which are not inhibited by sunitinib but are inhibited by imatinib/nolitinib at high concentrations. A RAF1 kinase inhibitor was examined and found to be without effect, suggesting that sorafenib is likely to be acting via inhibition of BRAF. Of note, in previous studies we did not find the V600E mutation or over expression of BRAF to be a feature of GCT (2). It may be that aberrant activation originates upstream of BRAF in the MAPK pathway, therefore we examined the effect of a selective SRC family inhibitor, SU6656. In the presence of SU6656 cell proliferation and cell viability assays dissociated the responses of COV434 and KGN cells, that is, whilst SU6656 inhibited the proliferation and viability of KGN cells in a dose dependent manner, it had no effect in COV434 cells. These findings strongly implicate BRAF in the activated signalling responsible for the growth and viability of GCT. In addition, given that COV434 and KGN cell lines represent juvenile and adult GCT, respectively (3), the observation that c-SRC inhibition is effective in KGN but not COV434 cells suggests divergent mechanisms of activation in juvenile and adult GCT. These studies suggest that TKI already in clinical use may be an effective therapeutic in the treatment of GCT and also provide further insights into the molecular mechanisms that contribute to the pathogenesis of GCT.

(1) Chu S et al., Gynecol Oncol 2008; 108:182
(2) Jamieson S et al., Gynecol Oncol 2004; 95:603
(3) Jamieson S et al., Mod Pathol 2010; 23:1477

Nothing to Disclose: SJ, PJF

Pub #	P2-215
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Ovarian & Endometrial Cancer (1:30 PM-3:30 PM)
Title	Analysis of TP53 and CTNNB1 Genes in Pediatric Ovarian Steroid Cell Tumors
Author String	JE Bezerra, L Lima, B Mariani, S Siqueira, BB Mendonca, AC Latronico Hospital das Clinicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Hospital das Clinicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil
Body	BACKGROUND: Steroid cell tumors are rare ovarian neoplasms composed of typical steroid hormone-secreting cells. The origin from adrenal rests was proposed but has been difficult to demonstrate. AIM: To study TP53 and Wnt /β-catenin pathways in two samples of steroid cell tumors from prepubertal patients with specific hormonal pattern similar to the adrenal cortex. PATIENTS AND METHODS: Case 1: 3 year-old child that presented with telarche, pubarche and increased growth velocity by the age of two. Pubertal development progressed despite cyproterone acetate treatment. By the age of 3 she had 106 cm (SD= +2.3), 17 kg (SD= + 1.3). On physical examination she had Tanner 3 of breast development and Tanner 2 of pubic hair. Clitoris was normal. Bone age was 5 years. Basal and stimulated gonadotropins were supressed. Estradiol, DHEA and androstenedione levels were markedly elevated. Testosterone and 17OH progesterone levels were slightly elevated. Pelvic ultrasound revealed a 4.4 cm mass in the right ovary. Pathological analysis revealed a steroid cell tumor with similar histological aspects to the glomerulosa layer from adrenal cortex. Case 2: 4.4 year-old child that presented with pubarche, facial hair and acne. Increased growth rate, muscle mass gain and deepening of the voice were also present. She had 115 cm (SD= + 2.75) and 25 kg. On physical examination she had Tanner 2 of breast development and Tanner 4 of pubic hair. Clitoris was enlarged (3.0 cm). Basal gonadotropins were supressed. Testosterone, androstenedione, estradiol, DHEA-S and 11-deoxycortisol levels were elevated. Basal cortisol and DHEA levels were in the normal range. Pelvic ultrasound revealed a 5.2 cm mass in the right ovary. Histological analysis revealed steroid cell tumor. Mutations of exon 3 of the CTNNB1 gene and R337H mutation of the TP53 were investigated by automatic gene sequencing. Immunohistochemical study of TP53, β-catenin, melan-A, and inibin-A were also performed. RESULTS: Both samples revealed negative immunohistochemical expression of TP53 protein, Melan-A and β-catenin. Inhibin-A was weakly positive. Somatic mutations of exon 3 of the CTNNB1 gene and R337H mutation in the exon 10 of the TP53 gene were not present in both cases. CONCLUSION: The tumorigenesis of the ovarian steroid cell tumors are different from tumorigenesis of adrenocortical tumors. Nothing to Disclose: JEB, LL, BM, SS, BBM, ACL

Pub #	P2-216
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Ovarian & Endometrial Cancer (1:30 PM-3:30 PM)
Title	The Role of Ghrelin and GHSR1a in Endometrial Cancer Cell Proliferation and Apoptosis
Author String	JN Fung, C Chen The University of Queensland, Brisbane, Australia
Body	Ghrelin is a peptide hormone produced in the stomach and a range of other tissues, where it has endocrine, paracrine, and autocrine roles in both normal and disease states. Ghrelin is expressed in a range of endometria cancer tissues, while its receptor, the growth hormone secretagogue receptor 1a (*GHSR1a*) is expressed at various levels in normal and cancer tissues. Ghrelin has been shown to have proliferative and anti-apoptotic functions in number of hormone dependent tumors, including endometrial cancer. The current study aims to examine the proliferative and anti-apoptotic function of ghrelin and GHSR1a in endometrial cancer using two human endometrial cancer cell lines: Ishikawa and KLE. The expression of GHSR1a in these two cell lines were silenced using shRNA lentiviral transduction approach with scrambled shRNA used as control. The 70% efficiency of GHSR1a knock-down in these two cell lines were confirmed using quantitative real-time PCR and western blotting. The effect of ghrelin on endometrial cancer cell proliferation in the transfected cell lines was determined using the MTS dye method (n=3). Moreover, flow cytometry was utilized in order to determine the effect of ghrelin treatment on cancer cell apoptosis in the transfected cell lines (n=3). We found that in transfected Ishikawa and KLE cell lines, ghrelin-stimulated cell proliferation was reduced by 3% and 4% when compared to scrambled controls respectively. Furthermore, we also found that cell apoptosis was no different in the GHSR1a shRNA transfected Ishikawa and KLE cell lines when compared to scrambled controls after ghrelin treatment. Taken together, these data indicate that ghrelin could promote the progressior of endometrial cancer cells *in vitro* by acting on alternative, not yet identified receptor(s) and not entirely through GHSR1a. Nothing to Disclose: JNTF, CC

Pub #	P2-217
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Ovarian & Endometrial Cancer (1:30 PM-3:30 PM)
Title	Overexpression of 17β-Hydroxysteroid Dehydrogenase Type 1 Increases the Exposure of Endometrial Cancer to 17β-Estradiol
Author String	K Cornel, B Delvoux, L Visconti, R Kruitwagen, A Romano Maastricht University Medical Centre, Maastricht, Netherlands
Body	Endometrial cancer (EC) is one of the most frequent gynaecologic malignancies. The American Cancer Society has estimated that deaths for EC have risen over 200% since early 1990s and EC incidence is expected to further increase in the future due to the longer life expectancy and augmented obesity. Novel therapies are therefore needed. Most endometrial cancers are estrogen-dependent and develop after menopause. Since at this time the ovaries are inactive, 17β-estradiol (the most potent estrogen) is produced locally from other circulating compounds. In particular, 17β-hydroxysteroid dehydrogenases (17β-HSDs) reduce estrone (with low activity) to 17β-estradiol and vice-versa. The balance between the 17β-HSDs maintains the correct exposure of healthy endometrium to 17β-estradiol. In the present study, we have applied a recently developed method to measure the metabolic activity of the various enzymes involved in the conversion of steroid hormones. The activity of the enzymes involved in estrone/17β-estradiol inter conversion was measured in postmenopausal endometrial biopsies and grade 1 ECs. The ratio between the activities of the 17β-HSDs synthesising 17β-estradiol versus those inactivating 17β-estradiol to estrone, which represents the potential of the tissue to synthesise 17β-estradiol, is increased in EC biopsies compared to controls. When the estrogen receptor status was taken into account, this difference results more pronounced. Most EC biopsies with low estrogenic potential are estrogen receptor negative. The high 17β-estradiol synthetic potential is confirmed by the higher expression of the estrogen-responsive gene TFF1 in ECs compared to controls which indicates higher exposure to 17β-estradiol compared to controls. Furthermore, we have assessed the expression level of the different 17β-HSDs in the same biopsies. We showed that specifically type 1 17β-HSD is up regulated in ECs compared to controls. The level of the other 17β-HSDs is similar in ECs and controls, therefore these enzymes are not involved in the hyper-estrogenism observed in EC. In vitro investigations confirmed the important role of this enzyme in the synthesis of 17β-estradiol. Conclusions: inhibition of 17β-HSD type 1 may be a novel therapeutic approach for EC. Nothing to Disclose: KC, BD, LV, RK, AR

Pub #	P2-218
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Ovarian & Endometrial Cancer (1:30 PM-3:30 PM)
Title	Effects of Gonadotropin-Releasing Hormone (GnRH) on Signaling Transduction Pathways in Endometrial Cancer Cells
Author String	M Cho-Clark, T-YJ Wu Uniformed Services University of the Health Sciences, Bethesda, MD
Body	The hypothalamic decapeptide, GnRH, is known primarily as a regulator of mammalian reproduction by modulating the secretion of hormones in the body. Its release from the hypothalamus and subsequent binding to its cognate receptor GnRH receptor (GnRHR) results in the release of luteinizing hormone (LH) and follicl stimulating hormone (FSH) in the pituitary. In addition to its key role on pituitary-gonadal axis, previous studies have suggested that LHRH and its analogs have an anti-proliferative effect on numerous types of cancer cells. The possibility of a tumor specific signal transduction pathway distinct from the classic PLC or PKC pathway in the pituitary has also been elucidated. Our studies have shown that the analog D-Ser-LHRH down-regulated ($p<0.05$) the expression of GPR30, a G-protein coupled receptor that has be implicated to tamoxifen resistance and carcinogenesis. Interestingly, the GnRH metabolite, GnRH-(1-5) had an inverse effect increasing ($p<0.05$) GPR30 expression by approximately two-fold. Thus, there may be a negative and positive feedback mechanism that LHRH and LHRH-(1-5) respectively may have in regulating expression of GPR30 in endometrial cancer cells. Other protein kinases involved in cell proliferation and division were also down-regulated at pAkt (Thr308) and pERK p42. Furthermore, there was an increase ($p<0.05$) in the phosphorylation of the EGF receptor at 3 sites (Tyr 992, 1045, and 1068). In summary, our data suggests that LHRH may have an anti-proliferative effect in endometrial cancer cell lines due to the down-regulation of receptors and kinases highly implicated in cell proliferation and division. Nothing to Disclose: MC-C, T-YJW

Pub #	P2-219
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Ovarian & Endometrial Cancer (1:30 PM-3:30 PM)
Title	Cortisol Metabolism in Patients with Benign and Malignant Uterine Tumors: Urinary Steroid Analysis Utilizing GC/MS/MS
Author String	O Abulafia, Y-C Lee, G Salame, T Shah, E Stevens, K Economos, C Gorelick, O Muneyyirci-Delale, VL Nacharaju SUNY, Downstate Medical Center, Brooklyn, NY
Body	The role of glucocorticoids in reproductive physiology was suggested by several investigators. The levels of active steroid cortisol are maintained by the conversion of cortisol (F) to cortisone (E) by 11β-Hydroxysteroid dehydrogenase (11β-HSD) or by converting into tetrahydrocortisol (THF) isomers (THF and alloTHF) in the liver with subsequent renal excretion. Measurements of urinary steroid levels of free cortisol, THF and THE enhance knowledge of cortisol metabolism. The ratio (THF + allo-THF)/THE represents 11β-HSD function and the ratio F/ (THF + allo-THF) represents hepatic clearance of free cortisol. We developed a GC/MS/MS method to determine these ratios in spot urine samples. Following approval by Institutional Review Board, spot urine specimens were collected from normal controls (n=14) and women with either uterine leiomyomata (n=10) or endometrial cancer (n=14) and kept frozen at -80o C. Urinary steroids were extracted using C-18 columns, hydrolyzed with glucoronidase enzyme, re-extracted, derivatized to yield MO-TMS (methoxime-trimethylsilyl) derivatives. A segmental GC/MS/MS method was developed to quantify THF, allo-THF and free cortisol in first injection and THE and free cortisone in second injection. The injections were made consecutively. The THE, THF, allo THF and cortisol peaks were identified in all samples, whereas cortisone peak was not identified in all samples. The ratios (THF+allo-THF)/THE, F/ (THF+allo-THF) were calculated and compared among control and study subjects. The (THF + allo-THF)/THE ratios (mean ± SD) in controls, leiomyoma and endometrial tumors were 1.01 ± 0.57, 0.70 ± 0.13 and 1.11 ± 1.21, respectively. The F/ (THF + allo-THF) ratios were 0.14 ± 0.08, 0.16 ± 0.21 and 0.50 ± 0.59, respectively. The F/ (THF + allo-THF) ratios were higher in endometrial cancer patients when compared to normal controls the $p = 0.032$, and (THF+allo-THF)/THE ratio was not different. All ratios in leiomyomata were not different from normal controls. Our results indicate higher levels of free cortisol in urine samples of patients with endometrial cancer in comparison with normal controls indicating decreased hepatic conversion of free cortisol to tetrahydrocortisol Nothing to Disclose: OA, Y-CL, GS, TS, ES, KE, CG, OM-D, VLN

Pub #	P2-220
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Ovarian & Endometrial Cancer (1:30 PM-3:30 PM)
Title	Determination of Urinary Cortisol Metabolites in Spot Urine of Patients with Uterine Cancer Utilizing Segmental GC/MS/MS
Author String	VL Nacharaju, Y-C Lee, G Salame, T Shah, E Stevens, K Economos, C Gorelick, O Muneyyirci-Delale, O Abulafia SUNY, Downstate Medical Center, Brooklyn, NY
Body	Urinary cortisol metabolites were studied intensively by several investigators using GC/MS method of analysis. In general 24-hour urine collection is used for steroid analysis. Measuring cortisol metabolites, especially urinary free cortisol in spot urine is difficult due to dilution effect. We developed a method using segmental GC/MS/MS analysis to achieve this goal. We previously described 11β-HSD enzyme activity in endometrial cancer (Manolis et al, Gynecol Obstet Invest, 2006). The ability to analyze cortisol metabolites in spot urine may provide valuable metabolic information in this disease. Spot urines samples from 14 patients with endometrial cancer were analyzed. Steroid conjugates were extracted from 5 ml of spot urine using C-18 columns and subjected to hydrolysis by glucoronidase enzyme for 48 hours at 37[deg] C. Steroids were re-extracted after hydrolysis by C-18 columns. The free steroids were derivatized to Methoxime-trimethylsilyl derivatives and one [micro]l of this mixture was injected into varian 2200 GC/MS instrument using auto-injector. Based on EI/MS mass spectra, conditions were determined to achieve optimal sensitivity by MS/MS isolation and collision induced dissociation of the molecular ion. The steroid metabolites of interest were determined in two consecutive injections. First injection was made to determine internal standard diethyl stilbestrol (DES) in a segment 4-15 minutes, THF and allo-THF in a segment 15-18 minutes and free cortisol in a segment between 18 to 25.5 minutes. The second consecutive injection was made to determine DES as above and THE in a segment 15-18 and free cortisone in a segment 18-25.5. Appropriate excitation storage levels were chosen for each segment based on parent molecular weight. The separation of interested steroids was achieved by using 2[deg] C/ min temperature increments between 250[deg] C and 290[deg] C. A method using segmental GC/MS/MS was developed to analyze cortisol metabolites from spot urine. We were able to measure free cortisol in all 14 specimens. The two consecutive injections facilitate determination of THE and THF levels without interference, as these compounds elute closely. This ratio of (THF + allo-THF)/THE in spot urine specimens of patients with endometrial carcinoma was 1.11± 1.21 (mean ± SD). Our data suggest that the ability to determine free cortisol levels in spot urine may assist in understanding cortisol metabolism in patients with gynecologic malignancies. Nothing to Disclose: VLN, Y-CL, GS, TS, ES, KE, CG, OM-D, OA

Pub #	P2-221
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Impaired Gallbladder Motility and Effect of Metformin Therapy in Patients with Polycystic Ovary Syndrome
Author String	S Isik, HN Ozcan, U Ozuguz, D Berker, YA Tutuncu, G Akbaba, S Guler Ministry of Health, Ankara Numune Research and Training Hospital, Ankara, Turkey; Ministry of Health, Ankara Numune Research and Training Hospital, Ankara, Turkey
Body	**Background**: Impaired gallbladder (GB) emptying was shown in patients with gallstones, and stasis of the GB bile plays an important role in the development of cholesterol stones. We aimed to evaluate GB motility ant the effect of metformin (MET) on GB motility in patients with polycystic ovary syndrome (PCOS). **Methods:** Thirty-six PCOS patients and 20 age and body mass index (BMI)-matched controls formed the study group. The GB volume is evaluated at baseline and at 10^{th}, 20^{th}, 30^{th}, 40^{th}, 50^{th}, 60^{th}, 75^{th} and 90^{th} minutes after standard liquid aliment consisting of 29.5% (19.68 g) fat, 16.7% protein, and 53.8% carbohydrate. Gallbladder ejection fraction (GBEF) was calculated for each measurement period. The patients are reevaluated after MET 1000 mg/day treatment for 12 weeks. **Results:** Insulin, LH, total and free testosterone (T and FT) levels, HOMA index and LH/FSH ratio values were significantly higher at baseline and decreased after MET treatment in patients with PCOS. While IR existed in 20 patients with PCOS (55.6%), there was no IR in the control group. The mean V0 was significantly higher in the study group than the controls (27.2±12.5 vs 13.3±7.0 cm^3, $p < 0.001$). Before MET treatment, GBEF values for each measurement, beginning from the 20^{th} minute, in PCOS group were lower compared to control group. Both of the groups reached the highest values at the 75^{th} minute (76.5±12.8% vs 86.7±14.9%, p=0.003), respectively]. A positive correlation was observed between V0 and age, fasting glucose, insulin, FT and T levels and HOMA-IR value [(r=0.322, p=0.043; r=0.472, p=0.01; r=0.389, p=0.04; r=0.425, p=0.07; r=0.528, p=0.08; r=0.493, p=0.008; and r=0.493, p=0.008), respectively]. A negative correlation was detected between GBEF75 and BMI, FT, T, LH/FSH ratio, and cortisol levels (r=-0.314, p=0.036; r=-0.299, p=0.033; r=-0.529, p=0.002; r=-0.281, p=0.043; r=-294, p=0.038). In regression analyses effects of presence of PCOS, age, LH/FSH ratio, T, FT, cortisol levels and V0 on 20^{th} minute GBEF values were persisting. Insulin, FSH, LH, E2, FT, androstenedion levels and HOMA-IR, LH/FSH values decreased significantly after MET treatment. GBEF values were also significantly improved with MET treatment. **Conclusion:** We, for the first time in the literature, showed that GB motility is impaired and can be improved significantly with MET treatment in PCOS patients. Nothing to Disclose: SI, HNO, UO, DB, YAT, GA, SG

Pub #	P2-222
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Hyperandrogenic Phenotypes Cannot Differentiate Non-Classical Form of 21-Hydroxylase Deficiency and Classic PCOS
Author String	VO Moura, LG Gomes, GAR Maciel, JAM Marcondes, SAY Yamashita, EC Baracat, BB Mendonca, TASS Bachega Unidade de São Paulo, São Paulo, Brazil; Universidade de São Paulo, São Paulo, Brazil
Body	**Context**: The nonclassical 21-hydroxylase deficiency (NCAH) and PCOS are the most frequent etiologies of hyperandrogenic diseases in reproductive-aged women. Both diseases present a wide range of clinical manifestations, including hirsutism (**H**), hyperandrogenemia, ovulatory dysfunction (**OD**) and polycystic ovarian morphology (**PO**). These diseases are clinically indistinguishable and PCOS diagnosis requires ruling out the NCAH. Recently, PCOS has been classified in 4 major phenotypes, which comprise different combinations of the aforesaid manifestations. However, it is not known if these phenotypes could discern PCOS and NCAH patients. **Objective**: To analyze the hyperandrogenic phenotype distributions and hormonal analysis in a large cohort of PCOS and adult NCAH patients. **Patients**: 101 PCOS patients diagnosed by the Rotterdam criteria and 59 NCAH patients by ACTH-17OHP levels [ge]10 ng/mL and molecular *CYP21A2* diagnosis. **Methods**: hyperandrogenic phenotypes were classified into 4 groups according to the Rotterdan criteria and their frequencies were compared between classic PCOS and NCAH patients. Androgens, basal and ACTH-17OHP levels were compared between patient groups. *t* test and Chi-square testes were used. **Results**: The type 1 (H+OD+PO) phenotype was observed in 53% and 17% of PCOS and NCAH groups ($P<0.05$), the type 2 (H+OD) in 29% and 28%, the type 3 (H+PO) in 6% and 3% and the type 4 phenotype (PO+H) in 13% and 3% of PCOS and NCAH groups, respectively. Mean Basal 17OHP levels in NCAH group were higher than PCOS group (13 ± 15 and 1.4 ± 2.5 ng/mL, respectively, $P<0.05$). However, 7% of NCAH patients had normal basal 17OHP levels (<2 ng/mL), whereas 13% of PCOS patients had increased basal 17OHP levels (>2ng/mL). Mean ACTH-17OHP levels were higher in genotyped NCAH group than PCOS group (48 ±34 and 3.1 ± 1.5 ng/mL, respectively, $P<0.05$). Mean testosterone levels did not differ between NCAH and PCOS groups (100 ±67 ng/dL and 103 ±78 ng/dL, respectively) as well as androstenedione levels (3.3 ± 2.4 ng/mL and 3.3 ± 1.2 ng/mL, respectively) and DHEAS levels (2,511 ± 1,77(ng/mL and 2,372 ± 1,199 ng/mL, respectively) (P>0.05). **Conclusion**: Although, there was a significant difference in the frequency of type 1 phenotype between PCOS and NCAH patient groups, we observed a great overlap of hyperandrogenic phenotypes as well as of basal 17OHP levels and androgen levels between PCOS and NCAH. Only ACTH-stimulated 17OHP levels were useful to distinguish both diseases. Sources of Research Support: FAPESP # 08/51624-6, FAPESP # 05/04726-0, CNPq # 305117/2009-2. Nothing to Disclose: VOM, LGG, GARM, JAMM, SAYY, ECB, BBM, TASSB

Pub #	P2-223
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	An HSD11[Beta]1 Variant with Reduced Activity Does Not Play a Protective Role for Metabolic Syndrome Development in PCOS
Author String	RPP Moreira, SAY Hayashida, GAR Maciel, JJM Marcondes, DDG Bugano, LG Gomes, BB Mendonca, EC Baract, TASS Bachega Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil
Body	Polycystic ovary syndrome (PCOS) is a metabolic disease affecting 6-10 % of women, associated with insulin resistance, obesity and adverse metabolic profile, which are risk factors for the Metabolic Syndrome (MetS). It is suggested that cortisol dysregulation due to increased pituitary-adrenal axis activity and cortisol action account for MetS development. The 11βHSD1 enzyme regulates cortisol action by way of its generation from cortisone. The HSD11B1-Ins4436A variant reduces its activity and it has been proposed to be a protective factor for diabetes/obesity in the general population. Objective: to evaluate the frequency of MetS in a large PCOS cohort and to analyze if the HSD11B1-Ins4436A allele influences the metabolic and hormonal profiles Methods: 111 patients (mean age 25.2 ± 5.2 yrs) diagnosed by AE-PCOS Society criteria. Overweight was defined by BMI [ge]25 and obesity by BMI [ge]30, insulin resistance by HOMA-IR and MetS by NCEPATPIII criteria. The Ins4436A allele was screened by sequencing. Results: The Ins4436A allele was found in 18% of alleles, in Hardy Weinberg equilibrium. Obesity, insulin resistance and MetS prevalence was 44%, 58% and 31%, respectively, and the ins4436A carrier group did not have a lower frequency of these disorders in relation to the wild type (wt) carrier group. MetS was not observed in patients with BMI < 25 kg/m[sup2] and it was identified in 26% of overweight patients, 51% obese and 63% morbidly obese. HOMA IR and waist circumference were higher in PCOS patients with MetS (P<0.001) and there were no differences between patient groups carrying mutated or wt alleles (P>0.05). Linear regression multivariate analysis showed that HOMA-IR was significantly correlated with BMI (P=0.003), but not with androgen levels or hirsutism Ferriman scores. There were no differences in Ferriman scores and menstrual patterns between patient groups carrying mutated or wt alleles. Testosterone and androstenedione levels were lower in the Ins4436A allele carrier group in relation to the wt carrier group (P=0.004 and P=0.03, respectively). Conclusion: We identified a high prevalence of obesity and MetS in PCOS. In contrast to what was observed in the normal population, the ins4436A variant is not a protective factor for the adverse metabolic profile in PCOS. On the other hand, we observed an influence of this allele on the severity of hyperandrogenemia. Sources of Research Support: FAPESP 09/54238-2, CNPq # 305117/2009-2. Nothing to Disclose: RPPM, SAYH, GARM, JJMM, DDGB, LGG, BBM, ECB, TASSB

Pub #	P2-224
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Heritability of Metabolic Syndrome in Families of Women with Polycystic Ovary Syndrome
Author String	P Vellanki, LL Armstrong, AJ Cooper, S Maraka, RS Legro, A Dunaif, MG Hayes Feinberg School of Medicine, Northwestern University, Chicago, IL; Pennsylvania State University College of Medicine, Hershey Medical Center, Hershey, PA
Body	Polycystic ovary syndrome (PCOS) is among the most common endocrine disorders of premenopausal women, affecting ~7% of the population. Familial clustering of PCOS, consistent with a genetic susceptibility to this disorder, is well documented. PCOS is associated with an increased risk for metabolic syndrome (MetS), which itself is highly heritable but also may be causally related to hyperandrogenemia. We tested the hypothesis that heritability of MetS is increased in women with PCOS compared to their unaffected sisters. A total of 1051 individuals were analyzed; 285 PCOS probands and 142 sisters with hyperandrogenemia (affected), 178 sisters with normal androgen levels and menses (unaffected), and 203 fathers and 243 mothers of these siblings were studied. MetS was defined using the American Heart Association Criteria. Heritability (h^2) of MetS and its defining criteria was calculated using SOLAR and adjusted for age. Estimated h^2 for MetS was 0.26 ($p= 8.0x10^{-7}$) when all family members were included. When only affected sisters and all parents were considered, h^2 was 0.31($p=5.3x\ 10^{-6}$), but for unaffected sisters and all parents h^2 was only 0.18 (p=0.06). Maternal contributions (including all family members but with MetS status of fathers changed to unknown) yielded h^2 of 0.24 ($p=2.1x10^{-5}$), which was similar to baseline. Complementary analysi to estimate paternal contributions (by changing MetS status of mothers to unknown) showed h^2 of 0.34 ($p=1.4x10^{-6}$). When component phenotypes of MetS were also examined, waist circumference had the highes h^2 at 0.86 ($p=1.7x10^{-11}$), followed by high density lipoprotein cholesterol levels at 0.58 ($p=7.2x10^{-9}$), serum triglyceride and fasting plasma glucose levels at 0.47 ($p=1.0x10^{-7}$ and $1.3x10^{-5}$ respectively), and blood pressure (BP) at 0.15 (p=0.10). When partitioned to affected and unaffected sisters, h^2 changed very little except for BP, which decreased to 0.06 (p=0.34) in affected sisters and increased to 0.88 ($p=2.9x10^{-3}$) in unaffected sisters. This study suggests greater heritability of MetS in affected than in unaffected women in PCOS families, consistent with the hypothesis that hyperandrogenemia increases MetS risk. MetS also seems to be more attributable to paternal than maternal contributions in PCOS families. Nothing to Disclose: PV, LLA, AJC, SM, RSL, AD, MGH

Pub #	P2-225
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Hypertension as a Marker of Life-Threatening Metabolic Disturbances in Adolescent Patients with Polycystic Ovary Syndrome (PCOS)
Author String	D Wiltgen, PM Spritzer Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil; Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil; National Institute of Hormones and Women's Health, Porto Alegre, Brazil
Body	PCOS is a common endocrinopathy affecting 5-10% of women in reproductive age and its classical phenotype is characterized by hyperandrogenism and ovulatory dysfunction. Adolescent patients may seem less affected by the metabolic and cardiovascular disturbances frequently associated with PCOS. However it's well described that even in early ages patients with PCOS are more insulin resistant than girls at same age without PCOS. Although hypertension is highly prevalent in PCOS, the role of hyperandrogenism in determining alterations in blood pressure is still controversial. We performed a cross-sectional study with 186 PCOS patients stratified into 4 groups according to age and presence of hypertension. Classical PCOS phenotype was defined as the presence of anovulatory cycles, clinical and/or laboratorial hyperandrogenism with or without polycystic ovarian morphology. Patients aged less than 19 years-old were classified as adolescents. Hypertension was defined as blood pressure levels of [ge]130/85 mmHg. Both hypertensive adolescents and adults PCOS (H-PCOS) were more obese than normotensive PCOS (N-PCOS) (BMI 33.8±6; 35.9±7, vs 26.2±6; 29.9±8, adolescents and adults, respectively; p=0.001). Insulin resistance measured by HOMA index was also higher in adolescents and adults H-PCOS [4.89 (2.87-12.39) and 6.4 (4.06-8.81), respectively] than N-PCOS [3.37 (2.25-5.43) and 3.36 (1.94-7.45) adolescents and adults respectively; p=0.01]. The presence of hypertension in the first evaluation of adolescents with PCOS was associated with similar prevalence of metabolic syndrome (54.5 vs 70.6%), impaired glucose tolerance (13 vs 15.7%) and type 2 diabetes (13 vs 13.7%) than adults H-PCOS and higher than adolescent N-PCOS. Although Ferriman-Galwey score for hirsutism was similar between the 4 groups, the androgen levels determined by the free androgen index was higher in H-PCOS patients, regardless of age [H-PCOS: 28.1 (18.1- 46.7); 15.1 (7.2-22.6) vs N-PCOS: 10.7 (6.8-20.3); 7.1 (3.8-12.9) adolescent and adult respectively; p=0.001]. In conclusion, our results suggest that the presence of hypertension in young PCOS patients is a strong marker of early and severe metabolic disturbances, similar to adults. Therefore the efforts to change lifestyle and to consider pharmacological intervention should not be delayed. The finding of higher androgen levels in the hypertensive group of PCOS patients may suggest a role for androgen action in the regulation of blood pressure. Sources of Research Support: National Institute of Hormones and Women's Health, Porto Alegre, Brazil. Nothing to Disclose: DW, PMS

Pub #	P2-226
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Persistence of Hyperinsulinemia, Positive Correlation of Insulin Resistance Indices with Androgen Levels, Increased Visfatin and Decreased PYY Levels in Women with Polycystic Ovary Syndrome after Menopause
Author String	MC Markopoulos, G Valsamakis, D Rizos, G Creatsas, G Mastorakos Aretaieion Hospital, Athens University Medical School, Athens, Greece; Aretaieion Hospital, Athens University Medical School, Athens, Greece; Aretaieion Hospital, Athens University Medical School, Athens, Greece

Body

Context: Hyperandrogenism and insulin resistance with compensatory hyperinsulinemia characterize premenopausal women with polycystic ovary syndrome (PCOS). We recently documented that hyperandrogenism persists in PCOS after menopause (1). The aim of this study was to investigate indices of carbohydrate metabolism, parameters of adipose tissue metabolism, and their correlation to androgens in women with PCOS after menopause.

Patients and Methods: Twenty four postmenopausal PCOS women and twenty four postmenopausal age- and BMI- matched healthy controls were studied. All subjects had fasting glucose, insulin, leptin, visfatin, pancreatic peptide YY (PYY) and high sensitivity C-reactive protein (hs-CRP) levels measured. Glucose and insulin levels were measured and corresponding AUC was calculated during a 75 gr OGTT, and insulin sensitivity (G/I, ISI, QUICKI), insulin resistance (HOMA-IR) and 1^{st} and 2^{nd} phase insulin secretion (1^{ST}PHIS and 2^{ND}PHIS) indices were calculated. Cortisol, 17-hydroxyprogesterone (17-OHP), [Delta]$_4$-androstenedione ([Delta]$_4$A), DHEAS, total testosterone (T), SHBG and free androgen index (FAI) levels were measured at baseline and after a CRH stimulation test.

Results: There was no significant difference in fasting glucose, insulin and leptin levels as well as in insulin resistance and insulin sensitivity indices between PCOS and control women. PCOS women had higher visfatin ($p<0.05$) and hs-CRP ($p<0.01$) levels, and lower PYY ($p<0.05$) levels. Baseline 17-OHP, [Delta]$_4$A, DHEAS, total T and FAI levels were higher and SHBG levels were lower in PCOS women ($p<0.05$). PCOS women had higher insulin AUC ($p<0.05$), 1^{ST}PHIS ($p<0.01$) and 2^{ND}PHIS ($p<0.01$) after OGTT. In PCOS women, baseline 17-OHP levels correlated positively to fasting insulin levels, glucose AUC and HOMA-IR, and negatively to G/I and QUICKI; [Delta]$_4$A levels after CRH stimulation correlated positively to glucose AUC and negatively to G/I and QUICKI.

Conclusions: Hyperandrogenism and hyperinsulinemia persist in postmenopausal PCOS women. Insulin resistance indices of the latter do not differ from controls while they correlate positively to baseline and post-CRH stimulation androgen levels. The elevated visfatin and decreased PYY levels in PCOS women after menopause may represent an intrinsic characteristic of PCOS or functional adaptation to PCOS metabolic defects.

(1) Markopoulos MC, Rizos D, Valsamakis G, Deligeoroglou E, Grigoriou O, Chrousos GP, Creatsas G, Mastorakos G. Hyperandrogenism in Women with Polycystic Ovary Syndrome Persists after Menopause.J Clin Endocrinol Metab. 2010 Dec 22. [Epub ahead of print]

Nothing to Disclose: MCM, GV, DR, GC, GM

Pub #	P2-227
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Omentin-1 Plasma Levels Are Reduced in Non-Obese Women with Normal Glucose Tolerance with Polycystic Ovary Syndrome
Author String	J-H Choi, E-J Rhee, K-H Kim, H-Y Woo, W-Y Lee, S-W Kim Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea; Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea; Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea
Body	**Introduction:** Omentin-1 is a novel adipokine expressed in visceral adipose tissue, which increases insulin sensitivity in human adipocytes. Polycystic ovary syndrome (PCOS) is associated with insulin resistance and obesity. The objective of this study was to determine the metabolic parameters that influence plasma omentin-1 levels in women with PCOS. **Research design and methods:** A cross-sectional study was performed in 87 women with PCOS and 53 body mass index (BMI)-matched healthy control, including 39 non-obese (NW) PCOS women with normal glucose tolerance (NGT) and 44 BMI- and HOMA-matched controls. Indices of insulin sensitivity, body composition and metabolic variables, circulating androgen levels, serum adiponectin and omentin-1 levels were measured. 75-g oral glucose tolerance test was performed in all participants. **Results:** The plasma omentin-1 level was significantly lower in women with PCOS compared with BMI-matched controls ($P<0.001$). Significant difference in the level of plasma omentin-1 was observed in non-obese PCOS women with normal glucose tolerance compared with BMI- and HOMA-matched control subjects ($P<0.001$). Plasma omentin-1 level was negatively correlated with BMI, indices of insulin sensitivity and circulating androgens and showed higher association with 2-hr postprandial glucose levels, C-peptide and insulin levels compared with fasting values. Within NW and NGT group, plasma omentin-1 levels remained negatively correlated with BMI, 2-hr postprandial C-peptide and circulating androgens and showed negative linear trend according to quartiles of free testosterone (P=0.028). **Conclusions:** Plasma levels of omentin-1 were reduced in non-obese PCOS women with normal glucose tolerance. Postprandial hyperinsulinemia and hyperglycemia contributed more to lower omentin-1 levels than fasting values in PCOS. Increased androgen levels contributed to decreased omentin-1 levels in PCOS patients. Sources of Research Support: IN-SUNG foundation for Medical Research. Nothing to Disclose: J-HC, E-JR, K-HK, H-YW, W-YL, S-WK

Pub #	P2-228
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Age-Associated Changes in Metabolic Risk Factors and Prevalence of Individual Rotterdam Criteria during Reproductive Age in 772 Caucasian Women with Hirsutism and PCOS
Author String	D Glintborg, H Mumm, P Ravn, M Andersen Odense University Hospital, Odense, Denmark; Odense University Hospital, Odense, Denmark
Body	**Objective:** Clinical manifestations and metabolic risk factors may differ according to age in patients with PCOS. **Design:** Retrospective trans-sectional study. **Patients:** 772 premenopausal, Caucasian women (age range 15-49 years) with the diagnoses hirsutism or PCOS were divided into four subgroups according to age: group 1 (15-19 years, n=72), group 2 (20-29 years, n=249), group 3 (30-39 years, n=330), group 4 (40-49 years, n=125). **Measurements:** Clinical evaluation (Ferriman-Gallwey score, BMI, waist, blood pressure), hormone analyses (testosterone, sex hormone binding globulin, DHEAS, fasting lipids, insulin, glucose), and transvaginal ultrasound. Oral glucose tolerance tests (OGTT) (n = 499) and ACTH tests (n = 434) were performed in a subgroup of patients. **Results:** **Clinical findings:** BMI, waist, Ferriman-Gallwey score, and blood pressure were increased in older vs. younger age groups. **Paraclinical findings:** Increased age group was associated with significantly decreased levels of androgens (total and free testosterone, 17 hydroxyprogesterone, and DHEAS). FSH and estradiol levels were significantly increased in group 4 vs. groups 1-3 ($p<0.05$). Measures of insulin resistance were unchanged (fasting insulin, HOMA-r, and AUC insulin) between age groups, but fasting and AUC glucose were significantly increased in group 4 vs. group 1 and 2 ($p<0.05$). Cholesterol, triglycerides, and LDL were significantly increased in older vs. younger age groups. **Rotterdam criteria**: PCO and anovulation were decreased in group 4 vs. groups 1-3 (Prevalence group 1-4 PCO: 58%, 55%, 50%, and 19%, respectively and prevalence of anovulation: 57%, 66%, 50%, and 29%, respectively, Chi-square test $p<0.001$). Paraclinical hyperandrogenism was significantly decreased in group 4 vs. 1-3 (prevalence group 1-4: 39%, 48%, 35%, and 18%, Chi-square test $p<0.001$) and clinical hyperandrogenism was significantly decreased in group 1 vs. groups 2-4 (prevalence group 1-4: 69%, 84%, 85%, and 86%, Chi-square test $p<0.05$). 314/772 patients fulfilled the Rotterdam criteria for PCOS. Data for these patients will also be presented at the ENDO meeting. **Conclusion:** The clinical and biochemical manifestations of PCOS vary according to age. Young patients are characterized by biochemical hyperandrogenism, anovulation, and PCO whereas older patients are more obese with more severe hirsutism and more cardiovascular and metabolic risk factors compared to young patients. Nothing to Disclose: DG, HM, PR, MA

Pub #	P2-229
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	The Cardiovascular Risk of Girls with Premature Pubarche: A Case-Control Study
Author String	F Satler, RDA Vieira, C Firpo, PM Spritzer Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil; Federal University of Rio Grande do Sul, Porto Alegre, Brazil; Institute of Cardiology of Rio Grande Do Sul, Porto Alegre, Brazil
Body	Premature Pubarche (PP) in girls is defined as the development of pubic hair before the age of 8 years. Although PP has been considered a benign normal variant of puberty, some authors have described it as an early clinical feature of the metabolic syndrome. It has been shown that girls with PP are also at increased risk of developing polycystic ovary syndrome (PCOS). PCOS has been associated with increased cardiovascular risk factors as well as left ventricular hypertrophy and diastolic dysfunction independently of weight (1). With the aim to evaluate the cardiovascular risk in girls with PP, we investigated metabolic and hormonal variables lipid profile and echocardiography in 20 girls with PP and 20 healthy age-matched girls, who did not differ in body mass index (20.09 ± 2.99 vs 19.43 ± 3.45 kg/m^2; p = 0.319), etnia [13 (65%) vs 12 (60%) Caucasian; p = 0.744] and pubertal stage [8 (35%) vs 7 (30%) prepubertal; p = 0.744]. The PP girls showed significantly higher levels of androgens: total testosterone [0.51 (0.39 - 0.83) vs 0.35 (0.25 - 0.47) ng/mL; p = 0.001]; free androgens index (FAI) [3.91 (1.80 - 6.32) vs 2.22 (1.21 - 5.38); p = 0.019]; S-DHEA [148.20 (74.95 - 197.37) vs 80.10 (23.70 - 117) ug/dL; p = 0.005]; androstenedione [1.87 (0.75 - 2.57) vs 1.55 (0.62 - 1.81) ng/mL ; p = 0.009]. No differences were found in the lipid profile, fasting glucose and insulin, homeostasis model assessment score of insulin sensitivity (HOMA-IR) and blood pressure. The ultrasensitive c-reactive protein was 0.6 (0.19 - 1.50) in PP girls vs 0.19 (0.11 - 0.50) ng/L in controls (p= 0.068). On echocardiography patterns, differences were found in left ventricular posterior wall thickness (LVPWT): (0.66 ± 0.09 vs 0.59 ± 0.07 cm; p = 0.004) and early to late mitral flow velocity (E/A ratio): (1.82 ± 0.27 vs 1.61 ± 0.24; p = 0.013), both higher on PP group. The LVPWT was correlated with HOMA-IR (r = 0.320; p = 0.044) and androgens levels: FAI (r = 0.718; p <0.001); androstenedione (r = 0.665; p <0,001) and S-DHEA (r = 0.501; p <0.001). The E/A ratio was correlated with systolic blood pressure (r = 0.393; p = 0.013). In conclusion, our findings show that PP group has greater LVPWT and diastolic dysfunction, independently of weight, suggesting that PP girls are at higher risk for early cardiovascular disease. (1) Orio Jr F, Palomba S, Spinelli L et al., J Clin Endocrinol Metabol 2004; 89:3696-3701 Nothing to Disclose: FS, RdAV, CF, PMS

Pub #	P2-230
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	The Effect of Atorvastatin on Dehydroepiandrosterone Sulfate and Androstenedione Levels in Patients with Polycystic Ovary Syndrome
Author String	T Sathyapalan, KA Smith, A-M Coady, ES Kilpatrick, SL Atkin Hull York Medical School, Hull, UK; Hull Royal Infirmary, Hull, UK; Hull & East Yorkshire Women's & Children's Hospital, Hull, UK
Body	**Context:** Hyperandrogenemia in polycystic ovary syndrome (PCOS) represents a composite of raised serum concentrations of testosterone, androstenedione, dehydroepiandrosterone (DHEA), and DHEA sulfate (DHEAS). In patients with PCOS, testosterone and androstenedione are primarily derived from the ovaries and DHEAS is a metabolite predominantly from the adrenals. It has been shown that atorvastatin reduces testosterone levels in patients with PCOS. **Objective:** To study the effect of atorvastatin on serum androstenedione and DHEAS concentrations in patients with PCOS. **Design:** Randomized, double blind placebo controlled study. **Participants:** Forty medication na[iuml]ve patients with PCOS. **Intervention:** Patients with PCOS were randomized to either atorvastatin 20mg daily or placebo for 3 months. Subsequently, a 3 month extension study for all patients was undertaken with metformin 1500mg daily. **Main outcome measure:** Changes in androstenedione and DHEAS concentrations. **Results:** The mean (SD) baseline androstenedione [5.6(0.9)vs.5.5(1.3) nmol/L; *P*=0.58] and DHEAS [7.1(1.0 vs.7.2(1.2) [micro]mol/L; *P*=0.72] levels were comparable between two groups. There was a significant reduction of androstenedione [5.6(0.9)vs.4.7(0.7) nmol/L;*P*=0.03] and DHEAS [7.1(1.0)vs.6.0(0.9)[micro] mol/L;*P*=0.02] with atorvastatin compared to placebo. Three months treatment with metformin maintained the reduction of androstenedione and DHEAS levels with atorvastatin compared to baseline. There were no changes in either DHEAS or androstenedione concentrations in the initial placebo group after 12 weeks of metformin. **Conclusions:** 12 weeks of atorvastatin significantly reduced both DHEAS and androstenedione contributing to the total reduction of androgen levels and indicating that the reduction of the hyperandrogenaemia is due to the action of atorvastatin at both the ovary and the adrenal gland in PCOS. Sources of Research Support: Grant from Pfizer. Pfizer has supplied atorvastatin 20mg tablets and placebo for the study. Otherwise sponsors had no input into study design, its execution, or interpretation of the findings. Nothing to Disclose: TS, KAS, A-MC, ESK, SLA

Pub #	P2-231
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Revisiting Ultrasonographic Criteria for Polycystic Ovarian Morphology: New Thresholds for Elevated Follicle Population
Author String	ME Lujan, ED Brooks, DR Chizen, RA Pierson, AK Peppin Cornell University, Ithaca, NY; University of Saskatchewan, Saskatoon, Canada; Guthrie Medical Group, Ithaca, NY
Body	The current sonographic criteria for polycystic ovarian morphology do not appear to adequately discriminate between normal and polycystic ovaries (PCO). A threshold of [ge]12 follicles throughout the entire ovary is likely too low, and leading to the misconception that PCO are a highly common finding in healthy women. One hundred women diagnosed with PCOS and 85 healthy female controls were prospectively evaluated by transvaginal ultrasonography. Both left and right ovaries were scanned using a 5 - 9 MHz transducer and images digitally archived for off-line processing. Follicle counts were performed using a validated grid systen which showed 0.82 and 0.93 levels of inter- and intra-observer agreement in achieving reproducible follicle counts. A receiver operator characteristic (ROC) curve analysis was performed to determine appropriate diagnostic thresholds for follicle counts. Mean follicle counts throughout the entire ovary (38.5 ± 14.7 vs. 15.1 ± 7.8, p<0.0001), and those in a single cross section (10.0 ± 3.8 vs. 5.6 ± 2.7, p<0.0001), were higher in the PCOS group than in controls. The area under the ROC curve was 0.934 for follicle counts made throughout the entire ovary and 0.830 for follicle counts made in a single cross-section indicating excellent to good diagnostic potential, respectively. A threshold at 26 follicles throughout the entire ovary had the best compromise between specificity (91%) and sensitivity (63%) when discriminating between normal and PCO. A threshold of 9 follicles in a single cross-section had 88% specificity and 62% sensitivity to distinguish between normal and PCO. In summary, follicle counts throughout the entire ovary have better diagnostic potential to distinguish between normal and PCO compared to follicle counts made in a single cross-section. The level of reliability when multiple observers implement these criteria should be evaluated to validate the appropriateness of the proposed criteria. Sources of Research Support: Strategic Training Initiative in Research in Reproductive Health Sciences (STIRRHS); Saskatchewan Health Research Foundation Fellowship (SHRF) Award; Canadian Institutes of Health Research (CIHR) Fellowship Award (Regional Partnership Program); Cornell Human Ecology Alumn Association (HEAA). Nothing to Disclose: MEL, EDB, DRC, RAP, AKP

Pub #	P2-232
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Increased Anti-Müllerian Hormone Serum Concentrations during Late Reproductive Age in Women with Polycystic Ovary Syndrome
Author String	T Sir-Petermann, A Ladron de Guevara, J Preisler, B Echiburu, M Maliqueo, N Crisosto University of Chile, Santiago, Chile
Body	Polycystic ovary syndrome (PCOS) is a common endocrine-metabolic dysfunction affecting premenopausal women. In healthy women, the late reproductive age starts around age 35 with a decline in ovarian function and an initial increase in FSH levels, with regular menstrual cycles. PCOS women exhibit an increased follicular mass and an increased ovarian steroidogenic capacity, which may prolong ovarian function later in life compared to normal women. The aim of this study was to assess ovarian steroidogenesis and ovarian reserve in a group of control women (n=30) and women with PCOS (n=30) matched for age and BMI. A secondary aim was to evaluate metabolic characteristics of these women compared with age- matched controls. PCOS patients were recruited at our clinical facility and diagnosed according to the NIH criteria. Control women were selected from women attending the preventive medical examination department of Obstetrics and Gynecology of our hospital. In both groups, the following procedures were performed: clinical history, anthropometry, transvaginal ultrasound and a leuprolide acetate test (10 ug/kg s.c.). Gonadotropins, steroid hormones, SHBG and AMH were assessed. In addition, an oral glucose tolerance test with measurement of glucose, insulin and lipids were performed. We observed that basal LH, FSH, and estradiol levels were not significantly different between both groups. Post-stimulated FSH levels were lower and estradiol levels were higher ($p<0.05$) in the PCOS group. As expected, basal and post-stimulated androgen concentrations were significantly higher in the PCOS group ($p<0.001$). Ovarian volume and antral follicular count were significantly higher in PCOS patients. AMH concentrations were significantly higher in the PCOS group (35.7 ± 30.8 vs 18.5 ± 11.4, mean ± SD, $p=0.032$). The metabolic syndrome was six times higher in the PCOS group compared to the control group. These preliminary observations suggest that in women with PCOS, AMH concentrations are higher than in normal women during late reproductive age, which might indicate that follicle pool exhaustion and menopause could be delayed in PCOS patients. The prevalence of metabolic syndrome was significantly higher in PCOS women compared to control women during late reproductive age. Sources of Research Support: SOCHED Grant 2009-05. Nothing to Disclose: TS-P, ALdG, JP, BE, MM, NC

Pub #	P2-233
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Ovarian Hyperandrogenism and Insulin Therapy in Young Women with Type 1 Diabetes
Author String	C Bizzarri, D Benevento, IP Patera, M Cappa Bambino Ges[ugrave] Children's Hospital, Rome, Italy
Body	Although hyperandrogenism is classically associated with type 2 diabetes, increasing evidence shows that women with type 1 diabetes (T1D) may also exhibit this abnormality. The trophic action of insulin on ovarian cells has been underlined, but the effects of substitutive insulin therapy on ovarian androgen production have not been clearly documented in T1D women up to now. We evaluated ovarian function in 54 T1D Caucasian adolescents and young women (age range: 15-25 years) without residual beta-cell mass (C peptide-negative). Aims of the study were: to define the prevalence of hyperandrogenism and polycystic ovary syndrome (PCOS); to investigate the impact of clinical characteristics on ovarian androgen levels and PCOS. Age, body mass index (BMI), waist circumference, duration of diabetes, insulin requirement and glycated hemoglobin (HbA1c) were considered as variables. Ten subjects (18.5%) showed high levels of testosterone (> 80 ng/dl) and delta-4-androstenedione (> 3.3 ng/ml), two subjects (3.7%) had reduced sex hormone binding globulin (SHBG) levels (< 13 nM/L). Five subjects (5.5%) could be considered affected by clinically evident PCOS, defined by the Rotterdam criteria, with polycystic ovaries at ultrasound in two of them (3.7%). Univariate analysis showed that testosterone levels were inversely related to HbA1c (*p*: 0.04). Multiple linear regression demonstrated a direct correlation between BMI and ovarian androgen levels (testosterone and delta-4-androstenedione, *p*: 0.04 and 0.03 respectively). SHBG levels were positively affected by the duration of diabetes (*p*: 0.02) and negatively influenced by the BMI (*p*: 0.03). No differences were evident between subjects on multiple daily injection therapy (MDI: 63%) and subjects using continuous subcutaneous insulin infusion (CSII: 37%), even if insulin requirement was significantly lower in subjects using CSII (MDI: 1.07 ± 0.16 U/kg/day - CSII: 0.64 ± 0.16 U/kg/day). No correlation was evident between insulin requirement and ovarian androgen levels. Our study confirms that high ovarian androgen levels are relatively frequent in T1D women, but PCOS prevalence is significantly lower than in previous reports. As in general population, overweight and obesity represent the main factors affecting the onset and progression of ovarian hyperandrogenism and PCOS. The daily dose of exogenous insulin, in completely insulin-dependent subjects, does not show a direct impact on ovarian function and androgen levels. Nothing to Disclose: CB, DB, IPP, MC

Pub #	P2-234
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Hyperandrogenism in Heterozygous Congenital Adrenal Hyperplasia Females with 21-Hydroxylase Deficiency
Author String	V Neocleous, C Shammas, E Andreou, M Picolos, M Toumba, K Kaffe, TC Kyriakides, N Skordis, LA Phylactou The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus; Makarios III, Hospital, Nicosia, Cyprus; Alithias Endocrinology Center, Nicosia, Cyprus; Iasis Hospital, Paphos, Cyprus; Makarios III, Hospital, Nicosia, Cyprus; Yale University, New Haven, CT
Body	There is disagreement as to whether heterozygous patients for the *CYP21A2* mutations are at increased risk fo development of hyperandrogenism. In selected populations, heterozygosity for 21-hydroxylase deficiency (21 OHD) seems to be associated with hirsutism, irregular menses, PCOS and premature acne. In the present study we attempt to provide new insight into the debate of whether carriers for *CYP21A2* mutations are at increased risk for symptoms of androgen excess. The present study was designed to seek evidence on the prevalence of heterozygous *CYP21A2* gene mutations in females with hyperandrogenism. The hormonal response to ACTH was evaluated in 130 female with clinical signs of hyperandrogenism patients along with direct DNA sequencing and MLPA analysis for mutations in the *CYP21A2* gene. The median plasma 17-hydroxyprogesterone (17-OHP) before and 60 minutes after ACTH stimulation were 6 nmol/l (range 1.3-17.6 and 16.8 nmol/l (range 5.0-60.6), respectively, suggesting heterozygosity based on the 17-OHP normogram. Sixty patients out of the 130 with signs of hyperandrogenism were identified as carriers of *CYP21A2* gene mutations. The most frequent mutations among the carriers were the mild p.V281L (51.6%), followed by p.Q318stop (16.6%), p.P482S (11.6%), p.V304M (6.6%), p.P453S (8.3%), p.A391T (1.7%), Del8bp (1.7%) and the large deletion (1.7%). Additionally, a very high allelic frequency (33.9%) of the N493S variant was observed in the group of 130 patients. The allelic frequency for this change was significantly different in the 70 hyperandrogenic patients identified with no mutation when compared to the group of 60 heterozygotes (50% *vs* 15%). Therefore, the presence of hyperandrogenic signs in the group of patients with no mutation in the *CYP21A2* gene could implicate N493S variant as a plausible disease causing mutation in the manifestatior of non-classic congenital adrenal hyperplasia (NC-CAH). The high prevalence of heterozygous *CYP21A2* mutations in females with distinct signs of hyperandrogenism such as acne, hirsutism, irregular menses and polycystic ovaries will prove the usefulness of both hormonal and genetic evaluation for the better clinical management of the disease. Nothing to Disclose: VN, CS, EA, MP, MT, KK, TCK, NS, LAP

Pub #	P2-235
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Androgen Measurement in Women: Comparable Performance of LC-MS/MS and Extracted Radioimmunoassay
Author String	F Janse, MMJC Eijkemans, BCJM Fauser, EGWM Lentjes, A Hoek, CB Lambalk, T Hickey, RJ Norman, AJ Goverde University Medical Center Utrecht, Utrecht, Netherlands; University Medical Center Utrecht, Utrecht, Netherlands; University Medical Center Utrecht, Utrecht, Netherlands; University Medical Center Groningen, University of Groningen, Groningen, Netherlands; VU University Medical Center, Amsterdam, Netherlands; School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, Australia
Body	Introduction: The measurement of serum testosterone (T) in women is challenging due to lack of precision and sensitivity o various available T assays, and because of the low T concentrations encountered in women. Accurate assessment of T in women is crucial especially in conditions associated with alleged over- or underproduction of T, such as in polycystic ovary syndrome (PCOS) or primary ovarian insufficiency (POI). Our study was aimed at measuring androgen concentrations in women with PCOS, POI and female controls, and to compare assay accuracy of extracted radioimmunoassay (RIA) and liquid chromatography- tandem mass spectrometry (LC-MS/MS) for T measurement in each of these groups. Methods: We included 208 women with POI, 200 women with PCOS, and 45 healthy, regularly cycling female control subjects. Serum concentrations of total T, androstenedione (AD), sex hormone-binding globulin (SHBG), Fre Androgen Index (FAI), dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulphate (DHEAS) were measured by LC-MS/MS and extracted RIA. A method comparison analysis was performed for T and FAI measurement by extracted RIA and LC-MS/MS using Bland-Altman and calculating the intra class correlation coefficient (ICC). Results: Androgen levels were significantly elevated in women with PCOS compared with POI patients and controls (< 0.05), yet women with POI showed androgen concentrations similar as controls. Compared with extracted RIA, androgen concentrations were measured systematically lower by LC-MS/MS. However, extracted RIA and LC-MS/MS were shown to have good agreement as assessed by Bland-Altman and ICC for T 0.967 (95% CI 0.960 - 0.972), and for FAI 0.967 (95% CI 0.960 - 0.972). Conclusions: With the current study we show that LC-MS/MS is of comparable quality to extracted RIAs for measuring female androgen concentrations. It is therefore a convenient assay for clinical and research purposes where androgen measurement in women is required. Nothing to Disclose: FJ, MRJCE, BCJMF, EGWML, AH, CNBL, TH, RJN, AJG

Pub #	P2-236
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Altered Granulosa Cell Function in Women with PCOS in Response to HCG Stimulation
Author String	RF Shayya, MA Rosencrantz, SS Chuan, H Cook-Andersen, RJ Chang University of California, San Diego, La Jolla, CA
Body	**CONTEXT**: In women with polycystic ovary syndrome (PCOS), excess ovarian androgen production is driven by increased LH secretion. Previously, we showed that in PCOS women enhanced androgen production following FSH administration was accompanied by corresponding increases of inhibin B (Inh B) compared to those of normal women. These findings suggest that excess androgen production in PCOS may involve a direct effect of theca cell stimulation by LH as well as an indirect mechanism involving altered granulosa-theca cell interaction. **OBJECTIVE**: To determine whether LH may exert an indirect effect on androgen production through granulosa cell stimulation, we assessed E2 and Inh B release following administration of hCG in women with PCOS and normal women. **DESIGN**: A prospective study was conducted to compare inhibin B and estradiol production in response to hCG in two groups of women. **SETTING**: The study was conducted in a General Clinical Research Center in a tertiary academic medical center. **PATIENTS**: Women with PCOS, 18-35 yr (n = 10), and normal ovulatory controls, 18-35 yr (n = 10), were recruited and participated in this study. **INTERVENTIONS**: Blood samples were obtained prior to and 24 hr after an iv injection of recombinant hCG at doses of 1ug, 25 ug, and 250 ug. **MAIN OUTCOME MEASURES**: The main outcome measures were serum inhibin B and estradiol responses after hCG administration. **RESULTS**: After iv hCG, PCOS women demonstrated no changes in the levels of circulating Inh B, whereas in normal women Inh B levels decreased significantly. Estradiol levels increased in both groups after hCG administration but to a larger extent in women with PCOS. **CONCLUSIONS**: These findings suggest that in PCOS women LH exerts a direct effect on theca cells to enhance androgen production with little, if any, stimulation of granulosa cell function. The lack of Inh B responsiveness after hCG in PCOS women compared to the significant decrement observed in normal women is consistent with abnormal granulosa cell function in this disorder. Sources of Research Support: Eunice Kennedy Shriver NICHD/NIH (U54 HD12303-28) as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research and NIH grant MO1 RR00827. Nothing to Disclose: RFS, MAR, SSC, HC-A, RJC

Pub #	P2-237
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Do Higher Androgens Enhance the Effect of Exercise Training on Cardiovascular Performance for Women with Polycystic Ovary Syndrome and Congenital Adrenal Hyperplasia?
Author String	AY Chang, P Snell, MD Palmer, S Thompson, BD Levine, RJ Auchus UT Southwestern Medical Center, Dallas, TX; UT Southwestern Medical Center, Dallas, TX; Presbyterian Hospital, Dallas, TX

Body

Background: Higher endogenous androgens have been implicated as a major cause for menstrual irregularities among female athletes, suggesting a competitive advantage to higher androgens in women. In contrast, previous studies have characterized lower peak oxygen uptake (VO_2max) in Polycystic Ovarian Syndrome (PCOS), the most common disorder of androgen excess in women, compared to controls. We sought to determine if higher androgen concentrations in women with PCOS and Classic Congenital Adrenal Hyperplasia (CAH) correlated with increases in left ventricular (LV) mass and improvements in cardiovascular (CV) performance after 6 months of exercise training.

Methods: Eight women with PCOS (n=5) or CAH secondary to 21-hydroxylase deficiency (n=3) consented t a 6 month exercise training program. Baseline tests included an incremental exercise treadmill protocol to measure VO_2max and cardiac output, cardiac MRI for LV mass, and the Frequently Sampled Intravenous Glucose Tolerance Test (FSIGTT) for insulin sensitivity (SI). Exercise training programs were customized to treadmill test results, and heart rate monitors were used to track exercise dose, duration and progress. Measurements were repeated at the end of 6 months.

Results: At baseline, women with PCOS were older (PCOS 36.6 ± 4.6, CAH 26.1 ± 3.7, p=0.02), but there was no significant difference in body mass index (38 ± 2.8 v 34.9 ± 12.7 kg/m^2), total testosterone (49.4 ± 24.1 v 61 ± 19.7 ng/dl), VO_2max (20.3 ± 3.2 v 23.8 ± 6.1 ml/kg/min) or LV mass indexed for body surface area (BSA) (64.1 ± 6.4 v 69.4 ± 6.6 g/m^2). Women with PCOS had significantly lower SI (2.37 ± 1.27 v 4.34 ± 0.49 $(mU/L)^{-1}min^{-1}$, p=0.046) and 17-hydroxyprogesterone (17OHP) [45 interquartile range (IQR) 37.8-54.8 v 912 IQR 293 - 8178 ng/dL, p=0.04). Five women completed 6 months of exercise training. VO_2 increased by 8.4 ± 14.3 % with a significant increase in LV mass/BSA (5.1 % ± 2.2, $p < 0.01$). There was no significant change in weight. Baseline 17OHP significantly correlated with increases in LV mass/BSA ($r = 0.92$, p=0.03) but not other changes in VO_2max, cardiac index or stroke index. Changes in LV mass/BSA did not correlate with other baseline measurements or changes in measurements of CV performance.

Conclusions: Higher endogenous androgens, reflected by serum 17OHP, might promote greater cardiac hypertrophy with exercise training in women with PCOS and CAH but might not result in improvements in cardiovascular performance.

Nothing to Disclose: AYC, PS, MDP, ST, BDL, RJA

Pub #	P2-238
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Evaluation of an ICD-9 Search Algorithm for Identifying Women with Polycystic Ovary Syndrome
Author String	D Purohit, S Thompson, J Choi, L Harrison, A Jones, AY Chang UT Southwestern Medical Center, Dallas, TX

Body

BACKGROUND: Polycystic Ovarian Syndrome (PCOS) is characterized by irregular, anovulatory, menstrua cycles and hyperandrogenism. Clinically, women with PCOS present with various conditions that include irregular menses, infertility, unwanted hair growth and acne. When conducting epidemiologic studies, multiple potential diagnoses make it difficult to efficiently identify PCOS cases. We sought to develop an algorithm that identifies PCOS cases from a clinical database using ICD-9 codes for conditions associated with PCOS.

METHODS: Outpatient visits to Parkland County Hospital over a 2 yr period for women between the ages of 18 to 50 were screened for the ICD-9 codes polycystic ovaries (PCO) 256.4, amenorrhea 626.0, infrequent menses 626.1, irregular menses 626.4, infertility 628.0, alopecia 704.0, hirsutism 704.1 and acne 706.1. Chart review was performed to classify women into three categories: PCOS, Possible PCOS, Not PCOS. PCOS was defined by Rotterdam criteria - at least 2 of the following: 1) oligomenorrhea (< 9 periods a year), 2) hyperandrogenism by exam findings or elevated serum androgens, 3) PCO morphology (Rotterdam ultrasoun criteria). Possible PCOS cases met 1/3 PCOS criteria but were missing data for another characteristic. The No PCOS category included women without 2 PCOS criteria or who had an alternative diagnosis.

RESULTS: Of the 640 charts reviewed, 5.8% were coded PCO, 47.2% amenorrhea, 7.5% infrequent menses, 32.2% irregular menses, 10.6% infertility, 0.6 % alopecia, 7.5% hirsutism, 0.6 % acne. 105 (16.4%) women were classified PCOS, 155 (24.2%) Possible PCOS, 290 (45.3%) Not PCOS and 88 (14.0%) could not be classified due to missing data. The ICD-9 codes with the highest sensitivity were amenorrhea (42.9%, 95% CI 33.4-52.9), hirsutism (24.8%, 17.1-34.3), PCO (21.9%, 14.7-31.2), and irregular menses (21.0%, 13.9-30.2). Although the amenorrhea code had the highest sensitivity, it also had the lowest positive predictive value (PPV, 15.2%, 11.4-19.9), requiring review of 275 additional charts to identify 34 more PCOS cases. The combination of PCO, hirsutism and irregular menses had the highest sensitivity (53.3%, 43.4-63.0), specificity (59.1%, 54.8-63.3) and PPV (20.7%, 16.1-26.1%).

CONCLUSIONS: An algorithm using ICD-9 codes for irregular menses, hirsutism and PCO had the highest sensitivity, specificity and PPV for identifying cases of PCOS. Although the amenorrhea code identified the most PCOS cases, it also had the lowest PPV.

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Nothing to Disclose: DP, ST, JC, LH, AJ, AYC

Pub #	P2-239
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Early Metabolic and Endocrine Perturbations in First-Degree Relative Adolescent Girls of Polycystic Ovary Syndrome Women
Author String	A Trottier, M-C Battista, J Simoneau-Roy, A Carpentier, DH Geller, J-P Baillargeon University of Sherbrooke, Sherbrooke, Canada; University of California (UCLA), Los Angeles, CA
Body	**Introduction:** Polycystic ovary syndrome (PCOS) is defined by hyperandrogenism and ovarian dysfunction, but is also characterised by insulin resistance (IR) and high levels of non-esterified fatty acids (NEFA). Previous results suggest that IR and/or NEFA spillover may contribute to the hyperandrogenemia of PCOS women. PCOS also seems to be genetically determined because of its higher frequency in relatives. The goal of this study was to verify whether metabolic and endocrine perturbations develop early in adolescent girls genetically linked to PCOS. **Methods:** We studied 9 adolescent girls having a relative (mother or sister) diagnosed with PCOS ($PCOS_r$) and 10 adolescent girls with no PCOS relative. Anthropometry and pubertal development (Tanner) stage were assessed. A 2h oral glucose tolerance test and a 3h frequently sampled intra-venous glucose tolerance test (FSivGTT) were performed. Insulin sensitivity (IS) was determined using the minimal model during FSivGTT ($IS_{FSIVGTT}$). Several adipokines, NEFA and androgens were assayed during these procedures. Insulin-induced NEFA suppression ($NEFA_{supp}$) was estimated by the negative slope of decreasing NEFA levels (on log scale) during the first 20 minutes of FSivGTT. Groups were compared with Wilcoxon tests and correlations were verified by Spearman tests. Adjustments were made by ANCOVA, using log-transformed variables when required. **Results:** In comparison to controls, $PCOS_r$ had higher body mass index (BMI) and waist circumference ($P<0.05$), increased fat mass ($P=0.09$) and decreased $IS_{FSIVGTT}$ ($P=0.003$). Leptin levels during OGTT were determined by adiposity. Levels of the androgen 17OH-progesterone (17Pg) were increased in $PCOS_r$ ($P=0.03$), independently of adiposity (Ps[le]0.05) or Tanner stage ([le]2 vs >2), but not of $IS_{FSIVGTT}$. Indeed, 17Pg was strongly correlated with $IS_{FSIVGTT}$ ($P=0.0003$) even after correction for Tanner stage ($P=0.0007$). Finally, $NEFA_{supp}$ was decreased in $PCOS_r$ ($P=0.004$) as compared to controls, independently of Tanner stage ($P=0.02$), adiposity (Ps[le]0.01), 17Pg ($P=0.050$) or BMI & 17Pg ($P=0.047$). $NEFA_{supp}$ was also correlated with $IS_{FSIVGTT}$ ($P=0.018$). **Conclusion:** Adolescent girls at high risk of developing PCOS demonstrate elevated adiposity, IR for glucose metabolism and hyperandrogenemia, but are mainly characterized by resistance to insulin-induced lipolysis, independently of adiposity. We thus report the first evidence that genetic predisposition to PCOS may be related to early adipocyte dysfunction. Nothing to Disclose: AT, M-CB, JS-R, AC, DHG, J-PB

Pub #	P2-240
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Free Testosterone Concentrations in Peripubertal Girls with Obesity: Association with Estimated 24-Hour LH and Insulin Concentrations
Author String	JP Beller, KL Knudsen, JS Collins, M Abshire, C Burt Solorzano, JC Marshall, CR McCartney University of Virginia Health System, Charlottesville, VA; Tidewater Physicians Multispecialty Group, Newport News, VA
Body	Peripubertal hyperandrogenemia (HA) can represent a precursor to the polycystic ovary syndrome. Many, but not all, peripubertal girls with obesity demonstrate elevated free testosterone (T); but the proximate causes of HA in these girls remain unclear. A recent analysis suggested that morning LH and fasting insulin concentrations (imprecise measures of overall LH secretion and insulin resistance/hyperinsulinemia, respectively) are independent predictors of free T in obese peripubertal girls (1). To assess these relationships further, we are pursuing a protocol that includes: (a) insulin every 30 min from 1 h before to 2 h after a standardized mixed meal (ingested at 1900 h); (b) LH every 10 min and T every 60 min from 1800-0900 h; (c) fasting insulin every 30 min from 0700-0900 h; (d) SHBG at 0700 h; and (e) a 2-h hyperinsulinemic euglycemic clamp (insulin dose: 80 mU/m2/min). To estimate 24-h insulin exposure, we assumed 2/3 of 24 h reflected by periprandial insulin measurements (i.e., mean insulin 1800-2100 h) and 1/3 of 24 h spent in a fasting state (mean insulin 0700-0900 h). To estimate 24-h LH exposure, we assumed 9 of 24 h reflected by mean LH from 2200-0700 h (overnight values) and 15 of 24 h reflected by mean LH from 1800-2200 h and 0700-0900 h (daytime values). Complete data are available for 9 obese (BMI-for-age percentile > 95) girls (two Tanner 2, one Tanner 3, one Tanner 4, five Tanner 5). All girls had evidence of insulin resistance with similar M values (94 to 155 mg/m2/min). However, free testosterone (T) values were markedly variable (mean ± SD, 39.3 ± 36.0; range, 5 to 99 pmol/liter). Spearman rank (non-parametric) partial correlation yielded a correlation coefficient of 0.69 ($p = 0.09$) between free T and estimated 24-h LH after controlling for estimated 24-h insulin and Tanner stage. The correlation between calculated free T and estimated 24-h insulin (after controlling for estimated 24-h LH and Tanner stage) was 0.50 ($p = 0.25$). Of interest, two subjects with estimated 24-h insulin > 100 [mu]U/ml had normal free T, perhaps because of lower LH values (i.e., estimated 24-h LH [le] 2 IU/liter). All subjects with estimated 24-h LH < 2 IU/liter (n = 4) had normal free T values for Tanner stage, including two late pubertal girls. In conclusion, early data suggest a critical role of LH in obesity-associated HA; and even in the presence of marked hyperinsulinemia, elevated free T may be dependent on a threshold amount of LH exposure. (1) Knudsen KL et al., Obesity (Silver Spring), 2010; 18:2118 Sources of Research Support: Eunice Kennedy Shriver National Institute of Child Health and Human Development/National Institutes of Health through cooperative agreement U54 HD28934 as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research (CM, JM, CBS, MA); F32 HD066855 (JC); General Clinical Research Center Grant M01 RR00847. Nothing to Disclose: JPB, KLK, JSC, MA, CBS, JCM, CRM

Pub #	P2-241
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Glucocorticoid Resistance (GR) as a Cause of Hyperandrogenism from Childhood to Adulthood
Author String	S Ghanny, P Philibert, L Nie, D Tan, I Predescu, S Bhandari, G Chrousos, C Stratakis, M New, J Michl, F Lacbawan, C Sultan, A Bhangoo, S Ten Infants and Children's Hospital of Brooklyn at Maimonides and SUNY, Brooklyn, NY; CHU de Montpellier, Montpellier, France; SUNY Downstate, Brooklyn, NY; Athens University Medical School, Athens, Greece; NICHD, Bethesda, MD; Mount Sinai Medical Center, New York, NY; SUNY Downstate, Brooklyn, NY

Body

Background:
Premature adrenarche(PA) and PCOS have previously been considered a rare sign of glucocorticoid resistance (GR). In the literature, there has been only been two reports of glucocorticoid resistance presenting as premature adrenarche[1,2]. There have also only been a few reports of glucocorticoid resistance and hypersensitivity causing PCOS.
Objective: To study glucocorticoid sensitivity in patients with PCOS and PA.
Methods: F-Dex binding assays were used to evaluate differential binding to the glucocorticoid receptor versus control. Glucocorticoid receptor gene (NR3C1) and FKBP4 were sequenced.
Patients:
Our group has screened 150 adolescents with PCOS and 127 children with premature adrenarche (PA) for elevated ACTH and/or cortisol levels. We have found fluctuating ACTH and/or cortisol levels in 13 patients out of 150 PCOS cases (9%) and 11 children from 127 (8.6%) PA cases and none of the controls. F-Dex binding analysis was done in this subgroup of 13 cases with PCOS and 11 cases of PA with fluctuating ACTH and/or cortisol levels, as well as 18 controls without fluctuating ACTH and/or cortisol levels.
Results:
9 females from 13 with PCOS and 8 from 11 children with PA had decreased binding in comparison to controls on F-Dex monocyte binding studies. Among the 9 PCOS cases with decreased F-Dex binding, 2 cases had gene variations in FKBP4, 1case was negative for gene variations in FKBP4 and the analysis of 6 cases for FKBP4 is pending. Among this same group, gene variations in NR3C1 were found in 6 cases, 1 case was negative gene variations in NR3C1 and 2 cases are pending. Among the 11 cases of PA with decreased F-Dex binding, we found 2 cases had gene variations in FKBP4 and in the same cases, 1 case had a gene variation in NR3C1 and the other case was negative for NR3C1. Mutational analysis of NR3C1 and FKBP4 for the other 9 cases is pending.
Conclusions:
Screening of our patients with PA and PCOS with fluctuating ACTH and/or cortisol demonstrated a higher incidence of GR by decreased F-Dex binding in comparison to previous understanding. Gene variations in NR3C1 and FKBP4 can be a cause of their decreased F-Dex binding. However further validation by RT PCR and expression studies are necessary.

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Nothing to Disclose: SG, PP, LN, DT, IP, SB, GC, CS, MN, JM, FL, CS, AB, ST

Pub #	P2-242
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Exenatide Reduces Body Weight but Not Hyperandrogenemia in Overweight Women with Polycystic Ovary Syndrome
Author String	AJ Dawson, T Sathyapalan, ES Kilpatrick, SL Atkin University of Hull, Hull, UK; Hull York Medical School, Hull, UK; Hull and East Yorkshire Hospitals NHS Trust, Hull, UK

Body

Objectives

Polycystic Ovary Syndrome (PCOS) is characterised by chronic anovulation, hyperandrogenism and polycystic ovaries on ultrasound scan. The condition is associated with increased insulin resistance and the prevalence of the metabolic syndrome and type 2 diabetes is higher in these women. Recognised treatments o PCOS include metformin to increase insulin sensitivity with improvement of metabolic parameters. Exenatide is a glucagon like polypeptide-1 analogue used in the treatment of diabetes and has been shown to reduce weight and insulin resistance.

Design

30 patients with PCOS diagnosed as per Rotterdam criteria were treated with exenatide initially the dose was 5 micrograms twice daily for one month increasing to 10 microgram twice daily for a further three months. The primary outcome was change in free androgen index; secondary outcomes were change in C-reactive protein (CRP) and fasting lipid profile.

Results

Nine patients withdrew from the study; 70% completed the study. There was no difference in free androgen index before (14.8±7.91) and after treatment (14.9±7.61) p=1.00. Weight improved from 112.9±19.1kg before to 109.23±19.34kg p=0.006 after treatment; BMI 41.48±7.96kg/m^2 to 40.12±7.80kg/m^2 p=0.0096; waist circumference 121.6±14.9cm to 118.4±16.6cm p=0.42; hip circumference 127.6±11.6cm to 127.8±14.8cm p=0.001. There was no difference in either systolic (119.5±12.7mmHg to 119.4±15.0mmHg p=0.71) or diastolic (73.9±6.9mmg Hg to 72.9±10.1mmHg p=0.82) blood pressure. CRP reduced from 7.13±4.65 mmol/L to 6.48±4.04 mmol/l p=0.004. There was no difference in fasting total cholesterol (4.57±0.74 mmol/L to 4.47±0.75 mmol/L p=0.66), HDL (1.10±0.36 to 0.98±0.29 p=0.39) LDL 2.92±0.69mmol/L to 2.96±0.57mmol/L p=0.83). There was a reduction in triglycerides from 1.33±0.55 mmol/L to 1.14±0.46 mmol/L p=0.006. There was not a correlation between either change in weight or change in CRP (r=0.438 p=0.15) or change in triglycerides and change in weight (r=-0.148 p=0.61).

Conclusion

Short term exenatide treatment had no effect on free androgen index but did reduce weight. There was also a reduction in CRP and fasting triglycerides in women with PCOS which is consistent with studies using exenatide in patients with type 2 diabetes(1) but is in contrast to previous study examining exenatide in PCOS (2).

1. Varanasi A, Chaudhuri A, Dhindsa S, Arora A, Lohano T, Vora M, Dandona P Durability of Effects of Exenatide Treatment on Glycemic Control, Body Weight, Systolic Blood Pressure, Crp and Triglyceride Concentrations. Endocr Pract:1-20

2. Elkind-Hirsch K, Marrioneaux O, Bhushan M, Vernor D, Bhushan R 2008 Comparison of single and combined treatment with exenatide and metformin on menstrual cyclicity in overweight women with polycystic ovary syndrome. J Clin Endocrinol Metab 93:2670-2678

Nothing to Disclose: AJD, TS, ESK, SLA

Pub #	P2-243
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Leucocytosis in Women with Polycystic Ovary Syndrome (PCOS) Is Incompletely Explained by Obesity and Insulin Resistance
Author String	N Phelan, A O'Connor, T Kyaw Tun, N Correia, G Boran, HM Roche, J Gibney The Adelaide and Meath Hospital Incorporating the National Children's Hospital, Tallaght, Ireland; The Adelaide and Meath Hospital Incorporating the National Children's Hospital, Tallaght, Ireland; UCD Conway Institute of Biomolecular and Biomedical Research, School of Public Health and Population Science, University College Dublin, Belfield, Ireland
Body	Introduction: Low-grade chronic inflammation predicts cardiovascular outcomes and is observed in women with polycystic ovary syndrome (PCOS). Whether this is entirely an effect of insulin resistance (IR) is not known. Methods: Seventy pairs of women with and without PCOS, matched for age, BMI and IR (HOMA, QUICKI and Avignon index), were generated from a larger cohort of 103 women with and 104 BMI-matched women without PCOS. Women with PCOS were studied in the follicular phase of the menstrual cycle. White cell count (WCC), high-sensitivity CRP (hsCRP) and a series of 12 cytokines and growth factors were measured. These inflammatory markers were also compared between women with PCOS and 10 normal women studied in the follicular, peri-ovulatory and luteal stages. Results: When all subjects were compared, WCC (6.99×10^9 vs 5.80×10^9 g/l, $p<0.005$), hsCRP (6.5 vs 4.8 mg/l, $p<0.05$), IL-6 (1.64 vs 1.12 pg/ml, $p<0.05$), IL-10 (0.68 vs 0.58 pg/ml, $p<0.05$) and TNFα (1.91 vs 1.55 pg/ml, $p<0.05$) were greater in women with PCOS. Pair-matching for IR eliminated between-group differences in hsCRP and cytokines but did not alter the difference in WCC (6.77×10^9 vs 5.72×10^9 g/l, $p<0.005$). WCC was greater in PCOS compared to normal women at all stages of the menstrual cycle. Summary and conclusions: Low-grade inflammation occurs in PCOS. Increased hsCRP and cytokines are associated with IR but increased WCC is observed even when IR is accounted for. The explanation for this and its clinical significance is unknown. Nothing to Disclose: NP, AO, TKT, NC, GB, HMR, JG

Pub #	P2-244
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Cardiovascular Disease Risk Markers in Women with Polycystic Ovary Syndrome: Systematic Review and Meta-Analysis
Author String	KA Toulis, G Mintziori, DG Goulis, E Kintiraki, E Eukarpidis, S-A Mouratoglou, A Pavlaki, S Stergianos, M Poulasouhidou, TG Tzellos, BC Tarlatzis Aristotle University of Thessaloniki, Thessaloniki, Greece
Body	**Introduction.** The relation between polycystic ovary syndrome (PCOS) and cardiovascular disease (CVD) remains unclear. The aim of the study was to systematically review the relevant trials that have studied CVD risk factors in woman with PCOS [CRP, Hcy, TNF-α, PAI-1, Lp(a), AGEs, VEGF, IL-6, ADMA and fibrinogen] and to meta-analyze the best evidence available. **Patients and Methods.** Search was conducted in the MEDLINE, EMBASE and CENTRAL d (last update June 2010). Eligible for the systematic reviews were studies, which reported on CVD risk markers levels in women with PCOS compared to controls. Weighted Mean Differences (WMD) and 95% Confidence Intervals (CI) were calculated in each of the CVD risk markers for all eligible studies and combined using random effects model. To ensure synthesis of the best available evidence, sensitivity analyses were performed. **Results.** 130 studies were including in 11 different meta-analyses, involving in total 6260 women with PCOS and 4546 controls. Women with PCOS demonstrated significantly elevated CRP [WMD (95% CI) = 0.96 (0.74 to 1.19)], Hcy [2.25 (1.46 to 3.03)], PAI-1 antigen [16.96 (7.65 to 26.28)], PAI-1 activity [0.70 (0.17 to 1.23)], VEGF [1.72 (0.96 to 2.48)], ADMA [0.19 (0.08 to 0.3)] and AGEs [3.91 (2.36 to 5.45)] levels as compared to controls, yet with significant between-study heterogeneity. Borderline significance was detected for TNF-α [0.75 (0.07 to 1.44)] and fibrinogen [0.20 (0.01 to 0.39)] whereas no significance was detected for IL-6 [0.71 (-0.16 to 1.59)]. **Conclusions.** Women with PCOS have increased serum concentrations of CVD risk factors as compared to controls. If this apparent risk is translated into increased incidence of CVD in later life remains to be elucidated. Nothing to Disclose: KAT, GM, DGG, EK, EE, S-AM, AP, SS, MP, TGT, BCT

Pub #	P2-245
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Sex Hormone Binding Globulin as an Independent Marker of Insulin Sensitivity in Women with Polycystic Ovary Syndrome (PCOS)
Author String	TM Barber, JAH Wass, S Franks University of Warwick, Coventry, UK; University of Oxford, Oxford, UK; Imperial College, London, UK
Body	**Objective:** To examine the clinical utility of serum sex hormone binding globulin (SHBG) as a marker of insulin sensitivity in women with PCOS and in control women. **Methods:** We recruited women with PCOS (n=50), who had polycystic ovarian morphology on MRI scan, and who fulfilled diagnostic (Rotterdam) criteria for PCOS. For comparison, we recruited 28 control women with normal ovarian morphology on MRI scan, and without any diagnostic features of PCOS. Fasting (9am) blood samples were analysed for serum SHBG, insulin and glucose. BMI and measures of insulin sensitivity (Homeostasis Model Assessment of Insulin Resistance [HOMA2 IR] values) were calculated. All participants were of UK British/Irish origin. We performed linear regression multivariate analyses and ROCs (Receiver Operator Curves) using SHBG as the test variable and HOMA2 IR as the state variable (HOMA2 IR [le]1.6 used to define insulin sensitivity). Measurements of cross-sectional areas of visceral fat depots were taken from T1-weighted axial MRI images (mid-L4 level). **Results:** SHBG had a significant inverse correlation with HOMA2 IR for PCOS cases (r^2=-0.52, P=0.001) and controls (r^2=-0.64, P=4.5x10^{-4}). On linear regression multivariate analyses (HOMA2 IR as the dependent variable), these associations remained nominally significant when BMI was added as an independent variable (PCOS cases: P=0.04, beta=-0.32; controls: P=0.02, beta=-0.40)**.** Although the addition of mid-L4 visceral area as an independent variable abolished the significance of the inverse SHBG/HOMA2 IR association for PCOS cases, this association remained significant in controls (P=0.02, beta=-0.39) and in the combined PCOS cases/controls group (P=0.02, beta=-0.25). ROC data show that SHBG levels >28mmol/l and >35mmol/l for PCOS cases and controls respectively are the best cut-offs for defining insulin sensitivity (HOMA2 IR [le]1.6 with positive predictive values at 73% and 85% respectively. **Conclusions:** SHBG is a sensitive and specific marker of insulin sensitivity in both women with PCOS and in control women, and appears to associate inversely with HOMA2 IR independently of BMI (and visceral fat cross-sectional area in combined analyses). SHBG can be regarded as a stable serum [ldquo]read-out[rdquo] of insulin sensitivity and could be used to identify women (including those with PCOS) with increased cardio-metabolic risk. These data highlight the potential use of SHBG as a simple alternative to the HOMA2 IR as a marker of insulin sensitivity. Nothing to Disclose: TMB, JAHW, SF

Pub #	P2-246
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Toward a Polymorphism Score Predicting the Response to Metformin: Evidence from Girls with Androgen Excess after Precocious Pubarche
Author String	M Diaz, A Lopez-Bermejo, D Sanchez-Infantes, J Bassols, F de Zegher, L Ibanez Hospital Sant Joan de Déu, University of Barcelona, Esplugues, Spain; Dr Josep Trueta Hospital and Girona Institute for Biomedical Research, Girona, Spain; University of Leuven, Leuven, Belgium
Body	**Context:** Single nucleotide polymorphisms [SNPs] in the organic cation transporter 1 [*OCT1*], serine-threonine kinase [*STK11*] and fat-mass- and obesity-associated [*FTO*] genes, as well as repeat numbers within the androgen receptor [*AR*] and sex-hormone-binding globulin [*SHBG*] genes, are each known to influence the phenotype of androgen excess and/or the response to metformin. **Aim:** To study the relationship between combinations of these genetic variants [reflected in a polymorphism score] and the responsiveness to metformin. **Setting:** University Hospital.[b]**Study Population, Design & Intervention:** SNPs and repeat numbers were assessed in 104 girls [mean age 15.5 yr, BMI 21.8 Kg/m^2] with androgen excess after precocious pubarche. The polymorphism score was the sum of 5 subscores: +0.5 per allele with *OCT1* rs628031 [G], *STK11* rs8111699 [G] and *FTO* rs9939609 [A]; +1.0 for >8 TAAAA repeats in *SHBG* and for >20 CAG repeats in *AR.* The girls were subgrouped by increasing polymorphism score [Score 1 to 4]. The response to metformin [850 mg/d] was judged by changes over 1 yr. **Main outcomes**: Fasting concentrations of circulating insulin, triacylglycerol, LDL- and HDL-cholesterol [and their ratio], and body composition by absorptiometry. **Results:** Beneficial changes in insulin, triacylglycerol, LDL- and HDL-cholesterol [and their ratio] and body composition differed markedly from Score 1 to Score 4 [0.0001Score 1 girls became more adipose on metformin, and Score 4 girls became much leaner without changing their body weight. **Conclusion:** Evidence from girls with androgen excess indicates that polymorphism scores may become clinically helpful to predict the responsiveness to metformin. Nothing to Disclose: MD, AL-B, DS-I, JB, FdZ, LI

Pub #	P2-247
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	EthinylEstradiol-CyproteroneAcetate vs Pioglitazone-Flutamide-Metformin in Adolescent Girls with Androgen Excess
Author String	L Ibanez, M Diaz, A Lopez-Bermejo, C Salvador, F de Zegher Hospital Sant Joan de Deu, University of Barcelona, Spain, Esplugues, Spain; Dr Josep Trueta Hospital and Girona Institute for Biomedical Research, Girona, Spain; University of Leuven, Leuven, Belgium
Body	Background Androgen excess is the most prevalent endocrinopathy of adolescent girls, often presenting with acne, hirsutism or irregular menses. Administration of an oral contraceptive containing an anti-androgenic progestagen is a common therapy for adolescents with androgen excess. Aim In adolescents with androgen excess (and without pregnancy risk), we compared the effects of EthinylEstradiol-CyproteroneAcetate (EE-CA; Diane 35 Diario) to those of a combination of pioglitazone (7.5 mg/d, Pio), flutamide (62.5 mg/d, Flu) and metformin (850 mg/d, Met). Study Design & Population 34 adolescent girls with androgen excess (mean age 16 yr, BMI 23 kg/m^2) were randomized to receive EE-CA (N=17) or low-dose PioFluMet (N=17) for 6 mo. Main Outcomes Anti-androgenic efficacy (hirsutism & acne scores; circulating testosterone, androstenedione & DHEAS), body composition (by absorptiometry and abdominal MRI), carotid Intima Media Thickness (cIMT) and highly sensitive assessment of C-reactive protein (hsCRP) at 0 & 6 mo. Circulating levels of AST and ALT were measured at 0, 2 & 6 mo. Results Anti-androgenic benefits were similar in the two treatment groups. Most girls on PioFluMet developed regular menses within 6 mo. All divergences between the treatment groups were to the advantage of low-dose PioFluMet, since the latter was associated with development of a leaner body composition (more lean mass; less visceral and hepatic fat), a lower cIMT and lower hsCRP levels. Transient ALT elevations were observed in two girls on EE-CA and in none on low-dose PioFluMet. Conclusion In adolescent girls with androgen excess and without pregnancy risk, the effects of low-dose PioFluMet on lean mass, visceral fat, hepatic fat, cIMT and hsCRP compared favorably to those of EE-CA. Low-dose PioFluMet may become a first-choice therapy for adolescent girls and young women with androgen excess and without pregnancy risk. Nothing to Disclose: LI, MD, AL-B, CS, FdZ

Pub #	P2-248
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Effect of Spironolactone on Endometrium in Patients with Polycystic Ovary Syndrome
Author String	C Fiore, M Zermiani, C Sabbadin, A Anddrisani, G Ambrosini, L Bordin, G Dona, G Clari, E Ragazzi, D Armanini University of Padua, Padua, Italy; University of Padua, Padua, Italy; University of Padua, Padua, Italy; University of Padua, Padua, Italy
Body	Polycystic ovary syndrome (PCOS) is a common endocrine-metabolic disease, affecting reproductive-age women. Spironolactone (SP) treatment improves clinical manifestations produced by hyperandrogenism and insulin-resistance and prevents long-term cardiovascular complications. The most important side effect of this therapy is the high prevalence of menstrual abnormalities in the middle-cycle. Aim of the study was to investigate the possible cause of metrorrhagias in the middle cycle period, assessing the thickness of the endometrium and the hormonal status. We have studied 15 consecutive patients with PCOS before and during treatment with SP at a daily dose of 100 mg. Serum progesterone, estradiol, FSH, LH and endometrial thickness were measured at the 14th and 16th day of the menstrual cycle before and during SP treatment. 11 patients had metrorrhagias at the middle cycle (14th-16th day) and 4 did not complain of any menstrual irregularity. The thickness of the endometrium measured in the 14th and 16th day of the menstrual cycle during treatment with SP was significantly lower; than before treatment in the same days (at the 14th before SP 8.2+/-2.7 mm and during SP 6.4+/-1.8 mm, p=0.0082 and at the 16th 9.4+/-3.3 before SP mm and 6.3+/-1.7 mm during SP, p=0.0020). In the 11 subjects with metrorrhagias the values of progesterone, LH, FSH remained unchanged before and during treatment, while estradiol decreased at day 14 of the menstrual cycle during SP compared to before therapy (at the 14th before SP 356+/-351 pmol/L and during SP 236+/-381 pmol/L, p=0.0225; at the 16th before SP 422+/-412 pmol/L and during SP 173+/-95 pmol/L, p=0.06). In the 4 cases without metrorrhagias the values of estradiol were higher both before than during SP ($p<0.05$). The decrease in endometrial thickness from the 14th to 16th days during treatment in PCOS patients with metrorrhagias is presumably correlated with the lowering of the concentration of estradiol in the follicular phase. The decrease in the concentration of estradiol measured at day 14 could be explained by a mechanism of inhibition of the ovarian biosynthesis operated by SP as demonstrated for aldosterone at the adrenal level. Nothing to Disclose: CF, MZ, CS, AA, GA, LB, GD, GC, ER, DA

Pub #	P2-249
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Macroprolactinemia in Polycystic Ovary Syndrome
Author String	SAY Hayashida, GAR Maciel, JM Soares, Jr, JA Marcondes, NKA Kobayashi, A Anzai, EC Baracat Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil
Body	**Introduction:** Hyperprolactinemia due to macroprolactin is a common laboratorial finding and may mislead the diagnosis of polycystic ovary syndrome (PCOS). However, the role of macroprolactin in PCOS is not well studied. **Objectives:** 1. To investigate the relationship between the presence of macroprolactinemia in PCOS subjects and the clinical and laboratorial parameters. 2. To assess the prevalence of macroprolactinemia in Brazilian women with PCOS. **Methods:** Two hundred seventy seven (277) PCOS women diagnosed according to AES-PCOS criteria (2006) were included in this study. All samples with hyperprolactinemia were treated with polyethylene glycerol (PEG) in order to exclude the presence of macroprolactinemia. Then, we compared clinical and laboratorial data of PCOS subjects with and without macroprolactinemia. The parameters analyzed were: age, body mass index (BMI), hirsutism score (Ferriman-Gallwey Index), menstrual cycles, FSH, LH, testosterone, DHEAS, 17OH-progesterone, androstenedione, glucose and insulin and SHBG. *t*-Student and chi-square tests were used for statistical analysis. **Results:** The overall prevalence of hyperprolactinemia was 12.3% of PCOS women. Macroprolactinemia was found in 5.8% (n=16) and the remaining cases were associated to stress or drug-induced hyperprolactinemia. In our population, PCOS women with macroprolactinemia presented smaller BMI (p=0.006) and HOMA-IR (p=0.03), when compared to the group without it. We found no differences between groups regarding age, hirsutism, menstrual pattern and androgens and hormonal profile. However, the small number of cases with macroprolactinemia does not allow us to draw a cause-effect interpretation of this data. **Conclusion:** The presence of macroprolactinemia in PCOS is a fairly common finding and must be remembered during the initial investigation. Similarly to non-PCOS women, macroprolactinemia does not seem to display biological activity of clinical impact. Nothing to Disclose: SAYH, GARM, JMS, JAM, NKAK, AA, ECB

Pub #	P2-250
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Testosterone Levels Are Associated with Increased Ovarian Volume and Follicle Numbers in Women with Polycystic Ovary Syndrome
Author String	H Lee, J-Y Oh, YS Hong, Y-A Sung, E-G Hong Ewha Womans University, School of Medicine, Seoul, Korea; Hallym University Kangnam Sacred Heart Hospital, Seoul, Korea
Body	Although the role of hyperandrogenism on preantral follicle development remains unclear, recent studies have reported that testosterone stimulates folliculogenesis, especially in small follicles, and promotes the gonadotropin responsiveness. We aimed to determine the relationship between testosterone and polycystic ovarian morphology. We recruited 489 women with PCOS (age: 24±5 yrs, BMI: 23.8±4.6 kg/m^2) diagnosed by NICHD criteria. The criteria for polycystic ovarian morphology required visualization of [ge] 12 follicles, 2 to 9 mm in diameter per ovary or ovarian volume > 10 cm^3 by transvaginal ultrasonography. Blood testosterone, sex hormone binding globulin (SHBG), LH, FSH, glucose, lipid, insulin levels were measured in the early follicular phase of the spontaneous menstrual cycle and whenever feasible in subjects without spontaneous menses. Polycystic ovarian (PCO) morphology was identified in 341 (69.7%) women with PCOS. PCOS with PCO morphology had significantly higher serum testosterone (78.9 vs. 71.4 ng/dl, $p<0.01$) and SHBG levels (50.1 vs. 43.4 nmol/L $p<0.01$) compared to PCOS without PCO morphology. No differences were found in plasma glucose, insulin, lipids and HOMA IR indices between two groups. Serum testosterone levels significantly correlated with ovarian volume ($r=0.225$, $p<0.01$) and follicle numbers ($r=0.160$, $p<0.01$). Multiple linear regression analysis showed that only testosterone levels are associated with ovarian volume ($\beta=0.052$, $p<0.05$).Our findings suggest that testosterone may have effects on ovarian folliculogenesis in women with PCOS but cause-effect relationship is not clear. Nothing to Disclose: HL, J-YO, YSH, Y-AS, E-GH

Pub #	P2-251
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	The Relationship between Visceral Fat and Insulin Resistance in PCOS Patients
Author String	E Cakir, N Colak, E Ozkaya, L Ozturk, A Gungunes, B Karbek, B Ucan, E Cakal, H Karakose, T Delibasi Diskapi Yildirim Beyazit Training and Research Hospital, Ankara, Turkey; Sami Ulus Training and Research Hosital, Ankara, Turkey
Body	**Objective:** A growing body of evidence indicates that accumulation of visceral (intra-abdominal) fat is strongly associated with insulin resistance, dyslipidemia, and atherogenic dyslipidemic state. Hyperinsulinism and insulin resistance are frequent findings in PCOS patients, and these traits have cause-consequence relationships with low-grade chronic inflammation, oxidative stress, and increased cardiovascular risk. Several studies have assessed the association between visceral fat areas, as measured using bioelectrical impedance analysis (BIA) and metabolic parameters. The reduction of visceral fat was closely associated with a decrease in the number of metabolic risk factors. The aim of the study was to evaluate the visceral fat area estimated by recently developed techniques such as abdominal bioelectrical impedance analysis in PCOS patients and healthy control women and its association between metabolic factors. **Research design and methods:** We studied 37 PCOS patients (22.4 ± 4.1 mean age, BMI, 25.7±6.2kg /m [sup2]) and age-BMI matched 30 healthy control women (23.2± 2.9 mean age, and BMI, 24.4±4.4kg/m [sup2]). PCOS was defined by the Rotterdam PCOS consensus criteria. The abdominal BIA measurement was conducted with subjects in prone position with voltage (detecting) electrodes located around the navel and the current (source) electrodes on both sides of the detecting electrodes by bioimpedans analyzator. **Results:** Abdominal and visceral fat was significantly higher in patients with PCOS than in control women (32,8± 30.1 and 8,1 ±4,1in PCOS patients; 37,8±9 and 5.8±2.2, respectively) (p:0.02, p:0.05; respectively). There was statistically significant difference between HOMA-IR level in PCOS patients (3.3 ± 2.1) and in control subjects (2.1 ± 0.85) (p:0.03). A significant positive correlation was found by pearson correlation test between measurement of abdominal-visceral fat and BMI, waist-hip ratio, HOMA-IR level, high sensitive C reactive protein, length of menstrual cycles, hirsutism score. (p<0,05). **Conclusion:** In our study, elevated insulin resistance and increased visceral fat area were observed in PCOS patients. Visceral fat might be the main reason of insulin resistance observed in PCOS patients. Nothing to Disclose: EC, NC, EO, LO, AG, BK, BU, EC, HK, TD

Pub #	P2-252
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	The Impact of Testosterone on the Outcome of Controlled Ovarian Hyperstimulation in Non-PCOS Women Undergoing Assisted Reproductive Treatment
Author String	T Sathyapalan, EH Dickerson, L Cho, SM Maguiness, S Killick, J Robinson, SL Atkin Hull York Medical School, Hull, UK; Women and Children's Hospital, Hull Royal Infirmary, Hull, UK
Body	**Introduction** Hyperandrogenemia has been suggested to have an important role in fertility even in women without polycystic ovary syndrome (PCOS). It has been shown that total testosterone measured by immunoassay is positively correlated with insulin resistance in women without PCOS undergoing in vitro fertilization (IVF), though there was no correlation of testosterone levels with oocyte fertilization rates. However, there is considerable cross reactivity for testosterone with other adrenal and ovarian androgens with immunoassay measurements that is not found when using isotope dilution liquid chromatography tandem mass spectrometry (LC/MS/MS). We conducted this analysis to study the relationship between total testosterone as measured byLC/MS/MS and fertilisation rates in an IVF programme. **Materials and methods:** A total of 49 infertile women without PCOS who were about to commence an IVF cycle were recruited to the study prior to cycle commencement. Serum testosterone was measured by LC/MS/MS. **Results:** There was a no correlation between total testosterone and oocyte fertilization rates (r=0.15, p value-0.23) for non insulin resistant patients (HOMA <2.5). However, for those women who were insulin resistant (HOMA >2.5) there was a significant negative correlation between total testosterone and fertilization rates(r= -0.73, p value - 0.02), total dose of gonadotrophin used (r=-0.60, p value-0.03) and days stimulated (r=-0.52, p value - 0.02). There was no significant correlation with androstenedione levels with body mass index, HOMA, follicular count or fertilization rates in women with or without insulin resistance. **Conclusion:** This study shows that there is a reduction in oocyte fertilization rates with an increase in testosterone levels in non-PCOS women undergoing assisted reproductive treatment who are insulin resistant. Nothing to Disclose: TS, EHD, LC, SMM, SK, JR, SLA

Pub #	P2-253
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Adiponectin in Obese PCOS Women -- Size Does Matter
Author String	L Leustean, S Fica, C Preda, MC Ungureanu, C Cristea, V Mogos, D Ungureanu, C Vulpoi University of Medicine and Pharmacy Gr T Popa, Iasi, Romania; University of Medicine and Pharmacy Carol Davilla, Bucuresti, Romania
Body	**Introduction.** Women with polycystic ovary syndrome (PCOS) have an increased rate of metabolic syndrome (1). Many studies demonstrated low levels of adiponectin in PCOS women, closely associated with MS (2). The aim of the study was to evaluate the association of adiponectin levels with MS in PCOS. **Patients and method**. Study group included 38 PCOS patients (Rotterdam criteria) compared to 30 healthy volunteers age matched, all with BMI>25 kg/m^2. Metabolic syndrome, serum adiponectin, and testosterone levels were performed in all subjects. **Results.** In the PCOS group, adiponectin was lower than in the control group, but the difference was not significant (p=0.2). In both groups adiponectin levels were significantly lower in younger (< 30 years old) vs. older women (p=0.03). There were no differences between adiponectin values in overweight versus obese women with PCOS (p=0.15), but the difference was significant in the non-PCOS group (p=0.018). When adjusted function of the BMI, adiponectin was significantly lower in overweight PCOS women compared to non-PCOS women (p=0.03) while the difference remained non-significant in the obese subgroups. Metabolic syndrome (MS) was more frequent in PCOS patients, and in women with metabolic syndrome adiponectin levels were lower, the difference being significant (p=0.012 in control group p=0.014 in PCOS group). Adiponectin levels were negatively correlated with waist circumference, fasting glycaemia, triglycerides, diastolic TA, and positively correlated with HDL. In the PCOS subjects insulin resistance had a significant negative correlation with adiponectin (r=-0.446) but not in the normal group (r=-0.08). **Conclusion**. Our results are similar to those of the literature. Adiponectin was not significantly lower in PCOS group probably because all patients were overweight. This supposition is sustained by the significant difference in the subgroups with BMI < 30 kg/m^2. Known to be correlated to body fat and an independent risk factor for MS, adiponectin seems also to be a marker of polycystic ovary syndrome. (1) Gulcelik NE et al, J Natl Med Assoc. 2008; 100:64 (2) Toulis KA et al, Hum Reprod Update. 2009; 15:297 Nothing to Disclose: LL, SF, CP, MCU, CC, VM, DU, CV

Pub #	P2-254
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Influence of the [ldquo]Normal[rdquo] Reference Range for Testosterone and Sex Hormone Binding Globulin in the Interpretation of Hyperandrogenemia
Author String	K Danilowicz, A Serra, D Mana, JA Rey, G Cross, OD Bruno Hospital de Clínicas, Buenos Aires, Argentina; Hospital de Clínicas, Buenos Aires, Argentina
Body	The hyperandrogenic syndrome (HS) is frequent in women of reproductive age. Abnormalities in serum androgens is cornerstone for the diagnosis and management. We aimed to analyze the influence of the definition of reference ranges for testosterone (T), sex hormone binding globulin (SHBG) and calculated free (FT) and bioavailable T (BT). Serum T and SHBG were measured in: **group A)** 20 healthy **carefully selected** normal weight and eumenorrheic women, without any clinical sign of androgen excess, **group B)** 15 **non-selected** normal weight women of reproductive age concurring to the hemotherapy bank, **group C)** 29 **hyperandrogenic** normal weight women of reproductive age with clinical evidence of hirsutism, acne, seborrhea and/or irregular menstrual cycles with hyperandrogenemia as defined through the kit references values and **group D)** 25 normal weight women of reproductive age with clinical evidence of hirsutism, acne, seborrhea and/or irregular menstrual cycles **without hyperandrogenemia.** Results in **group A** were: SHBG 79.5 ± 15 nmol/l, T 29.6 ± 14 ng/dl, FT 0.2 ± 0.09, BT 4.7 ± 2.2. G**roup B**: SHBG 60.1 ± 9.2, T 24.9 ± 9.3, FT 0.21 ± 0.07, BT 4.9 ± 1.7.G**roup C**: SHBG 42.4 ± 15.3, T 74.1 ± 27.4, FT 0.9 ± 0.4, BT 21.4 ± 10.4. **Group D:** SHBG 56.7 ± 17.9, T 36.5 ± 12.6, FT 0.34 ± 0.12, BT 7.9 ± 2.8. SHBG concentrations were significantly different among all groups. T levels differed significantly as expected between group **C and A or B or D** without difference between **A, B and D**. Considering **group A**, the reference suggested values (mean ± 2SD) would be: SHBG 49.5-109.5, T 1.6-57.6, FT 0.02-0.38, BT 0.3-9.1. Considering group b, SHBG 41.7-78.5, T 6.3-43.5, FT 0.07-0.35, BT 1.5-8.3. The suggested normal values se by the kit for SHBG are for SHBG 18 to114, T, ND to 80, and FT 0.21-0.56 ng/dl and BT 5-13 ng/dl. When analyzing **group D**, 12 out of 25 (48%) patients would have been categorized as hyperandrogenic taking into consideration the reference values obtained from group **A**.We observed that when we carefully selected the group of women defined as normal, the serum SHBG and T reference ranges narrowed. Specially, statistically significant differences were detected in mean SHBG. By using the selected range for SHBG previous patients defined as [ldquo]normals[rdquo] were re-defined as hyperandrogenic. In this observational study we show reference SHBG and T levels for a population of premenopausal women from Buenos Aires. By using these suggested values, the diagnosis of HS is improved. Nothing to Disclose: KD, AS, DM, JAR, GC, ODB

Pub #	P2-255
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Obesity, but Not PCOS, Is Associated with Increased IL-6 and hs-CRP in Young Women without Glucose Disturbances, Arterial Hypertension and Severe Abnormalities of Lipid Profile
Author String	CR Barcellos, MP Rocha, S Hayashida, E Baracat, W Dantas, G Maciel, JA Marcondes Hospital das Clínicas - Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Hospital das Clínicas - Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil
Body	Introduction. Pro-inflammatory cytokines, such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), are associated with metabolic syndrome and CV disease (CVD), while high sensitive c-reactive protein (hs-CRP) is involved in the pathogenesis of atherosclerosis and can predict CVD. The polycystic ovary syndrome (PCOS) and obesity are related to high concentrations of IL-6, TNF-α, hs-CRP and the increase of CV risk. However it is not known yet if this fact is due to metabolic disturbances commonly related to these two conditions or the presence of PCOS *per se* or obesity itself. Objective. The aim of this study was to determine the impact of PCOS and obesity, separately, in the concentrations of IL-6, TNF-α and hs-CRP in young women without glucose disturbances, arterial hypertension and severe abnormalities of lipid profile. Methods. We selected 25 women with PCOS (anovulatory cycles, hyperandrogenism and/or hyperandrogenemia and polycystic ovaries) aged between 18 and 35, subdivided according to BMI in lean (< 25 kg/m^2) and obese (30 40 kg/m^2) and 23 control women matched to age and BMI. Insulin resistance (IR) was estimated by HOMA-IR and AUCi. To suggest the impact of PCOS and obesity, the parameters studied were compared between women with PCOS and controls, regardless of BMI, and between lean and obese women, regardless of the presence of PCOS. Results. Values expressed as median (min-max). The median ages were similar between PCOS group and Control group and between Obese group and Lean group. IR parameters were higher in PCOS group than in Control group and were higher in Obese group than in Lean group. TNF-α was similar between PCOS group and Control group and between Obese group and Lean group. IL-6 and hs-CRP were similar between PCOS group and Control group [3.8 (0.4-62.0) vs 5.7 (0.9-38.8) pg/ml, p=NS and 0.9 (0.0-5.7) vs 0.5 (0.0-4.6) mg/l, p=NS, respectively] and higher in Obese group than in Lean group [8.7 (1.3-62.0) vs 2.0 (0.4-14.9) pg/ml, $p<0.05$ and 1.4 (0.2-5.7) vs 0.2 (0.0-1.0) mg/l, $p<0.05$, respectively]. The hs-CRP was positively correlated with BMI ($r=0.61$; $p<0.05$), waist circumference ($r=0.66$; $p<0.05$), HOMA-IR ($r=0.46$; $p<0.05$) and AUCi ($r=0.37$; $p<0.05$). Conclusion. Neither PCOS nor obesity were associated with increased TNF-α. Obesity itself, but not PCOS, was associated with increased IL-6 and hs-CRP in young women without glucose disturbances, arterial hypertension and severe abnormalities of lipid profile.

Sources of Research Support: Fundacao de Amparo a Pesquisa do Estado de Sao Paulo.

Nothing to Disclose: CRB, MPR, SH, EB, WD, GM, JAM

Pub #	P2-256
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Polycystic Ovary Syndrome and Obesity Do Not Influence Plasma Visfatin in Young Women without Glucose Metabolism Disturbances and Hypertension
Author String	CR Barcellos, MP Rocha, S Hayashida, E Baracat, W Dantas, G Maciel, JA Marcondes Hospital das Clínicas - Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Hospital das Clínicas - Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil
Body	Introduction. Polycystic ovary syndrome (PCOS) and obesity were associated with insulin resistance (IR). Visfatin, a protein secreted by adipose tissue, is suggested to play a role in pathogenesis of IR and is related to visceral fat mass and, in PCOS patients, with markers of hyperandrogenism. Objective. The aim of this study was to determine the influence of PCOS and obesity, separately, in the plasmatic concentrations of visfatin in young normotensive women without impaired fasting glucose, impaired glucose tolerance and type 2 diabetes. Methods. We selected 25 women with PCOS (anovulatory cycles, hyperandrogenism and/or hyperandrogenemia and polycystic ovaries) aged between 18 and 35, subdivided according to body mass index (BMI) in lean (< 25 kg/m^2) and obese (30-39 kg/m^2) and 23 control women matched to age, BMI and waist circumference (WC). We performed OGTT to evaluate glucose disturbances and to determine IR. IR was estimated by homeostatic model assessment of IR (HOMA-IR) and area under the curve of insulin (AUCi). To suggest the influence of PCOS and obesity, the parameters studied were compared between women with PCOS and controls, regardless of BMI, and between lean and obese women, regardless of the presence of PCOS. Results. Values were expressed as median (min-max). The median ages were similar between PCOS group and Control group and between Obese group and Lean group. HOMA and AUCi were higher in PCOS group than in Control group [2.2 (0.7-7.5) vs 1.2 (0.5-3.6), $p < 0.05$ and 258.5 (32.6-1,927.1) vs 39.4 (8.6-404.6) [mu]IU/ml/min.10^{-2}, $p < 0.05$, respectively] and were higher in Obese group than Lean group [2.6 (0.7-7.5) vs 0.9 (0.5-3.7), $p < 0.05$ and 261.1 (13.6-1,927) vs 44.2 (8.6-402.6) [mu]IU/ml/min.10^{-2}, $p < 0.05$, respectively]. Plasmatic visfatin was similar between PCOS group and Control group [5.5 (3.9-13.7) vs 6.0 (2.6-11.8) ng/ml, p=NS] and between Obese group and Lean group [5.8 (2.6-13.7) vs 5.9 (3.9-11.8) ng/ml, p=NS]. Conclusion. In our series, neither PCOS nor obesity, alone or in combination, were associated with increased concentrations of visfatin. Thus, visfatin was not associated with IR and markers of hyperandrogenism. Sources of Research Support: Fundacao de Amparo a Pesquisa do Estado de Sao Paulo. Nothing to Disclose: CRB, MPR, SH, EB, WD, GM, JAM

Pub #	P2-257
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Prevalence of Metabolic Syndrome, Insulin Resistance and Glucose Intolerance in North Indian Women with Polycystic Ovary Syndrome -- A Prospective Case-Control Study
Author String	V Bhatia, B Bansal Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India
Body	Consecutive patients with PCOS (n=106) aged 15 to 40 years, attending endocrinology clinic in our hospital were recruited over a 2.5 year period. Non - hirsute, regularly menstruating, age-matched (within 5 year blocks) controls (n=117) were recruited from the community. For comparing insulin resistance, BMI - matched (within 3 kg/m^2 blocks) cases (n=70) and controls (n=74) were taken. Evaluation included history, examination, standard oral glucose tolerance test and tests to exclude disorders with presentation similar to PCOS. Results Subjects with PCOS had mean $\pm$ sd age 22.9 $\pm$ 6.4 years and BMI 27.3 $\pm$ 6.3 kg/m^2). Controls had age 24.5 $\pm$ 6.5 years and BMI 23.6 $\pm$ 4.6 kg/m^2. Family history of diabetes was not different between cases and controls (42.5 % vs. 40.2 %). Significantly more subjects with PCOS had acanthosis nigricans as compared to the controls (60.9 % vs. 22.2 %, $p<0.001$). Eighty (75.5 %) PCOS subjects versus 60 (51.3%) controls were overweight by taking the Indian cut-off of BMI of 23 kg/m^2 ($p <0.001$). Diabetes was diagnosed in 8 PCOS subjects (6 overweight and 2 normal weight), and in none of the controls ($p < 0.005$). Abnormal glucose tolerance was seen in 16/76 overweight subjects with PCOS vs 5 of 58 overweight controls ($p<0.058$). All components of metabolic syndrome were significantly more prevalent in PCOS subjects except low HDL cholesterol, which was equally prevalent in patients and controls. PCOS status and waist- hip ratio were independent predictors of abnormal glucose tolerance on multivariate regression analysis. Insulin resistance as measured by HOMA-IR and fasting glucose / insulin was similar in PCOS subjects compared to controls. Conclusions North Indian women with PCOS, including non-obese and younger subjects, were at significantly increased risk of developing metabolic syndrome and abnormal glucose tolerance. PCOS subjects were not more insulin resistant than age and BMI matched controls. Nothing to Disclose: VB, BB

Pub #	P2-258
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	The Assessment of Lymphocyte T Subsets in Women with Polycystic Ovary Syndrome
Author String	W Foltyn, B Kos-Kudla, J Blicharz-Dorniak, A Zemczak, L Sieminska, B Marek, D Kajdaniuk Medical University of Silesia, Katowice, Poland
Body	**Introduction:** Sex hormones are potent immune modulators. Estrogens are known from immunostimulatory effect whereas androgens are considered to be immunosupressive. **The aim of the study** was the assessment of lymphocyte T subsets: CD3+, CD3+CD16+CD56+ (NK cells), CD4+, CD8+, TCR alfa/beta, TCR gamma/delta in peripheral blood in women with polycystic ovary syndrome (PCOS). **Material and methods:** 35 patients with PCOS, in the age of 18-42, and 15 age-machted healthy women were included for the study. The lymphocyte T subpopulations: CD3+, CD4+, CD8+, NK cells,TCR alfa/beta TCR gamma/delta were determined in peripheral blood by fow cytometry. Collected data were analyzed by using computer-based statistic program. **Results:** The percentages of CD3+, CD4+, TCR alfa/beta T cells and immunomodulatory index (CD4+/CD8+ ratio) were significant lower in women with PCOS then in healthy subjects (respectively $p<0,001$, $p<0,05$, $p<0,05$, $p<0,05$). The percentage of NK cells was significant higher in hiperandrogenic women then in controls ($p<0,05$). We found a positive correlation between androstenedione serum level and percentage of NK cells ($p<0,05$) and negative correlation between androstenedione serum level and percentages of CD3+ ($p<0,05$), CD4+ Tcells ($p<0,001$) and CD4+/CD8+ratio ($p<0,05$) in women with PCOS. It was a positive correlation between free testosteron serum level and percentage of CD8+ ($p<0,05$) in patients with PCOS. **Conclusion**: Hyperandrogenemia modulates cellular immunity in women with PCOS by quantitative changes in lymphocyte T subsets. Nothing to Disclose: WF, BK-K, JB-D, AZ, LS, BM, DK

Pub #	P2-259
Session Information	POSTER SESSION: CLINICAL - Reproductive Endocrinology: Polycystic Ovary Syndrome & Female Androgenic Disorders (1:30 PM-3:30 PM)
Title	Cardiovascular Risk Factors in Patients with Polycystic Ovary Syndrome
Author String	G Hart, R Redorat, R Monte, MFM Pinheiro, FL Conceicao UFRJ, Rio de Janeiro, Brazil; Laboratório Sérgio Franco, Rio de Janeiro, Brazil
Body	The polycystic ovary syndrome (PCOS) is a disorder characterized by hyperandrogenism and anovulation associated with insulin resistance and a variety of cardiovascular risk factors, as well as an increased risk of nonalcoholic steatohepatitis. The aim of this study was to compare cardiovascular risk factors and levels of transaminases'>nases in patients with PCOS seen in our day care facility with a group of control women. Methods: Patients and controls were submitted to evaluation of lipid profile, glucose metabolism (fasting insulinand glucose, HOMA-IR, transaminases (glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT)) activities and ultrasound/Doppler of carotid arteries. At the time of the investigation patients were not taking any medication. We evaluated 58 women with SOP (age 27.95 ± 5.93 years, BMI 31.37 ± 9. 14) and 31 controls (age 40.13 ± 9.15 years, BMI 27.16 ± 6.53). Patients presented higher levels of glucose (90.81 ± 21.41 vs. 86.43 ± 9.16, p= 0. 003) and a tendency of higher levels of insulin (18.22 ± 24.63 vs. 10.69 ± 12.35, p= 0,080). The control group presented a tendency towards higher levels of triglycerides, with similar levels of total cholesterol, LDL and HDL cholesterol. Transaminases activities and carotids intima media thickness were similar between patients and controls. There was a positive correlation with age and total cholesterol and LDL cholesterol, and age with carotids intima media thickness; BMI and glucose, insulin, triglycerides and carotids intima media thickness, and a negative correlation with BMI and HDL cholesterol; a positive correlation with glucose and carotids intima media thickness; and lipid profile and carotids intima media thickness. Conclusions: Patients with PCOS had a higher BMI and glucose/insulin levels showing a tendency to be more insulin resistant than controls. However,total cholesterol, LDL and HDL cholesterol, transaminases levels and carotids intima media thickness were similar between patients and controls, what might explained by the fact that the control group was older. To corroborate this, we found a positive correlation between age and carotids intima media thickness. On the other hand, measures of insulin resistance were positively correlated with BMI that was higher in the patients group. Nothing to Disclose: GH, RR, RM, MFMP, FLC

Pub #	P2-260
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	NK3 Receptor Signaling Maintains Tonic Activation of the Hypothalamic-Pituitary-Gonadal Axis
Author String	GL Fraser, Z Khoja, M-O Roy, HR Hoveyda, S Blanc, GS Tannenbaum Euroscreen SA, Gosselies, Belgium; McGill University and Montreal Children's Hospital Research Institute, Montreal, Canada
Body	Missense mutations in TACR and TACR3 (encoding NKB and its receptor NK3, respectively) are found in patients with idiopathic hypogonadotropic hypogonadism [1], whereas administration of NK3 receptor (NK3R) agonists in male monkeys and ewes stimulates lutenizing hormone (LH) release [2,3]. In total, these data implicate NK3R signaling in the modulation of the hypothalamic-pituitary-gonadal (HPG) axis. The aim of the present study was to evaluate the relevance of tonic, endogenous NK3R activation on circulating LH and testosterone (T) levels. ESN-364 is a proprietary, selective, small-molecule NK3 ligand with an *in vitro* pharmacology profile demonstrating high receptor affinity (K_i = 10 nM, radioligand binding assay) and poten antagonist activity (IC_{50} = 40 nM, aequorin assay); both assays were performed on CHO cells expressing the cloned rat NK3R. *In vivo*, intravenous (iv) administration of ESN-364 (20 mg/kg, bolus injection; pharmacokinetic $t_{1/2}$ = 5h) to freely-moving, adult male Sprague-Dawley rats (N=6) significantly inhibited both LH and T plasma levels throughout the 7-h duration of the sampling period. Conversely, iv administration of the selective NK3R peptide agonist, senktide (0.5 mg/kg) and the kisspeptin receptor (a.k.a. GPR54) agonist KP-10 (0.05 mg/kg) increased circulating T 4-fold and 9-fold, respectively. Pre-injection of ESN-364 selectively antagonized senktide-induced T release, but not KP-10 induced T release. This study provides the first demonstration that senktide (iv) increases T in the rat. Moreover, two key conclusions are drawn from this investigation: (1) NK3R activation plays a role in maintaining tonic signaling in the HPG axi based on the finding that exogenous administration of a selective antagonist elicits a profound, sustained decrease in circulating levels of LH and T, and (2) NK3R signaling is positioned either superior to or independent of kisspeptin receptor signaling in the modulation hierarchy of the HPG axis. Our findings suggest that NK3 antagonists can provide a therapeutic alternative to GnRH antagonists in the treatment of conditions where decreased gonadal steroid production is desired. 1. Topaloglu AK et al., (2009) Nat Genet 41: 354 2. Ramaswamy S et al., (2010) Endocrinol 15: 4090 3. Billings HJ et al., (2010) Endocrinol 15: 3836 Disclosures: GLF: Employee, Euroscreen. M-OR: Employee, Euroscreen. HRH: Employee, Euroscreen. SB: Employee, Euroscreen. GST: Researcher, Euroscreen. Nothing to Disclose: ZK

Pub #	P2-261
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Evidence from Studies of the Male Rhesus Monkey (*Macaca mulatta*) for the View That the Action of Neurokinin B to Trigger GnRH Release Lies Upstream from the Kisspeptin Receptor
Author String	S Ramaswamy, SB Seminara, TM Plant University of Pittsburgh, Pittsburgh, PA; Massachusetts General Hospital, Boston, MA
Body	Human genetics have revealed that kisspeptin (KP) and neurokinin B (NKB) signaling are both required for generating pulsatile GnRH release (1-3), and therefore for initiation of puberty and maintenance of adult gonadal function. How these two peptides, which are co-synthesized by the same subset of neurons in the arcuate nucleus (ARC), interact to affect GnRH pulse generation remains a mystery. In the case of KP, evidence is emerging to suggest that intermittent release of this peptide from axonal projections terminating ir the median eminence and originating in ARC is the proximal signal for intermittent GnRH discharges; ie, an intermittent KP signal provides the output of the GnRH pulse generator. To address whether the site of NKB signaling to elicit GnRH release lies upstream to KP, two experiments were conducted using agonadal, juvenile male monkeys. Pituitary responsiveness to GnRH was first heightened by a pulsatile iv infusion of synthetic GnRH in order to use the *in situ* pituitary as a bioassay for GnRH release as described previously (4). In the first experiment (N=4), the ability of Senktide (250 [micro]g), an NKB agonist, to elicit GnRH release was first confirmed, before the KP receptor (KISS1R) was down regulated by a continuous 99 h iv infusion of KP-10 (100 [micro]g/h) also as previously described (5). During the last 4 h of continuous KP-10 infusion, downregulation of KISS1R was confirmed by the failure of an iv bolus of KP-10 (10 [micro]g) to elicit GnRH release. Interestingly, downregulation of KISS1R was associated with a markedly blunted GnRH response to Senktide (peak LH levels of 1.8±0.2 vs 9.3±3.0 ng/ml, cont KP-10 vs vehicle; N=3). The response to Senktide was progressively restored during the first 48 h following termination of continuous KP-10 exposure. An analogous design was employed in the second experiment (N=3) to downregulate the NKB receptor by administration of a continuous 48 h iv infusion of Senktide (200 [micro]g/hr). While a bolus injection of Senktide during the last 4 h of the continuous Senktide administration failed to elicit GnRH release, the ability of KP-10 to stimulate release of GnRH at this time was unimpaired (peak LH levels of 13.0±0.6 vs 10.3±0.2 ng/ml; cont. Senktide vs vehicle). The foregoing findings support the view that NKB stimulation of GnRH release is upstream from KISS1R. While the locus of NKB stimulated release of GnRH remains to be established, KP cell bodies in the ARC are considered the most likely. 1) Seminara et al, 2003 NEJM 349:1614; 2) de Roux et al, 2003 PNAS 100:10972; 3) Topaloglu et al, 2009 Nat Genet 41:354, 4) Plant et al, 2006 Endocrinology147:1007 5) Seminara et al, 2006 Endocrinology 147:2122 Sources of Research Support: HD 008160 (TMP), HD 013254 (TMP) and HD 028138 (SS). Nothing to Disclose: SR, SBS, TMP

Pub #	P2-262
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	The Arrest of GnRH Pulsatility during Infancy That Guarantees the Quiescence of the Primate Gonad during Juvenile Development Is Correlated with a Reduction in Immunopositive Kisspeptin Neurons in the Arcuate Nucleus of the Male Rhesus Monkey (*Macaca mulatta*)
Author String	K Dwarki, S Ramaswamy, RB Gibbs, TM Plant University of Pittsburgh, Pittsburgh, PA; University of Pittsburgh, Pittsburgh, PA
Body	Pulsatile gonadotropin-releasing hormone (GnRH) secretion is increased at the time of puberty, providing the drive to the pituitary-gonadal axis at this stage of development. Interestingly, in primates, robust GnRH pulsatility is also found in infancy. Thus, two hypothalamic postnatal switches control the timing of primate puberty: one to turn off GnRH pulsatility during infancy and the second to reactivate pulsatility late in juvenile development. In man, mutations of GPR54 are associated with hypogonadotropic hypogonadism and delayed or absent puberty. The ligand for GPR54 is kisspeptin (KP), which is encoded by *KISS1* and is expressed in the arcuate nucleus (ARC). In monkeys, expression of *KISS1* and release of KP increase at puberty. KP neurons in ARC also express neurokinin B (NKB), and mutations in this ligand and its receptor also result in hypogonadotropism in man. The present purpose was to begin to determine whether decreased expression of hypothalamic KP and/or NKB underlies the first postnatal switch determining the timing of puberty in the monkey. Six male macaques were used: 5 were castrated within one week of birth to amplify the developmental pattern in GnRH release. LH was measured in weekly blood samples to track the arrest of GnRH pulsatility. Three of these 5 monkeys were killed at 7-8 weeks of age when GnRH pulsatility was robust, and two were perfused at 32 and 42 weeks of age after establishing the arrest of GnRH pulsatility. The sixth animal was castrated at 37 weeks of age and studied 32 weeks later. Serial coronal 25[mu]m hypothalamic sections were prepared, and 1 in every 10 serial sections (ie every 250[mu]m) were stained for KP and NKB using a cocktail of primary antibodies (GQ2[Bloom] at 1:120K[Ciofi] and IS681 at 1:6K, respectively) and the neuropeptides were detected with fluorescently tagged secondary antibodies. KP and NKB perikarya were counted using a fluorescent microscope and mobile stage allowing for systematic scanning of ARC. The number of KP perikarya/section throughout the infant ARC ranged from 50-250, while only 15-150 perikarya/corresponding section were seen in juveniles. NKB was co-expressed in about 20% of KP neurons but demonstrated no developmental change. These findings are consistent with the view that arrest of GnRH pulsatility during infancy, which leads to quiescence of the pituitary-gonadal axis during childhood and juvenile development, is the result of a decrease in activity of KP neurons in ARC. Sources of Research Support: NIH Grants R01 HD013254 and U54 HD008610. Nothing to Disclose: KD, SR, RBG, TMP

Pub #	P2-263
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Studies of the GnRH-Releasing Activities of Intravenously Administered Mutant (KP-P74S) or Wild-Type (KP-54WT) Kisspeptin in the Rhesus Monkey (*Macaca mulatta*)
Author String	S Ramaswamy, LG Silveira, UB Kaiser, AC Latronico, TM Plant University of Pittsburgh, Pittsburgh, PA; São Paulo University Medical School, São Paulo, Brazil; Brigham and Womens Hospital, Boston, MA

Body

A recent clinical finding has linked central precocious puberty with two mutations in *KISS1* (1), the gene encoding kisspeptin (KP), which is expressed by a subset of neurons in the arcuate/infundibular nucleus of the hypothalamus and is a potent secretagogue for GnRH release. One of the two *KISS1* variants has been proposed to generate a mutant peptide, KP-P74S, with resistance to degradation. Specifically, after incubation of KP-P74S or wildtype KP (KP-54WT) in human sera, the mutant retained greater inositol phosphate stimulating activity when tested in a GPR54 expressing cell line than that of the wildtype peptide. In order to test the foregoing hypothesis *in vivo*, we used 4 agonadal juvenile male rhesus monkeys (16-18 months of age 2-4 kg body weight); a phase of primate development during which spontaneous GnRH release is arrested. The pituitary responsiveness to GnRH was heightened by an intermittent iv infusion with synthetic GnRH in order to use LH secretion as a bioassay for GnRH release as previously described (2). In one experiment, we compared dose-responses to KP-P74S or KP-54WT at 3 doses (3, 10, and 30 [micro]g given as a bolus iv injection). To place the responses to these peptides into perspective, the animals were also given a 2 [micro]g bolus iv injection of kisspeptin-10 (KP-10), which is known to elicit an LH discharge in this model that is comparable to that elicited spontaneously in adult castrates (2). It is to be noted that 2 [micro]g KP-10 is approximately equimolar to 10 [micro]g KP-P74S or KP-54WT. Peak mean LH responses to the 3, 10, and 30 [micro]g doses were 3.6±1.2, 12.2±2.4, and 16.4±2.8 ng/ml for KP-P74S and 3.0±0.3, 13.0±2.7, and 14.6±3.7 ng/ml for KP-54WT. KP1-0 at 2 [micro]g induced an LH peak of 9.8±1.4 ng/ml. Vehicle injection was without effect. In a second experiment, the same animals were treated with 5 repetitive bolus iv injections of KP-P74S (10 [micro]g), KP-54WT (10 [micro]g), KP-10 (2 [micro]g) or vehicle. As previously shown for KP-10 (2), this mode of administration elicited a sustained train of GnRH pulses as reflected by the LH profiles, but differences between KP-P74S and KP-54WT were not revealed. Notably, however, KP-10 induced LH discharges were more discrete than those elicited by either KP-P74S or KP-54WT. These results fail to provide evidence that KP-P74S is a more potent *in vivo* GnRH secretagogue than KP-54WT.

1) Silveira et al., 2010 JCEM 95:2276; 2) Plant et al., 2006 Endocrinology 147:1007.

Sources of Research Support: HD 08160 (TMP), HD13254 (TMP), FAPESP grants # 2005/04726-0 (ACL), HD28138 (UBK) and HD61577 (UBK).

Nothing to Disclose: SR, LGS, UBK, ACL, TMP

Pub #	P2-264
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Effects of Chronic Administration of a Metastin Analog, TAK-683, on the GnRH Pulse Generator Activity in Ovariectomized Goats
Author String	Y Wakabayashi, T Yamamura, S Ohkura, T Tanaka, M Kusaka, H Okamura National Institute of Agrobiological Sciences, Tsukuba, Japan; Nagoya University, Nagoya, Japan; Tokyo University of Agriculture and Technology, Tokyo, Japan; Takeda Pharmaceutical Company, Ltd, Tukuba, Japan
Body	Metastin/kisspeptin plays critical roles in the control of gonadotropin-releasing hormone (GnRH) secretion. It has been suggested that metastin neurons in the arcuate nucleus (ARC) participate in the generation of the GnRH pulse generator activity. TAK-683 is a metastin analogue, and chronic administration of the compound potently suppresses testosterone secretion in male rats or pulsatile luteinizing hormone (LH) secretion in goats. To clarify the role of ARC metastin neurons, effects of chronic administration of TAK-683 on the GnRH pulse generator activity was examined in goats. Four ovariectomized goats were implanted with recording electrodes aimed at close proximity of ARC metastin neurons, and multiple unit activity (MUA) was recorded. Characteristic bursts in MUA (MUA volleys) were considered to the electrophysiological manifestation of the GnRH pulse generator. An osmotic pump containing TAK-683 (500 nmol/kgBW/week) was subcutaneously implanted. On one day before and 5 days after the implantation, blood samples were taken for 4 hrs, while monitoring MUA. In addition, the goats received a bolus intracerebroventricular injection of human metastin (10 nmol) at the midpoint of the experimental period on each day. Plasma LH concentrations were determined by RIA. Prior to the TAK-683 treatment, periodic fluctuations of LH secretion (LH pulses) were observed with a constant interpulse interval of approximately 25 min. In each goat LH pulses were invariably associated with MUA volleys, confirming that the MUA volley represents the GnRH pulse generator activity. The injection of human metastin markedly increased LH secretion. After the TAK-683 treatment for 5 days, LH secretion was completely suppressed and no LH pulse was detected, whereas periodic MUA volleys were observed with similar frequency and magnitude as prior to the treatment Although the metastin injection resulted in a slight rise in LH secretion in the TAK-683 treated goat as well, the stimulatory effect of metastin on LH secretion was remarkably reduced. The present results suggest that chronic administration of TAK-683 potently attenuates the responsiveness of GnRH neurons to metastin by acting at the downstream of the GnRH pulse generator. The apparent dissociation between the MUA volley and LH pulse indicates that the MUA volley is not a consequence of activity of GnRH neurons, and suggests that ARC metastin neurons may be the intrinsic source of the GnRH pulse generator activity. Nothing to Disclose: YW, TY, SO, TT, MK, HO

Pub #	P2-265
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Vesicular Glutamate Transporter 2 (VGLUT2) on GnRH Secretory Vesicles
Author String	W Yin, Z Sun, DM Walker, PD Riha, JM Mendenhall, AC Gore University of Texas at Austin, Austin, TX; University of Texas at Austin, Austin, TX; University of Texas at Austin, Austin, TX; Tianjin Medical University, Tianjin, China
Body	The pulsatile release of GnRH is crucial for normal reproductive physiology across the life cycle, a process that is regulated by hypothalamic neurotransmitters. Several studies have shown that GnRH cells are glutamatergic through co-expression of vesicular glutamate transporter 2 (vGluT2), a presynaptic glutamatergic marker. The current study sought to elucidate the relationship between glutamate and GnRH in the nerve terminals in the median eminence, the site of GnRH release into the portal capillary vasculature. We examined the subcellular localization of vGluT2 in neuroterminals. We also determined whether vGluT2-GnRH terminal co-expression may change during reproductive senescence, and whether steroid hormones, which affect responsiveness of GnRH neurons to glutamate, may alter this co-localization. Female Sprague-Dawley rats were ovariectomized at young, middle-aged and old ages (~4, 11, and 22 mo, respectively) and treated 4 weeks later with sequential vehicle + vehicle (VEH + VEH), estradiol + vehicle (E2 + VEH), or estradiol + progesterone (E2+P4) given 48 hrs apart. Rats were perfused 24 hrs after the second hormone treatment. Confocal microscopy was used to determine colocalization and labeling intensity of GnRH and vGluT2 immunofluorescence in the median eminence. Post-embedding immunogold labeling, serial sectioning transmission electron microscopy (ssTEM) and three-dimensional (3D) reconstruction computer analysis was used to determine the ultrastructural site of vGluT2 in the median eminence. Concentrations of LH, estradiol and progesterone in serum were measured. We found that 1) GnRH and vGluT2 immunofluorescent labeling are extensively colocalized in the median eminence. Surprisingly, ssTEM and 3D reconstruction analysis showed that vGluT2 immunogold labeling is associated with virtually all GnRH immunolabeled secretory vesicles, suggesting that GnRH and glutamate may be co-released from neuroterminals. 2) There are no significant changes in GnRH and vGluT2 immunoreactivity in the median eminence although there is a robust age-related decline in pituitary response as viewed by LH serum concentration. This suggests that GnRH terminals are capable of storing GnRH and glutamate despite age and hormone changes. Our results suggest that glutamate may be involved in GnRH release and that they may be co-released. Measures of co-release of GnRH and glutamate, and whether they are altered with age and hormone treatment, are needed. Sources of Research Support: NIH Grant AG16765. Nothing to Disclose: WY, ZS, DMW, PDR, JMM, ACG

Pub #	P2-266
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Characterization of an Immortalized GnRH-GFP Neuronal Cell Line FAC-Sorted from Adult-Derived GnRH-GFP Mouse Primary Culture
Author String	SA McFadden, SS Dhillon, JA Chalmers, M-L Centeno, DD Belsham University of Toronto, Toronto, Canada; University of Toronto, Toronto, Canada; University of Toronto, Toronto, Canada
Body	Gonadotrophin releasing hormone (GnRH) has been widely studied for its involvement in the regulation of reproductive function. Yet, the underlying mechanisms triggering the direct cellular regulation of GnRH neurons are still not completely understood. GnRH cell lines currently available are clonal and have been isolated from hypothalamic tumors, thus it is difficult to judge their overall representation of GnRH neurons *in vivo*. Therefore, our lab has recently generated and immortalized an adult-derived GnRH cell line consisting of the entire population of GnRH neurons from GnRH-GFP mice, wherein primary hypothalamic cultures were isolated from 2-month-old mice. The primary cultures were treated with 10 ng/ml ciliary neurotrophic factor to induce neurogenesis and immortalized using simian virus (SV40) large T antigen with a neomycin resistance gene. Following immortalization and selection, cells were FAC-sorted based on GFP fluorescence with greater than 95% purity. These cells represent the entire population of mHypoA-GnRH/GFP neurons, and have not been further subcloned. Using specific antibodies, the neurons have been examined for GnRH and GFP protein using double label immunocytochemistry to confirm cell phenotype and purity. All neurons in culture co-expressed both GnRH and GFP. ELISA revealed that the cells undergo KCl-induced depolarization to enhance GnRH secretion. Subsequently, RT-PCR was utilized to confirm that the cells endogenously express neural markers. These cells also express Otx2, a putative marker of differentiated GnRH neurons. RT-PCR also confirmed the presence of estrogen receptor β (ER-β), GPR130, GPR54 (Kiss1R), GPR147 (GnIHR), and the active form of the leptin receptor (Ob-Rb). Whether this new line will be a more representative model of the entire adult GnRH neuronal population than the clonal cell lines available is yet to be determined. Studies are underway to determine hormonal regulation of these cells; and we have found that they are responsive to both estrogen and leptin. Although it is still debated whether native GnRH neurons express leptin receptors, we have found the expression of ERs and Ob-Rb in FAC-sorted GnRH-GFP neurons from native GnRH-GFP transgenic mouse hypothalamus. Characterization of the mHypoA-GnRH/GFP cell line is ongoing and this information will be used to analyze the direct hormonal regulatory mechanisms on a model of the entire isolated population of GnRH neurons from the adult mouse hypothalamus. Sources of Research Support: Canadian Institutes for Health Research (CIHR), Canadian Diabetes Association (CDA), Canada Foundation for Innovation (CFI), the Canada Research Chairs (CRC). Nothing to Disclose: SAM, SSD, JAC, M-LC, DDB

Pub #	P2-267
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Hypothalamic Insulin-Like Growth Factor-1 Receptors Are Necessary for Gonadotropin-Releasing Hormone Neuronal Activation during Steroid-Induced LH Surges in Female Rats
Author String	Y Sun, K Thornton, K Kyei, J Shu, AM Etgen, GS Neal-Perry Albert Einstein College of Medicine, Bronx, NY; Albert Einstein College of Medicine, Bronx, NY; College o Staten Island, Staten Island, NY

Body

Brain insulin-like growth factor-1 receptors (IGF-1R) are required for maintenance of estrous cycles and for steroid-induced luteinizing hormone (LH) surges in female rats. Circulating and hypothalamic IGF-1 levels decrease with aging, suggesting a role for IGF-1 in the initiation of reproductive senescence. Our previous studies demonstrated that intracerebroventricular (icv) infusion of the selective IGF-1 receptor antagonist JB- delayed and attenuated the LH surge in young, ovariectomized (OVX) rats primed with estradiol (E2) and progesterone (P). Infusion of IGF-1 also partially restores LH surge amplitude in middle-aged rats. To test the hypothesis that IGF-1Rs are necessary for gonadotropin releasing hormone (GnRH) neuronal activation during steroid-induced LH surges in young rats, and that IGF-1 increases GnRH neuron activation in middle-aged rats, we used osmotic minipumps to infuse JB-1 (100 [micro]g/ml) or IGF-1 (2 [micro]g/ml) icv in OVX young adult (3-4 months) or middle-aged (9-11 months) female rats, respectively. All females were primed with E2 and P and killed in the late afternoon of the expected LH surge. Double label immunohistochemistry for GnRH and c-fos was used to detect activated GnRH neurons in the preoptic area. ANOVA and Tukey's multiple comparison tests were used for statistical analysis of the total number of singly labeled GnRH and c-fos neurons and the percent of doubly labeled GnRH and c-fos neurons. In young females, blockade of IGF-1Rs with JB-1 significantly reduced the LH surge and the percent of GnRH neurons expressing c-fos when compared with controls (51.0±2.59 vs.15.1±4.2%, $P<0.01$). JB-1 infusion also significantly decreased the number of c-fos expressing neurons in the preoptic area under estrogen positive feedback conditions (JB-1: 329.2±54.4 vs. 527.4±77.4, $P<0.05$). Control middle-aged females had significantly fewer GnRH neurons tha expressed c-fos on the day of the LH surge than young females (14.66±4.58 vs. 51.0±2.59 %), and infusion of an IGF-1 dose that should increase LH surge amplitude did not increase the percent of GnRH neurons with c-fos (12.5±4.48%) in this age group. These data suggest that IGF-1R signaling is necessary for GnRH neuronal activation during steroid-induced LH surges. However, the facilitation of LH surges by chronic infusion of IGF-1 in middle-aged rats does not reflect increased GnRH neuronal activation.

Sources of Research Support: Eunice Kennedy Shriver National Institute of Child Health; Human Development/National Institutes of Health through cooperative agreement HD058155 as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Reasearch.

Nothing to Disclose: YS, KT, KK, JS, AME, GSN-P

Pub #	P2-268
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Evidence for Neuroplasticity in Synaptic Inputs to Arcuate Kisspeptin Cells and GnRH Neurons across the Ovine Estrous Cycle
Author String	CM Merkley, LN Coolen, RL Goodman, MN Lehman University of Western Ontario, London, Canada; University of Michigan, Ann Arbor, MI; University of Michigan, Ann Arbor, MI; West Virginia University, Morgantown, WV
Body	Kisspeptin neurons, located in the arcuate nucleus (ARC) and preoptic area (POA), are critical mediators of steroid feedback onto GnRH neurons. Kisspeptin neurons of the ARC are distinguished from those in the POA by their extensive reciprocal connections, as well as their colocalization with dynorphin, neurokinin B (1). Further, recent work in the sheep has also shown that ARC kisspeptin neurons colocalize the vesicular glutamate transporter-2 (vGlut2) and receive glutamatergic inputs (2). The ARC in rodents has long been known to be a site of hormone-induced neuroplasticity, and changes in synaptic inputs to ARC neurons have been demonstrated over the course of the estrous cycle (3). Based on this evidence, we examined possible changes in synaptic inputs to kisspeptin and GnRH neurons across the estrous cycle, using the sheep as a model. Brain sections from gonadal-intact breeding season ewes, perfused during either the luteal (n=4) or follicular (n=4) phase of the estrous cycle, were processed for triple label immunodetection of kisspeptin/vGlut2/synaptophysin, or kisspeptin/vGlut2/GnRH. The total number of synaptic contacts onto ARC and POA kisspeptin neurons were examined, as were the number of reciprocal contacts among ARC kisspeptin cells. In the ARC, the total number of synaptic inputs onto kisspeptin neurons, as well as those that were vGlut2-positive, was significantly higher during the follicular than luteal phase. By contrast, we found no evidence for changes across the estrous cycle in synaptic inputs onto POA kisspeptin neurons. Significant changes were also seen in synaptic inputs to GnRH neurons, specifically an increase in vGlut2-positive inputs to GnRH cells in the POA and dual labeled kisspeptin/vGlut2 inputs to GnRH neurons in the mediobasal hypothalamus (MBH) during the follicular phase. The results suggest that synaptic plasticity, at the level of inputs onto ARC kisspeptin cells and GnRH neurons, may contribute to changes in steroid feedback control of GnRH secretion across the estrous cycle. 1. Goodman et al., Endocrinology 2007, 148 (12): 5752-5760 2. Merkley et al., ICN Abstract 2010, P2-10 3. Naftolin et al., Reproductive Sciences 2007, 14: 101-116 Sources of Research Support: NIH RO1 HD39916 (MNL and RLG). Nothing to Disclose: CMM, LNC, RLG, MNL

Pub #	P2-269
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Homo- and Hetero-Oligomerization and Cellular Signaling of the GnRH-Receptor and GPR54 in Immortalized GnRH Neurons
Author String	P-K Leung, LZ Krsmanovic, KJ Catt PDEGEN, NICHD, NIH, Bethesda, MD
Body	The gonadotropin-releasing hormone receptor (GnRH-R) and G protein-coupled receptor 54 (GPR54), and their endogenous ligand, GnRH and kisspeptin, are essential for activation and regulation of the hypothalamic-pituitary-gonadal axis in mammals. Analysis of RNA extracts from individually identified hypothalamic GnRH neurons and immortalized GnRH neurons (GT1-7) has revealed the expression of GnRH GnRH-R and GPR54 and kisspeptin. Constitutive and an agonist-induced bioluminescence resonant energy transfer (BRET) between the Renilla luciferase (Rluc)-tagged GnRH-R and GPR54 and their peers tagged with green fluorescent protein (GFP), expressed in GT1-7 neurons, revealed homo- and hetero-oligomerization of the two receptors. Activation of endogenous GnRH-R in intact GT1-7 neurons caused a dose-dependent monotonic increase in cytosolic calcium ($[Ca^{2+}]_i$). In GT1-7 neurons transfected with Rluc-tagged GnRH-R, basal and maximal levels $[Ca^{2+}]_i$ were significantly higher in comparison to intact GT1-7 neurons. Also, transition from the dose-dependent monotonic increase of $[Ca^{2+}]_i$, in intact GT1-7 neurons, to a biphasic $[Ca^{2+}]_i$ response was observed in GT1-7 neurons expressing Rluc-tagged GnRH-R, where low GnRH concentrations caused inhibition of $[Ca^{2+}]_i$ and high nanomolar and micromolar GnRH concentrations increased $[Ca^{2+}]_i$. Activation of endogenous GPR54 with kisspeptin-10 (kiss-10) caused a monotonic dose-dependent inhibition of $[Ca^{2+}]_i$ that was not pertussis toxin (PTX) sensitive. In GT1-7 neurons transfected with Rluc-tagged GPR54, treatment with kiss-10 caused a biphasic $[Ca^{2+}]_i$ response. Inhibition of $[Ca^{2+}]_i$ was observed at low nanomolar kiss-10 and stimulatory effect of kiss-10 on $[Ca^{2+}]_i$ was observed at high nanomolar and micromolar kiss-10 concentrations. The inhibitory actions of kiss-10 on $[Ca^{2+}]_i$ in both intact GT1-7 neurons and GT1-7 neurons expressing Rluc-tagged GPR54 were abolished during concomitant activation of the GnRH-R with GnRH. The stimulatory of effect of high kiss-10 concentrations on $[Ca^{2+}]_i$ was potentiated during concomitant treatment with GnRH. In summary, the formation of GnRH-R and GPR54 homo- and hetero oligomers in hypothalamic GnRH neurons, and the modulation of $[Ca^{2+}]_i$ by receptor number and agonist concentration may provide for the fine tuning of hypothalamic GnRH neurons and the regulation of reproductive function. Nothing to Disclose: P-KL, LZK, KJC

Pub #	P2-270
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Colocalization of FM1-43, Bassoon and GnRH-1: Possible GnRH-1 Release from the Cell Body and Their Neuroprocesses
Author String	LC Fuenzalida, KL Keen, E Terasawa University of Wisconsin, Madison, WI; University of Wisconsin, Madison, WI
Body	Gonadotropin-releasing hormone (GnRH-1) release is a Ca2+ dependent process triggered by synaptic and non-synaptic inputs. Despite considerable amounts of information regarding the input, the cellular mechanism of exocytotic events is still unclear. In this study, we examined the exocytotic process using time-lapse image acquisition followed by immunocytochemistry with confocal microscopy. Cultured GnRH-1 neurons derived from monkey embryos were labeled with FM1-43 or a fixable form of FM1-43 (FM1-43Fx) in the presence o absence of depolarization signals and changes in vesicles labeled with FM1-43 were analyzed. The results showed FM1-43 was taken up into the cell forming puncta in the soma and neuroprocesses in the absence of depolarization signals, indicating that GnRH-1 neurons were spontaneously active. Depolarization of GnRH-1 neurons with high K+ or veratridine challenge increased the intensity and size of puncta in both soma and neuroprocesses with a similar timing, and the veratridine-induced changes in puncta were blocked by TTX, indicating that changes in the puncta intensity and size reflect neurosecretion. Subsequent double immunocytochemistry for GnRH-1 and the synaptic vesicle marker, vesicle-associated membrane protein (VAMP), demonstrated that the FM1-43 labeled puncta were indeed synaptic vesicles with the GnRH-1 peptide. Additional double immunocytochemistry for GnRH-1 and the marker of the neurosecretory active zone, Bassoon, indicated that the FM1-43 labeled puncta were located at the sites of active exocytosis in GnRH-1 neurons. Collectively, the results suggest that GnRH-1 neurons have a capacity to release the peptide from the soma and neuroprocesses and may play a role in synchronized GnRH-1 activity. Sources of Research Support: NIH grants HD15433 and HD11355 awarded to ET. Nothing to Disclose: LCF, KLK, ET

Pub #	P2-271
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Estradiol and Endogenous Circadian Clock Activation of GPR54 Transcription in GnRH-Secreting GT1-7 Cells
Author String	KP Tolson, CP Goodall, A Patron, KJ Tonsfeldt, PE Chappell Oregon State University, Corvallis, OR
Body	Ovulation requires surges of gonadotropin-releasing hormone (GnRH) released from specialized hypothalamic neurons. Surge release is mediated by ovarian estradiol (E2) positive feedback, which stimulate robust increases in GnRH secretion, confined to proestrus afternoon, implicating the influence of a circadian clock. Endogenous circadian clocks, comprised of transcriptional feedback loops, are present in a portion of native GnRH neurons and immortalized GT1-7 cells. We are investigating the role of the circadian clock in GnRH surge generation, and what effects E2 exerts on gene expression timing in GnRH neurons. We demonstrated that >24h exposure to 100pM E2 elicits dramatic increases in GnRH secretion from GT1-7 cells in static incubation and perifusion. Also, we found that E2 exposure induces gene and protein oscillations of the Kisspeptin receptor in GT1-7 cells, inducing peaks of sensitivity to Kiss-1 with a circadian period. Kiss-1 is an essential part of the reproductive axis, stimulating GnRH secretion through its receptor, GPR54. We observed that GPR54 expression levels in GT1-7 cells oscillate over time, but only robustly in the presence of elevated E2, and that amplitude of clock oscillations can be modulated by E2. We have observed that clock transcription factors CLOCK and BMAL1 regulate GPR54 expression in GT1-7 cells using a luciferase reporter, and are exploring to what extent this regulation is modulated by E2. To further explore the role of the clock in E2-stimulated GnRH release, we also created subcloned GT1-7 cell lines overexpressing the mutant form of CLOCK, CLOCK-[Delta]19, a dominant negative disruptor of clock function. Clock gene expression and protein patterns are blunted in these cells, and GPR54 expression is constitutively elevated and arrhythmic. Interestingly, constitutive overexpression of CLOCK-[Delta]19 results in significantly altered GnRH secretion from perifused cells exposed to long-term E2, and *decreases* GnRH secretion in response to Kiss-1, suggesting that endogenous clocks are important for direct effects of E2 on GnRH secretion. These results support a model in which E2 positive feedback induces changes in receptor expression patterns by coupling to endogenous oscillators, thus increasing GnRH sensitivity to Kiss-1 timed to occur prior to the period of optimal sexual receptivity. Elevated ovarian E2 may thus increase kisspeptidergic tone while also increasing GnRH neuronal sensitivity for maximal surge release. Sources of Research Support: R01 HD065331. Nothing to Disclose: KPT, CPG, AP, KJT, PEC

Pub #	P2-272
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Essential Role of PI3K p110α Isoform in GnRH Neuron Migration and Olfactory Bulb Neurite Outgrowth
Author String	Y Hu, S Poopalasundaram, PM Bouloux UCL Medical School, Royal Free Campus, London, UK
Body	FGF signalling is essential in olfactory bulb (OB) morphogenesis and GnRH neuron fate specification, migration, axonal extension and targeting. Defective FGFR1 signalling causes idiopathic hypogonadotropic hypogonadism either with normal sense of smell (nIHH) or in association with anosmia (Kallmann syndrome, KS). Mutations in anosmin-1, FGFR1, FGF8, and other KS genes associated with FGFR1 signalling account for 20-30% of KS cases. However, the signalling pathway downstream of FGFR1 specific for both olfactory and GnRH system development is hitherto unknown. PI3K is one of three typical signalling pathways responding to FGFR1 activation. Class I PI3K are heterodimers containing α, β, γ, and [delta] isoforms in p110 catalytic subunit. Using pharmacological PI3K isoform specific inhibitors we found that anosmin-1 induced human embryonic GnRH neuroblast (FNC-B4) migration occurred specifically via the PI3K p110α isoform. Anosmin-1 and FGF2 induced phosphorylation of the PI3K downstream effectors, Akt and GSK-3β, in an α isoform dependent manner. PI3K-dependent migration of GnRH neurons was further confirmed in explant cultures of olfactory placodes from E4 chick embryos in which GnRH neuron migration was blocked by LY294002, a broad-spectrum PI3K inhibitor. Furthermore, neurite outgrowth and elongation of OB neurons in OB explant cultures from E10 chick embryos was also dependent on the PI3K p110α isoform. Taken together, these observations strongly suggest that the PI3K p110α isoform is a key signalling pathway downstream of FGFR1 in both GnRH neuron ontogeny and OB development. Nothing to Disclose: YH, SP, PMB

Pub #	P2-273
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Effects of Metastin/Kisspeptin Analog, TAK-683, on Luteinizing Hormone Secretion in Peripheral Plasma, and Gonadotropin-Releasing Hormone Secretion in the Pituitary Portal Circulation in Goats
Author String	S Ohkura, T Tanaka, T Kuroiwa, Y Wakabayashi, T Ohtaki, M Kusaka, H Okamura Nagoya University, Nagoya, Japan; Tokyo University of Agriculture and Technology, Fuchu, Japan; National Institute of Agrobiological Sciences, Tsukuba, Japan; Takeda Pharmaceutical Company Limited, Tsukuba, Japan
Body	Metastin/Kisspeptin is a potent secretagogue of gonadotropin-releasing hormone (GnRH)/luteinizing hormone (LH) secretion. The present study aims to determine if a metastin/kisspeptin analogue, TAK-683, has a stimulatory effects on LH secretion in peripheral plasma and GnRH release in the pituitary portal circulation in castrated male or ovariectomized female goats. In experiment 1, castrated (n=5) or ovariectomized (n=5) goats were received a bolus intravenous (iv) injection of TAK-683 (3.5 pmol/head - 3.5 nmol/head). A single injection of TAK-683 stimulated LH secretion in a dose-dependent manner in both castrated and ovariectomized goats. The effective dose (3.5 nmol) of TAK-683 was much lower than that (380 nmol) of the metastin/kisspeptin C-terminal active decapeptide, kisspeptin-10 (1), indicating that TAK-683 is a potent analogue to induce LH secretion. In experiment 2, portal blood sampling techniques were applied to monitor change in GnRH release in pituitary portal circulation. A bolus iv injection of TAK-683 (35 nmol/head) strongly stimulated GnRH release in castrated male goats (n=6). The duration of elevated GnRH/LH secretion lasted for more than 4 hours after the injection. This observation provides direct evidence that TAK-683 induces LH secretion by stimulating hypothalamic GnRH release. In experiment 3, a chronic application of TAK-683 was achieved by subcutaneous implantation of an osmotic pump containing TAK-683 solution (50 nmol/kg BW/week) and pulsatile GnRH/LH secretion was examined in castrated male goats (n=5). By contrast to a bolus iv injection of TAK-683, a chronic treatment of this analogue for 5 to 6 days caused severe suppression of GnRH and LH pulses. This [ldquo]paradoxical effect[rdquo] on GnRH/LH secretion was achieved by 3 days after the initiation of TAK-683 application. The present findings demonstrate that TAK-683 is a potent secretagogue of GnRH/LH secretion and that continuous exposure to this metastin/kisspeptin analogue attenuates the responsiveness of GnRH neurons, which in turn decreases LH secretion. These series of experiments using a metastin/kisspeptin analogue suggests that endogenous metastin/kisspeptin is a potent stimulator of LH secretion via GnRH release from the hypothalamus and would be critical for the control of reproductive functions in goats as in other mammalian species. (1) Ohkura S et al., J Neuroendocrinol 2009; 21:813 Nothing to Disclose: SO, TT, TK, YW, TO, MK, HO

Pub #	P2-274
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Transcriptional Regulation of the Mouse GnRH Gene by Kisspeptin
Author String	HJ Novaira, D Fadoju, S Radovick Johns Hopkins University School of Medicine, Baltimore, MD
Body	**Introduction:** Kisspeptins, as well as their GPR54 receptor, have been shown to be key components in the regulation of GnRH secretion. In vitro studies have demonstrated an increase in GnRH gene expression associated with an elevated secretory response to kisspeptin administration. However, the cellular targets and intracellular mechanisms mediating kisspeptin effects in the central reproductive axis are unclear. Objectives: 1) To identify specific regions of the mouse GnRH (mGnRH) promoter that mediate kisspeptin action on GnRH gene expression in neuronal cell lines and transgenic mice. 2) To determine which transcriptional factors mediate kisspeptin action on target gene expression. **Methods and Results:** Transient transfection studies in GT1-7 and Gn11 cells using sequential deletions of the mGnRH gene promoter have demonstrated that kisspeptin is able to significantly increase LUC activity when cells were treated with 10^{-9}M kisspeptin for 4h (*n*=5, *p*[le]0.01), thus localizing a kisspeptin-response element between -3446 bp and -2806 bp of the mGnRH gene upstream from the transcription start site. In addition, transgenic mice containing sequential deletions of the mGnRH gene promoter linked to the luciferase reporter demonstrated that kisspeptin (1nmol via IP) treatment also increased LUC activity in the hypothalamus of male and female mice by 2-fold (*n*=3, *p*[le]0.01) and again a kisspeptin-response element was located between -3446 bp and -2806 bp of the mGnRH gene. Furthermore, analysis of this region have localized an OTX-2 transcription factor binding site within this element. Additionally, 10^{-9}M kisspeptin treatment of GT1-7 cells increased mRNA levels of OTX-2 in a time-dependent manner; induced opening of chromatin at the mGnRH promoter documented by the FAIRE assay, and increased binding of OTX-2 to this region documented by the ChiP assay. **Conclusion:** These in vitro and in vivo studies demonstrate that the elements between -3446 bp and -2806 bp of the GnRH gene are responsible for GnRH induction by kisspeptin. In addition, we show for the first time that the OTX-2 transcription factor is regulated by kisspeptin, and mediates the transcriptional response of the mGnRH gene to kisspeptin. Considering the critical nature of kisspeptin induction of GnRH for pubertal development and successful reproduction, kisspeptin not only induces secretion of GnRH, but we propose also increases GnRH gene expression to potentially replenish peptide stores. Nothing to Disclose: HJN, DF, SR

Pub #	P2-275
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Seasonal Variation in GnRH Response to Kisspeptin in Sheep: Possible Kisspeptin Regulation of the Kisspeptin Receptor
Author String	Q Li, A Rao, IJ Clarke, JT Smith Monash University, Melbourne, Australia
Body	The *Kiss1* gene encodes the kisspeptin family of peptides, the endogenous ligands for the kisspeptin receptor, Kiss1r (GPR54). The kisspeptin/Kiss1r system in the hypothalamus appears critical for the onset of puberty and has a major role in driving the reproductive axis. In sheep, reproduction is seasonal, being activated by short-days and inhibited by long days. During the non-breeding season, gonadotropin-releasing hormone (GnRH) and gonadotropin secretion is reduced due to these effects of photoperiod. We recently showed expression of Kiss1 mRNA in the brain is reduced during the non-breeding season, but the LH response to kisspeptin during this time was greater. To determine whether the GnRH response to kisspeptin is increased during the non-breeding season, we utilized hypophysial portal blood sampling. Ewes were studied in the non breeding and breeding (luteal phase of the estrous cycle) seasons and received kisspeptin (50 [mu]g YNWNSFGLRY-NH_2) or vehicle (iv). Paired portal and jugular blood samples were collected every 10 min for 2 h before and 2 h after kisspeptin treatment. The GnRH and LH responses to kisspeptin were greater ($P<0.05$) in non-breeding season ewes. To ascertain whether this difference reflects a change in Kiss1r, we measured its expression on GnRH neurons using double-label *in situ* hybridization (ISH). The majority of GnRH cells expressed Kiss1r and the level of expression was greater during the non-breeding season vs breeding season. To further examine the mechanism underlying the change in Kiss1r, we examined Kiss1r/GnRH expression in ovariectomized (OVX) ewes (controlling for sex steroids) during breeding and non-breeding seasons as well as in OVX non-breeding season ewes with or without estrogen replacement. In both these experiments, the level of Kiss1r expression on GnRH neurons was unchanged. Finally, we examined the effect of kisspeptin treatment on Kiss1r. OVX ewes in the non-breeding season were given kisspeptin (5 [mu]g/h for 20 h) or vehicle via icv infusion and brains prepared for ISH. Kiss1r expression on GnRH neurons was reduced ($P<0.05$) by kisspeptin treatment. These studies indicate that the kisspeptin response is indeed greater during the non-breeding season and this may in part be due to increased Kiss1r expression on GnRH neurons. Interestingly, we show that kisspeptin may regulate the expression of its own receptor. Sources of Research Support: Australian NHMRC. Nothing to Disclose: QL, AR, IJC, JTS

Pub #	P2-276
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Axl and Met: Crosstalk of Tyrosine Kinase Receptor Signaling in GnRH Neurons
Author String	S Salian Mehta, M Xu, M Schaller, ME Wierman University of Colorado Denver, Aurora, CO; Veterans Affairs Medical Center, Denver, CO
Body	Gonadotropin releasing hormone (GnRH) neurons undergo a precise migration from the olfactory placode int the forebrain during embyrogenesis. Disruption of migration or survival of these neurons results in abnormal sexual maturation in mice and humans. Reproductive defects observed in Axl/Tyro3 null mice in our earlier studies showed the significance of Gas6/Axl/Tyro3 pathway to mediate GnRH neuronal migration, survival and *in vivo* fertility. The cytokine hepatocyte growth factor (HGF) activates its tyrosine kinase receptor Met and plays a role in olfactory axon outgrowth and GnRH neuron development. Microarray analysis revealed that NLT migratory GnRH neurons express higher levels of Met transcripts (734 versus 18) and HGF (450 versus 28) as compared to GT1-7 post-migratory cells. Since both Axl and Met are activated by heparan sulfate proteoglycans tethered ligands, Gas6 and HGF, respectively, we postulated that their pathways may interact. Gas6/Axl induces GnRH neuronal cell survival via ERK MAP kinase and PI3 kinase to Akt signaling. Similar pathways are activated downstream of HGF/Met signaling in other systems. Thus, we hypothesized that Axl may cross-talk with Met to mediate survival and/or migration. In NLT GnRH neurons, Met protein was detected by immunoprecipitation and Axl was detected by co-immunoprecipitation suggesting that the two tyrosine kinase receptors, Axl and Met directly interact. Of interest, Tyro3 was not detected in co-immunoprecipitation assays with Met, suggesting the receptor interaction is specific for Axl and Met. HGF activated Met phosphorylation and downstream effectors ERK and Akt in NLT GnRH neurons To ask if Gas6 or HGF may activate each other's receptor, we silenced Axl in NLT GnRH neurons and asked if HGF activation of Met was altered. Axl silencing (50-80% by siRNA) resulted in a decreased ability of HGF to phosphorylate Met and Akt as compared to the scrambled controls (12.3 and 0.8 fold respectively), with no change in ERK phosphorylation, suggesting a pathway specific interaction. In contrast, Gas6 did not cross-phosphorylate Met. Together, these data suggest that Axl and Met to directly heterodimerize and that HGF signaling may be modulated by Axl in early GnRH neuron development. Studies are ongoing to map the downstream components of the signaling pathways to impact GnRH neuronal cell migration and survival. Sources of Research Support: NIH Grant R01 HD031191-14A2 to MEW. Nothing to Disclose: SSM, MX, MS, MEW

Pub #	P2-277
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Spectrum of Phenotypes Associated with Various Levels of Mutational Burden in Humans with Isolated GnRH Deficiency Due to Defects in the *GNRHR* Gene
Author String	E Gianetti, JE Hall, MG Au, L Plummer, R Quinton, JA Stewart, DL Metzger, N Pitteloud, V Mericq, PM Merino, LL Levitsky, L Izatt, M Lang Muritano, RG Dluhy, WF Crowley, Jr, SB Seminara Massachusetts General Hospital, Boston, MA; Institute for Human Genetic Medicine - University of Newcastle-upon-Tyne, Newcastle-upon-Tyne, UK; International Centre for Life, Newcastle-upon-Tyne, UK; British Columbia's Childrens' Hospital, Vancouver, Canada; Le CHUV, Lausanne, Switzerland; Faculty of Medicine - University of Chile, Santiago de Chile, Chile; Faculty of Medicine - University of Chile, Santiago de Chile, Chile; Massachusetts General Hospital, Boston, MA; Guy's Hospital, London, UK; University Children's Hospital, Zurich, Switzerland; Brigham and Women's Hospital - Harvard Medical School, Boston, MA
Body	Introduction: *GNRHR* mutations have been identified in individuals with a broad spectrum of GnRH deficient phenotypes. In this study, the number of affected alleles & the functional severity of *GNRHR* mutations were hypothesized to correlate with the severity of the reproductive phenotype. Subjects: 863 probands with GnRH deficiency (375 normosmic hypogonadotropic hypogonadism [nIHH], 360 Kallmann syndrome [KS], 51 constitutional delay of puberty [CDP], 77 hypothalamic amenorrhea [HA]). 46 family members & 422 healthy controls were screened for mutations in *GNRHR*. Sequence variants were categorized according to previously published *in vitro* studies or *in silico* prediction programs. Probands & family members harboring [ge] 1 deleterious variant(s) were divided in 4 groups: G1 = both alleles carry a complete loss of function (LOF) mutation (n = 4); G2 = 1 allele has a complete LOF, & the other a partial LOF mutation (n = 4); G3 = both alleles carry partial LOF mutations (n = 10); and G4 = only 1 allele carries mutation(s) (complete or partial LOF, n = 52). Results: The prevalence of heterozygous nucleotide variants was significantly different between probands and controls (2.5% vs. 0.5%, $p < 0.01$). All 18 patients belonging to G1, G2, or G3 (biallelic mutations) presented with nIHH, except for 1 patient with KS. Severe mutations were associated with complete GnRH deficiency & less disabling mutations were identified in patients with fertile eunuch syndrome & reversible hygogonadotropism. Three women had an abnormal response to either pulsatile GnRH or exogenous gonadotropins, & one woman had 5 miscarriages. In contrast, the 52 subjects of G4 (monoallelic mutations only) demonstrated a wide-range of phenotypes including KS, CDP, HA, adult onset IHH & normal reproductive function. Conclusions: Mutation burden in *GNRHR* is not predictive of GnRH deficient sub-phenotypes. While the severity of biallelic mutations does appear to correlate with the hypogonadotropic phenotype spectrum, monoallelic mutations are not associated with milder disease. Rather, heterozygous patients demonstrate a paradoxically wide spectrum of GnRH deficient states, suggesting the presence of as yet unidentified genetic factors (oligogenicity) or environmental triggers working in combination with mutated *GNRHR* alleles. Moreover, *GNRHR* mutations are present in patients with abnormal ovarian responsiveness and multiple miscarriages, suggesting a role for GNRHR beyond the level of the pituitary. Nothing to Disclose: EG, JEH, MGA, LP, RQ, JAS, DLM, NP, VM, PMM, LLL, LI, MLM, RGD, WFC , SBS

Pub #	P2-278
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Mutations in the FGF8 Genetic Network Underlie a Large Proportion of Isolated Human GnRH Deficiency
Author String	H Miraoui, B Feng, G Sykiotis, L Plummer, R Quinton, V Hughes, VV Guy, JP Chanoine, WF Crowley, Jr, A Dwyer, Y Sidis, M Mohammadi, N Pitteloud Massachusetts General Hospital, Boston, MA; University of Lausanne - Le CHUV, Lausanne, Switzerland; University of Patras Medical School, Patras, Greece; University of Newcastle, Newcastle, UK; CHU Sainte-Justine - University of Montreal, Montreal, Canada; British Columbia Children's Hospital, Vancouver, Canada; New York University School of Medicine, New York, NY
Body	**Background:** The discovery of FGFR1c and FGF8 mutations in patients with isolated GnRH deficiency (diagnosed as idiopathic hypogonadotropic hypogonadism, IHH) has demonstrated the critical role of FGF8 signaling in GnRH neuron ontogeny. The timing, duration, and location of FGF8 signaling are tightly regulated during development and are modulated by a large number of co-expressed proteins (the [ldquo] FGF8 syn-expression group[rdquo]). These include inhibitors, enhancers, co-ligands, and heparin sulfate-modifying enzymes. We hypothesized that this conserved genetic network as a whole could underlie IHH. **Methods:** We screened the coding sequences and intron-exon boundaries of 6 genes in the FGF8 synexpression group (the inhibitors SEF, SPROUTY4, and DUSP6; the enhancer FLRT3; the ligand FGF17, and the heparin sulfate-modifying enzyme HS6OST1 in 400 well-phenotyped IHH patients and 200 ethnically-matched controls. The 3 genes, FGFR1, KAL1, and FGF8, members of the FGF8 synexpression group were previously screened in the same cohort of patients and controls, (Sykiotis et al 2010 PNAS). Rare variants were defined as occurring in < 1% of controls. The potential damaging effect of each variant was evaluated using prediction programs, structural modeling, previously established functional assays, or in vitro functional studies for novel IHH genes. **Results:** As many as 29% of IHH patients harbored a rare variant within the FGF8 syn-expression group; as such, <50% of IHH cases remain without a known gene defect. Moreover, of the patients harboring a mutation within these 9 genes, 16 (12%) had an additional rare variant within the FGF8 synexpression group. The identified KAL1, FGFR1, and FGF8 mutants have been previously characterized (Sykiotis et. al, 2010 PNAS). The SEF mutants (p.P306S, p.Y379C, p.S468L, p.P577A) were loss-of-function in expression studies, transcripitional assays, and maturation assays. The FGF17 mutants (p.I108T, p.R177H) were loss-of-function in structural modeling and transcriptional assays. All 5 HS6OST1 mutants (p.R296W, p.R296Q, p.R313Q, p.R372W, p.M394V) (p.P306S, p.Y379C, p.S468L, p.P577A) were loss-of-function based on reduced HS 6O-sulfotransferase activity in vitro. Ongoing studies are characterizing functionally the SPROUTY4, DUSP6, and FLRT3 mutants. **Conclusion:** The FGF8 synexpression group is rich in novel IHH loci, with interacting mutations contributing to disease in an oligogenic architecture. Sykiotis et al., 2010;15140-4. Sources of Research Support: National Institutes of Health Grants U54 HD028138, HD015788-23, HD056264, and GM061354. Nothing to Disclose: HM, BF, GS, LP, RQ, VH, VVG, JPC, WFC, AD, YS, MM, NP

Pub #	P2-279
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Olfactory Function in GnRH Deficiency Displays a Phenotypic Spectrum: Potential Implications for Genetic Screening and Pathophysiology
Author String	HM Lewkowitz-Shpuntoff, R Balasubramanian, V Hughes, L Plummer, MG Au, SB Seminara, N Pitteloud, RL Doty, WF Crowley Massachusetts General Hospital, Boston, MA; University of Pennsylvania, Philadelphia, PA; University of Lausanne, Lausanne, Switzerland
Body	Context: Humans with isolated Gonadotropin-Releasing Hormone (GnRH) deficiency characteristically present as Kallmann syndrome (KS) when anosmic or when normosmic as Idiopathic Hypogonadotropic Hypogonadism (nIHH). However, the true spectrum of olfactory function in these disorders and their underlying genetic architecture is unclear. Objective: Define the olfactory function and genetic etiologies in a large series of GnRH deficient subjects and compare to a large base of age- and sex-appropriate normals with standard olfactory testing that is widely available as part of a standardized phenotyping effort. Design: The standardized 40-item University of Pennsylvania Smell Identification test (UPSIT) using large numbers of age- and gender-based normative UPSIT data and mutational screening for KAL1, PROK2, PROKR2, FGFR1, FGF8, KISS1R, GNRHR, and NELF were performed in a population of GnRH deficient subjects. Patients: 287 GnRH deficient patients were tested (85F/202M; ages 15- 54 years) and age- and sex-matched random population (1,172 F/1,011M) from the U.of Pennsylvania Smell & Taste Center served as control subjects. Results: Rather than being bimodal in their olfactory function, GnRH deficient subjects displayed a continuous spectrum of olfactory abilities (Anosmia: 31 %: Hyposmia: 34%: Normosmia: 35%) with a distribution of olfactory function that was significantly different from controls and defined by SD scores (Anosmia: 0.5%: Hyposmia:10.7%: Normosmia 88.8%, [chi][sup2] test, p= 0.001). When expressed as age and gender-matched olfactory function percentiles, the majority (90%) of GnRH deficient subjects tested below the 50th centile of age and sex adjusted normals. Mutations in PROK2 and KAL1 were only seen in subjects testing below the 15th centile while all subjects with FGFR1 mutations tested <35th centile. In contrast, mutations in PROKR2, FGF8, GnRHR and NELF were seen across multiple olfactory centiles. Conclusions: 1) Contrary to some prior studies, olfactory function in GnRH deficiency is not a bimodal trait but rather a continuing spectrum including a significant hyposmic phenotype; 2) The majority of GnRH deficient patients demonstrated some degree of impaired olfaction; 3) Accurate olfactory function assessment may enable targeted genetic screening in GnRH deficiency; and 4) These findings may have implications for the biologic defects underlying defective GnRH neuronal network development and function. Disclosures: RLD: Consultant, Sensonics Inc. Nothing to Disclose: HML-S, RB, VH, LP, MGA, SBS, NP, WFC

Pub #	P2-280
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Defects in Genetic Pathways Controlling Hypothalamic Differentiation Are Correlated with GnRH1 Cell Loss
Author String	H Kim, C Maturana, KE Whitlock Cornell University, Ithaca, NY; Universidad de Valparaiso, Valparaiso, Chile
Body	Failure of reproductive function in human Kallmann Syndrome is due to deficits in gonadotropin-releasing hormone (GnRH) and can be associated with anosmia (loss of sense of smell). To date mutations in several known genes result in Kallmann Syndrome including *anosmin1* (KAL1) and *fibroblast growth factor receptor 1*, (*fgfr1*; KAL2). Previously we demonstrated that loss of endocrine GnRH cells is correlated with loss of the anterior pituitary and not olfactory organs. Through lineage tracing we show that, like in other vertebrates, the anterior hypothalamus and anterior pituitary arise from adjacent tissues. Thus the loss of GnRH endocrine cells of the hypothalamus in Kallmann Syndrome patients may result from to malformation of the hypothalamus during early development. We have disrupted the function of the *kal1a* and *fgfr1* genes using morpholinos (MO) in zebrafish. Strikingly we find that in *kal1a* and *fgfr1* MO treated embryos *oxytocin-like* expression is decreased or absent. Additionally, the hypothalamus is present but malformed as judged by *otp* gene expression. Thus, decrement in *kal1a* (KAL1) and *fgfr1* (KAL2) function results in the loss of two hypothalamic cells types: GnRH cells and *oxytocin-like* cells, suggesting that defects in endocrine GnRH cell development, like those observed in Kallmann patients, may result from disruptions in the development of the hypothalamus. Clinical studies have shown that adult males suffering from hypogonadic-hypogonadism (HH) can regain GnRH function after hormone treatment, raising the question of the origin of the endocrine GnRH secreting cells in adults. Our data, and recent studies culturing cells of the hypothalamus (1,2), suggest that there are GnRH progenitors in the hypothalamus. Currently we are developing an *in vivo* tissue culture system to test whether treatments analogous to those used in HH patients can trigger differentiation of GnRH cells in the hypothalamus of adult brains. (1) Salvi R et al., PLoS One 2009;4(2):e4392. (2) Markakis EA et al., J Neurosci.2004; Mar 24;24(12):2886-97 Sources of Research Support: NIH/ HD050820; FONDECYT 1071071; Center for Genomics of the Cell, Millenium Science Initiative Program awarded to KEW. Nothing to Disclose: HK, CM, KEW

Pub #	P2-281
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	Neuronendocrine Regulation of Kisspeptin and Gonadotropin-Releasing Hormone Gene Expression in Goldfish Brain Cell Culture
Author String	C Lin, C Sun, W Ko, A Wong The University of Hong Kong, Hong Kong, China
Body	Kisspeptin (Kiss) plays a key role in reproductive functions mainly by regulating gonadotropin-releasing hormone (GnRH) secretion in the hypothalamus. However, the evidence for Kiss induction of GnRH expression is restricted to mammals, which is largely based on whole animal experiments and *in vitro* studies of GnRH neuronal cell lines. To our knowledge, no attempt has been made to examine the direct effects of Kiss on GnRH expression in primary culture of brain cells. Recently, two Kiss isoforms, Kiss1 and Kiss2, have been identified in goldfish and Kiss1 was shown to induce luteinizing hormone (LH) secretion and gene expression at the pituitary level. To further investigate Kiss and GnRH regulation in the central nervous system (CNS) in fish model, primary cultures of brain cells composed mainly of neuronal cells and glial cells were prepared from male and female goldfish, respectively. Treatment of these brain cell cultures with human chorionic gonadotropin (hCG) reduced Kiss1 and Kiss2 mRNA levels with concurrent drop in salmon GnRH (sGnRH) but not chicken GnRH -II (cGnRH-II) transcript expression. The inhibitory effects of hCG on Kiss1 Kiss2 and sGnRH gene expression was mimicked by LH but not follicle-stimulating hormone. In brain cell cultures prepared from female fish, Kiss1 and Kiss2 were both effective in elevating sGnRH but not cGnRH-II mRNA levels, and these stimulatory actions could be blocked by hCG. In parallel studies, transcript expression of Kiss1, Kiss2 and sGnRH could be induced by the neuropeptide pituitary adenylate cyclase (AC -activating polypeptide (PACAP) and these stimulatory effects were mimicked by the AC activator forskolin and cAMP analog CPT-cAMP but abolished by hCG treatment. These results, as a whole, provide evidence that (i) Kiss induction of GnRH expression can be observed in fish model, (ii) PACAP may serve as a novel stimulator of sGnRH expression in female goldfish via up-regulation of Kiss1 and Kiss2 gene expression through cAMP-dependent pathways, and (iii) LH produced at the pituitary level may exert a [ldquo]short-loo feedback[rdquo] in female fish to reduce sGnRH production in the hypothalamus via blockade of Kiss1/Kiss2- and PACAP-induced sGnRH gene expression. Sources of Research Support: Grants from Research Grant Council, Hong Kong. Nothing to Disclose: CL, CS, WK, AW

Pub #	P2-282
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Gonadotropin-Releasing Hormone (1:30 PM-3:30 PM)
Title	The Prevalence of Digenic Mutations in Patients with Hypogonadotropic Hypogonadism and Kallmann Syndrome
Author String	SD Quaynor, H-G Kim, EM Cappello, LP Chorich, LC Layman Medical College of Georgia, Augusta, GA; Medical College of Georgia, Augusta, GA; Medical College of Georgia, Augusta, GA
Body	Patients with idiopathic hypogonadotropic hypogonadism (IHH) are characterized by delayed puberty with low sex steroids and low gonadotropins--follicle stimulating hormone (FSH) and luteinizing hormone (LH). When IHH occurs in association with anosmia, it is termed Kallmann Syndrome (KS). The molecular basis of IHH/KS is known for 30-40% of patients, and at least 17 causative genes are known, mostly affecting GnRH migration and/or GnRH action. Recent evidence indicates that digenic, or even oligogenic, mutations may occur, but the prevalence taking into account the most commonly involved genes is unknown. The purpose of the present study was to determine the prevalence of digenic mutations in IHH/KS. Two groups of IHH/KS patients were studied: 24 patients with one known mutation in an IHH/KS gene (Group 1); and 23 patients without any previously known mutation (Group 2). The protein coding and splice junctions for 12 known IHH/KS genes (KAL1, GNRHR, FGFR1, KISS1R, FGF8, TAC3, TACR3, CHD7, PROKR2, PROK2, GNRH1, and WDR11) were subjected to PCR-based capillary DNA sequencing. In Group 1, 7/24 (29%) had a putative mutation in a second gene. For the 23 individuals without a known mutation in Group 2, no patient had a mutation in two genes, although 13 (57%) had a mutation in one gene. None of the mutations were seen in controls or the SNP database. The overall prevalence from our study revealed that 7 of 47 (15%) IHH/KS patients had mutations in more than one gene. These findings indicate that although mutation in more than one gene can occur in IHH/KS, most cases were monogenic, as determined by sequencing of 12 known, common genes. Sources of Research Support: LCL funded by NICHD grant HD33004. Nothing to Disclose: SDQ, H-GK, EMC, LPC, LCL

Pub #	P2-283
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Genetically Mediated Reversal of Obesity by Eutopic Proopiomelanocortin Expression Is Associated with Normalization of Food Intake and Locomotor Activity but Not Oxygen Consumption
Author String	M Yamashita, V Otero-Corchon, VF Bumaschny, M Rubinstein, MJ Low University of Michigan, Ann Arbor, MI; Consejo Nacional de Investigaciones Cientificas y Técnicas, Buenos Aires, Argentina
Body	Body weight is remarkably stable under normal physiological conditions, suggesting the existence of an intrinsic set-point. We generated a reversible genetic mouse model of early onset obesity to examine the role of brain proopiomelanocortin (POMC) peptides in the set-point mechanism. Mice selectively lacking Pomc expression in hypothalamic arcuate (ARC) neurons (ARC-PomcKO) were crossed with transgenic mice expressing a tamoxifen-inducible Cre recombinase. Compound ARC-PomcKO:Cre-ERT mice are hyperphagic and obese due to central melanocortin deficiency. The mice recover expression of ARC Pomc after tamoxifen (50 mg/kg i.p. x 5 daily doses) to similar levels regardless of their age at treatment, however the improvement in body weight is progressively attenuated as treatment age is delayed from weaning to adulthood. In this study, we evaluated the energy balance of ARC-PomcKO:Cre-ERT mice before (age 7 wk) and after (age 12 wk) tamoxifen treatment at age 8 wk to determine the basis for incomplete restoration of normal body weight. Body composition, food intake, locomotor activity and metabolic rate were evaluated serially. BW of ARC-PomcKO:Cre-ERT male and female mice were 57% and 81% greater, respectively, than WT:Cre-ERT controls. These differences dropped to 12% and 21%, respectively, after tamoxifen treatment mainly due to the loss of fat. Male mice reduced their food intake from 5.7 ± 0.3 to 4.5 ± 0.3 g/day ($P<0.01$), which was equivalent to WT (4.8 ± 0.4 g/day). Female mice reduced their food intake from 5.7 ± 0.3 to 3.7 ± 0.3 g/day ($P<0.01$), which was also equivalent to WT (4.1 ± 0.1 g/day). ARC-PomcKO:Cre-ERT mice of both sexes showed significant increases following treatment in their originally low locomotor activity to levels that were not different from WT mice. In males, there was no difference between ARC-PomcKO:Cre-ERT and WT mice in oxygen consumption corrected by lean mass (VO2, ml/kg/hr) either before or after treatment. However, there was a significant ~10% reduction of VO2 in obese female ARC-PomcKO:Cre-ERT compared to WT mice before treatment ($P<0.01$) and this difference remained after treatment. These data suggest that an impairment of metabolic rate persists despite the normalization of food intake and locomotor activity following genetic rescue of ARC Pomc expression. Future studies are needed to explain the disassociation between these variables for their normalization once an obesity state has been established. Sources of Research Support: NIH Grant DK066604; NIH Grant DK068400. Nothing to Disclose: MY, VO-C, VFB, MR, MJL

Pub #	P2-284
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	The Bone Morphogenetic Protein (BMP) Receptor Type 1A, BMPR1A, in POMC Neurons Is Required for Proper Energy Balance
Author String	KL Townsend, T Huang, L McDougall, M Diakow, Y Mishina, Y-H Tseng Joslin Diabetes Center, Boston, MA; University of Michigan, Ann Arbor, MI; SUNY Downstate College of Medicine, Brooklyn, NY
Body	The bone morphogenetic proteins (BMPs) are a family of growth factors which fulfill diverse roles in development and morphogenesis, and recent emerging evidence has implicated these factors in the regulation of energy balance. We have previously demonstrated that BMP7 is able to exert effects on differentiation and function of brown adipocytes, as well as affecting whole body energy expenditure. However, it was unknown if BMP7 could also affect energy intake. Others have shown that in C. elegans BMPs can regulate satiety, and we have shown that intracerebroventricular (i.c.v.) injection of BMP7 in mice leads to an acute reduction in food intake, accompanied by a reduction in hypothalamic pro-opiomelanocortin (POMC) expression. POMC neurons in the arcuate nucleus of the hypothalamus are key anorectic neurons which mediate signals leading to decreased food intake and increased energy expenditure, in response to signals such as leptin. The BMPs signal through a receptor heterodimer comprised of a type 1 and a type 2 transmembrane receptor complex. BMPR1a, one of the type 1 receptors known to mediate effects of BMP7, appears to be co-localized with POMC neurons in the hypothalamus. Therefore to determine if BMP signaling is required for proper energy balance in anorectic POMC neurons, we created a mouse model with a deletion of BMPR1a specifically in POMC neurons, using Cre-Lox technology and the POMC-cre mouse. We found that this deletion results in altered energy balance including hyperphagia and increased energy expenditure on a high fat diet, with no net effect on body weight. This hyperphagia was accompanied by increased expression of hypothalamic orexigenic neuropeptides (such as NPY) and a reduction in anorexigenic POMC expression, even in the basal (ie: chow-fed) state. Additionally, there was increased expression of UCP1 and mitochondrial genes (such as PGC1α, NRF1/2) in brown adipose tissue (BAT), a trend for increased BAT mass, and the mice were better able to maintain their body temperature during cold-exposure. Thus, the increased oxygen consumption measured by indirect calorimetry may be due to increased BAT thermogenesis. This may be mediated by increased sympathetic innervation of BAT via POMC neurons, which is the focus of current experiments. In summary, these studies provide evidence that central BMP signaling plays an important role in energy balance, including the regulation of energy intake and energy expenditure. Nothing to Disclose: KLT, TH, LM, MD, YM, Y-HT

Pub #	P2-285
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Leptin Binding Proteins in Mouse Cerebrovasculature: Identification of a Potential Leptin Transporter
Author String	VG Eranki, P Patrick, WA Banks, GN Shah St Louis University, St Louis, MO; Veterans Administration Puget Sound Health Care System (VAPSHCS), Seattle, WA

Body

Introduction: The role of leptin as an adiposity signal is well recognized and its resistance is implicated in obesity. One mechanism of resistance is an impaired transport of leptin across the blood-brain barrier (BBB). The short form of the leptin receptor (LR) is considered to be the leptin transporter protein. However, leptin crosses BBB in Koletsky rats, which lack functional leptin receptors, thus raising the possibility that other proteins may be involved in the transport of leptin across the BBB.

Objective: To identify leptin binding proteins in mouse cerebrovasculature and ascertain their role in leptin transport.

Methods and Results: Mouse brain microvessels (MV) were prepared and extracted in a buffer containing 1% Triton X-100. The MV extract was incubated with a leptin affinity column generated by covalent binding of the lysine residues on leptin to the activated aldehyde groups on the agarose beads. After extensive washing, bound proteins were eluted with 0.2M glycine, pH 2.5 and 0.05% Triton X 100.

The eluted proteins were separated by 2D gel electrophoresis. Three Coomassie stained bands were extracted from the gel and evaluated by tandem mass spectrometry (MS/MS). Four proteins with the ion scores of 76, 88, 118 and 510 were identified. The amino acid sequence of the protein with the ion score of 510 showed significant homology with ATP synthase α (ATPS). We focused on this protein for further analysis.

An Immunoprecipitation (IP)/ Western analysis was performed to further ascertain the binding of leptin to ATPS. The MV extract was incubated with leptin and IP with anti-leptin Ab. The resulting IP was resolved or a SDS-PAGE and probed with anti- ATPS Ab. An immunoreactive band in the IP comigrated with the positive control for ATPS.

Conclusions: ATPS binds leptin and is possibly involved in the transport of leptin across the BBB.

Discussion: H±ATP synthase has been found in all energy-transducing membranes e.g. mitochondria and chloroplasts. Several reports have shown that some of its subunits including ATPS localize in the plasma membranes of a variety of cells including endothelial cells. More recently, H±ATP synthase has been proposed as a potential molecular target for anti-obesity drugs. Whether ATPS is involved in the transport of leptin in the brain needs to be investigated.

1. Leptin transport across the blood brain barrier of the Koletsky rat is not mediated by a product of the leptin receptor gene. Banks WA, et al. Brain Research, 950:130-136, 2002.
2. Cell-surface H±ATP synthase as a potential molecular target for anti-obesity drugs. Arakaki N, et al. FEBS Letters 581:3405-3409, 2007.

Nothing to Disclose: VGE, PP, WAB, GNS

Pub #	P2-286
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Physiological Role of Urocortin 3 and Its Receptor in the Ventromedial Hypothalamus in Regulating Lipid Metabolism
Author String	H Chao, P Chen, M Digruccio, C Li University of Virginia, Charlottesville, VA
Body	Hypothalamus plays a critical role in regulating energy metabolism. In particular, electrical stimulation of ventromedial nucleus of the hypothalamus induces lipolysis in adipose tissue. Currently the molecular mediators underlie this effect remain unknown. Urocortin 3 (Ucn 3) is a member of the corticotropin-releasing factor (CRF) Peptide family identified in rodents and humans. Ucn 3 binds selectively to the type 2 CRF receptor (CRFR2) with high affinity while displaying minimal binding to the type 1 CRF receptor (CRFR1). Ucn 3 nerve fibers heavily innervate the VMH and abundant CRFR2 mRNA is expressed in the nucleus. Thus, it is conceivable Ucn 3 signaling through CRFR2 is a molecular mediator in the VMH to modulate lipolysis. In the present study we tested this hypothesis by using a lentiviral vector expressing small hairpin RNA (shRNA) against mouse CRFR2 to suppress the expression of CRFR2 in the VMH in vivo. CRFR2 shRNA viral vector or control vector were bilaterally injected into the VMH of male adult C57BL/6J mice and the mice were allowed to recover for one week after the surgery. Mice with VMH CRFR2 knockdown gained significantly more weight than the controls during the experimental period. The weight gain of the receptor knockdown mice was mainly due to accumulation of white fat pad and this was accompanied with an increase in adipocyte size in white adipose tissue (WAT) (Ctrl: 1318.8±91.4 vs CRFR2 knockdown: 1804±173 [mu] m^2, p=0.03) and decrease in plasma levels of free fatty acids (Ctrl: 1.56±0.06 vs. CRFR2 knockdown: 1.1±0.07 mmol/l, p<0.01) and glycerol (Ctrl: 62.9±6 vs. CRFR2 knockdown: 60.1±3.4 mg/dl, p<0.01). On the other hand, no differences were observed in basal plasma levels of triglyceride and glucose between the two groups. Indirect calorimetry analysis showed respiratory quotient of the receptor knockdown mice were significantly elevated compared to the control mice, suggesting that body fat oxidation rate was significantly reduced in VMH CRFR2 knockdown mice. Furthermore, the expression of a number of genes including hormone sensitive lipase and peroxisome proliferator-activated receptor-γ in WAT of the knockdown mice was significantly lower compared to that of control mice. Taken together, our results argue endogenous Ucn 3 through CRFR2 in the VMH plays an important role in regulating lipolysis in white adipose tissue. The underlying mechanism by which Ucn 3 in the VMH regulates lipolysis remains to be elucidated. Sources of Research Support: NIH Grant DK078049 awarded to CL. Nothing to Disclose: HC, PC, MD, CL

Pub #	P2-287
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Hypothalamic Phosphodiesterase-3B Pathway of Leptin Signaling Is Impaired during the Development of Diet-Induced Obesity
Author String	A Sahu, M Sahu University of Pittsburgh School of Medicine, Pittsburgh, PA
Body	Leptin signaling in the hypothalamus is critical for normal energy homeostasis. Obesity is usually associated with resistance to the effect of leptin on food intake and energy homeostasis. Although central leptin resistance is thought to be involved in the development of diet-induced obesity (DIO), the underlying mechanism behind this phenomenon is not clearly understood. Rodent models of DIO, in which animals become obese and hyperleptinimic with a high-fat diet (HFD), appear to provide compelling models for human obesity. We have recently shown that phosphatidyinositol 3-Kinase (PI3K) pathway of leptin signaling in the hypothalamus is impaired during the development of DIO in mice (1). Because phosphodiesterase-3B (PDE3B) signaling in the hypothalamus plays an important role in transducing anorectic and body wt reducing effects of leptin (2), and PI3K is upstream of PDE3B signaling, we tested if the PDE3B-pathway of leptin signaling was impaired during the development of DIO. To this end, 4-wk-old male FVB/N mice were fed with either a low-fat diet (LFD, 6% kcal as fat] or a HFD (58% kcal as fat) for a period of 4 wk. At the end of dieting, the animals were fasted overnight, followed by leptin (3.5 mg/kg, ip) or saline injection. Thirty minutes later, animals were killed. The medial basal hypothalamus (MBH) were dissected out and processed for protein extraction to measure PDE3B activity by an enzymatic assay. Epididymal (E) fat, retroperitoneal (RP) fat, and brown adipose tissue (BAT) were dissected out and weighed. The results showed an increase in body wt in both the LFD- and HFD-fed groups during the 4 wk period. However, there was more increase in body wt in the HFD as compared to that in the LFD group (5 g in LFD vs 8.5 g in HFD, $p < 0001$). Weights o E-fat ($p < 0001$), RP-fat ($p < 0001$), and BAT ($p < 0001$) were significantly increased in the HFD group. Cumulative 7-day food intake measured during 19- to 26-d was significantly increased in the HFD group ($p = 0.0004$). These results suggest the development of DIO in the HFD group. In addition, whereas leptin significantly ($p < 0.01$) increased PDE3B activity in the MBH of the LFD-fed mice, it failed to do so in the MBH of the HFD-fed mice, suggesting impairment in the PDE3B pathway of leptin signaling in the hypothalamus during the development of DIO. In conclusion, a defective PDE3B pathway of leptin signaling in the hypothalamus may be one of the mechanisms of central leptin resistance and DIO. (1) Metalakunta AS et al., Endocrinology 2008; 149:1121 (2) Zhao A et al., Nature Neuroscience 2002; 5:727 Sources of Research Support: NIH RO1 DK78068 to AS. Nothing to Disclose: AS, MS

Pub #	P2-288
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Desensitization of Hypophagia during Prolonged Inflammatory Challenge Is Associated with Leptin Resistance
Author String	BC Borges, R Rorato, Y Avraham, LECM Silva, M Castro, L Vorobiav, E Berry, J Antunes-Rodrigues, LLK Elias School of Medicine of Ribeirao Preto, University of São Paulo, Ribeirao Preto, Brazil; School of Medicine of Ribeirao Preto, University of São Paulo, Ribeirao Preto, Brazil; Hadassah Medical School, Jerusalem, Israel
Body	Acute exposure to bacterial lipopolysaccharide (LPS) is a potent inducer of immune response, as well as hypophagia. Nevertheless, desensitization of responses to LPS occurs during long-term exposure to endotoxin We induced endotoxin tolerance, injecting repeated (6LPS) LPS doses (100[micro]g/kg) in comparison with single (1LPS) treatment. 1LPS group, but not 6LPS group, showed a decreased food intake and body weight, associated with an increased plasma leptin concentrations and higher mRNA expression of OB-Rb, MC4R and SOCS3 in the hypothalamus. We also evaluated the effects of leptin on the brain 2-arachydonoyl glycerol (2-AG) content and phosphorylation of STAT-3 and activity of AMP-activated protein kinase (AMPK) in the hypothalamus. Hypophagia induced by 1LPS was associated with lower levels of 2-AG, increased number of p-STAT-3 expressing neurons and decreased AMPK activity. Desensitization of hypophagia in 6LPS group was associated with high 2-AG, no changes in p-STAT-3 and increased p-AMPK. Leptin injection decreased food intake, body weight, 2-AG levels and AMPK activity, and enhanced p-STAT-3 in control rats. However, leptin had no effects on 2-AG, p-STAT-3 and p-AMPK in single or repeated LPS-treated animals. Therefore, desensitization of hypophagia in response to repeated exposure to endotoxin is related to an inability of leptin to inhibit AMPK phosphorylation and 2-AG production and to activate STAT-3. SOCS3 is unlikely to underlie this resistance to leptin signaling in the endotoxin tolerance. The present model of prolonged inflammatory challenge may contribute to further investigations on mechanisms of leptin resistance. Sources of Research Support: FAPESP. CNPq, FAEPA. Nothing to Disclose: BCB, RR, YA, LECMS, MC, LV, EB, JA-R, LLKE

Pub #	P2-289
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Chronic Peripheral Administration of PK2 Causes a Potent Reduction in Food Intake and Body Weight in Diet-Induced Obese Mice
Author String	KEL Beale, K Hostomska, JV Gardiner, GA Bewick, MA Ghatei, SR Bloom, WS Dhillo Imperial College London, London, UK
Body	Prokineticin 2 (PK2) is a neuropeptide expressed in hypothalamic nuclei known to be involved in the regulation of energy homeostasis. It has previously been demonstrated that acute central and peripheral administration of PK2 potently reduces food intake in rodents. We aimed to investigate the effect of chronic peripheral administration of PK2 on body weight and food intake in diet-induced obese mice to determine the therapeutic potential of PK2 as a novel anti-obesity agent. Diet-induced obese mice weighing 45-50g were implanted with a subcutaneous osmotic mini-pump containing either saline, PK2 delivered at 60nmol/kg/hour or PK2 delivered at 120nmol/kg/hour (n=10-11 per group). Additionally, two groups of mice were implanted with a mini-pump containing saline and pair-fed to either the low dose or high dose PK2-treated animals (n=10-11 per group). Food intake and body weight were measured daily for 13 days. Body weight was significantly and dramatically reduced in both PK2-treated groups from day 2 to 13 compared to *ad libitum* fed animals treated with saline. At the termination of the study there was no attenuation of the effect of PK2 on body weight, suggesting that tachyphylaxis to the anorectic effect of PK2 did not occur. A significant reduction in food intake was observed in animals treated with 60nmol/kg/hour PK2 from day 1 to day 3 and also in animals treated with 120nmol/kg/hour PK2 between days 1 and 9. The effect of PK2 on body weight was independent of changes in energy expenditure, as mice pair-fed to the PK2 treated groups lost a similar amount of weight to mice given PK2. In conclusion, this work has demonstrated that chronic peripheral administration of PK2 robustly reduces body weight and food intake in a mouse model of human obesity. PK2 therefore represents a novel therapeuti agent for the treatment of obesity. Sources of Research Support: Astra Zeneca; NIHR Career Development Fellowship awarded to WSD; Jean Shanks Studentship awarded to KELB; Integrative Mammalian Biology Capacity Building Award; FP7-HEALTH-2009-241592 EurOCHIP grant; NIHR. Nothing to Disclose: KELB, KH, JVG, GAB, MAG, SRB, WSD

Pub #	P2-290
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Neuroendocrine Regulatory Peptide-2 Regulates Feeding Behavior Via the Orexin System in the Hypothalamus
Author String	T Matsuo, K Toshinai, H Kageyama, H Yamaguchi, K Sasaki, S Shioda, N Minamino, M Nakazato University of Miyazaki, Miyazaki, Japan; Showa University School of Medicine, Tokyo, Japan; National Cerebral and Cardiovascular Center Research Institute, Osaka, Japan
Body	Peptides are an important class of cell-to-cell signaling molecules. We have used a peptidomics approach to profile a complete set of secretory peptides from cultured human endocrine cells and identified two novel carboxy-terminally amidated peptides, Neuroendocrine regulatory peptide (NERP)-1 and NERP-2, derived from a neurosecretory protein VGF (J Biol Chem, 282, 26354-60, 2007). *Vgf* null mice exhibit dwarfism and hypermetabolic rates, suggesting that VGF or VGF-derived peptides play important roles in energy metabolism. We attempted to identify NERPs expressing neurons in rats by immunohistochemistry and explore the effects of intracerebroventricular (icv) administration of NERP-2 on feeding, body temperature, oxygen consumption and locomotor activity in rats and mice. Icv administration of NERP-2, but not NERP-1 or a form of NERP-2 bearing a COOH-terminal glycine extension, increased food intake in rats. We investigated the downstream signal of NERP-2 on the basis of studies of NERP-2-induced feeding with neutralization of orexins, neuropeptide Y, or agouti-related protein. NERP-2 expression localized to the lateral hypothalamus (LH) and the dorsomedial perifornical hypothalamus in rats, colocalizing with orexins that activate feeding behavior and arousal. NERP-2 administration induced Fos protein, a marker of neuronal activation, in the orexin-immunoreactive neurons. *Vgf* mRNA levels were upregulated in the rat LH upon food deprivation. Icv administration of NERP-2 also increased body temperature, oxygen consumption, and locomotor activity in rats. Treatment with anti-NERP-2 IgG decreased food intake. NERP-2-induced bioactivities were abrogated by administration of anti-orexins IgG or orexin receptor antagonists. NERP-2 did not induce food intake or locomotor activity in orexin-deficient mice (Am J Physiol Endocrinol Metab, 299, E394-401, 2010). Our findings indicate that hypothalamic NERP-2 plays a role in the control of food intake and energy homeostasis via the orexin pathway. Thus, VGF serves as a precursor of multiple bioactive peptides exerting a diverse set of neuroendocrine functions. Further studies of NERPs and their receptors will pave the way for elucidating unknown extracellular signaling mechanisms as well as developing novel therapeutics for endocrine diseases or eating disorders. Nothing to Disclose: TM, KT, HK, HY, KS, SS, NM, MN

Pub #	P2-291
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Leptin Increases Sympathetic Nerve Activity by Acting in the Dorsomedial Hypothalamus of Leptin Resistan Mice
Author String	PJ Enriori, SE Simonds, P Sinnayah, C Garcia Rudaz, RD Brown, MA Cowley Monash University, Clayton, Australia; Oregon Health and Science University, Beaverton, OR
Body	Activation of central leptin receptors increases the sympathetic nervous activity (SNA), which stimulates energy expenditure in intrascapular brown adipose tissue (iBAT). Obese mice (diet induced obese mice, DIO) and humans are resistant to the anorectic actions of leptin. However, a positive association exists between increased leptin levels and hypertension in humans. We evaluated whether leptin still stimulated sympathetic outflow in DIO mice measuring iBAT temperature. We also tested the hypothesis that hyperleptinemia in obesity contributes to increased blood pressure (BP) and heart rate (HR) through increased stimulation of the SNA. Radiotelemetry probes where implanted into iBAT to measure temperature or into the carotid artery to measure BP and HR. We demonstrated that hyperleptinemic mice have higher iBAT temperature than mice on regular diet. Conversely, leptin deficient ob/ob mice have lower iBAT temperature. We also found that central and peripheral leptin administration increases SNA in obese (DIO and ob/ob) and control mice. Leptin also induced pStat3 expression, a well known mediator of leptin activation, in dorsomedial (DMH), but not in the arcuate nucleus of the hypothalamus of DIO mice. Moreover, we demonstrated that these neurons mediate the thermogenic responses to hyperleptinemia in obese mammals because blockade of leptin receptors in the DMH prevented the thermogenic effects of leptin. Although both DIO and ob/ob mice where significantly heavier than lean mice, only DIO mice exhibited significantly higher mean arterial pressure (MAP) and HR. Chronic intra-DMH administration (seven days) o leptin antagonist decreased MAP in all DIO mice and was no longer significantly higher compared to lean mice. These studies strongly suggest that DMH could mediate the sympathetic responses to hyperleptinemia. As the sympathetic nervous system contributes in regulating blood pressure, heart rate and hepatic glucose production, selective leptin resistance may be a crucial mechanism linking adiposity and metabolic syndrome. Sources of Research Support: NHMRC 606662, Pfizer Australia, Monash University, the National Heart Foundation of Australia G 09M 4306, and the US NIH RR0163 and DK62202. Nothing to Disclose: PJE, SES, PS, CGR, RDB, MAC

Pub #	P2-292
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Studying Physiologic Signals for Ghrelin Secretion Using a Novel Genetic Mouse Model of Hyperghrelinemia
Author String	PK Piper, I Sakata, J-C Chuang, S Rovinsky, S Osborne-Lawrence, C Clemens, J Zigman UT Southwestern Medical Center, Dallas, TX; UT Southwestern Medical Center, Dallas, TX

Body

Objective: The peptide hormone ghrelin mediates food intake and reward-based eating behaviors, body weight, blood glucose levels, and the coordinated response to chronic stress. Ghrelin is released from a distinct group of ghrelin cells before meals and upon caloric restriction or stress. Yet, despite all that is now known about these actions of ghrelin and when ghrelin levels are high, little is known about the detailed physiology of ghrelin secretion. Here, we have investigated the role that heterotrimeric G-protein signaling cascades play in stimulating ghrelin release.

Research Design & Methods: We employed a quantitative PCR approach to determine the G-protein alpha-subunit complement of ghrelin cells isolated from ghrelin-GFP reporter mice. Next, we tested the ability of various modulators of G-protein signaling cascades to stimulate ghrelin secretion from primary cultures of ghrelin cell-containing gastric mucosal cell preparations. Finally, we studied ghrelin secretion and certain ghrelin-mediated processes in a novel mouse line with altered G-inhibitory signaling targeted specifically to ghrelin cells. This was achieved by crossing the recently described *ROSA26*^{PTX} mouse with our ghrelin-Cre (GCre) mice, yielding offspring that produce pertussis toxin (PTx) only in ghrelin cells. PTx inactivates members of the G-inhibitory alpha-subunit family.

Results: Several G-alpha subunits were highly expressed within ghrelin cells, and several modulators of G-protein signaling cascades were shown to enhance ghrelin secretion from isolated ghrelin cells. GCre+/PTX+ mice developed markedly elevated serum ghrelin levels by 6 weeks of age. Despite the differences in circulating ghrelin, we observed no phenotypic differences in body weight, body length, body composition, or glucose tolerance. Furthermore, the mice showed no differences in stress-induced depression-like behaviors.

Conclusions: G-proteins play a pivotal role in ghrelin secretion. PTX-induced inhibition of Gai subunits within ghrelin cells leads to marked hyperghrelinemia, although no obvious physiological consequences of this elevated ghrelin have yet been revealed.

Nothing to Disclose: PKP, IS, J-CC, SR, SO-L, CC, JZ

Pub #	P2-293
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	β-Endorphin Antagonizes the Effects of α-MSH on Food Intake and Energy Metabolism
Author String	R Dutia, K Meece, SV Dighe, SL Wardlaw Columbia University College of Physicians & Surgeons, New York, NY
Body	Proopiomelanocortin (POMC) is posttranslationally processed to several peptides including α-MSH, a primary regulator of energy balance that inhibits food intake and stimulates energy expenditure. However another POMC-derived peptide, β-Endorphin (β-EP), is reported to stimulate food intake. We therefore examined the interaction of these two POMC peptide products on energy balance in male rats cannulated in the right lateral ventricle. In the first experiment we examined the ability of β-EP (1 ug) to attenuate the effects of α-MSH on energy balance in rats studied after a 16h fast. We had previously found that 1 [micro]g of β-EP transiently increased food intake (FI) during the dark cycle 2 and 4h after injection (157% and 132% vs saline (SAL), $p<.01$) but did not affect food intake during refeeding after a 16h fast. Rats received 1 [micro]g of the α-MSH analog, NDP-MSH, 1 [micro]g NDP-MSH+1 [micro]g β-EP, or SAL ICV in the morning after a 16h fast. MSH suppressed FI 2, 4 and 8h after refeeding ($p<.05$). This effect was partly reversed in the MSH+β-EP group such that FI was not different from SAL at 2 and 4h and was higher than MSH alone at 2h ($p=.05$). At 4h, SAL gained 9.2 ± 1.7g while MSH lost 0.5 ± 1.2g ($p<.001$). In contrast, MSH + β-EP gained 5.5 ± 1.2 g ($p<.01$ vs MSH). At 8h, MSH suppressed both FI and weight gain, however, β-EP no longer reversed these effects. To examine chronic interaction between these peptides rats received an ICV minipump for 7d with MSH (0.1 [micro]g/h), MSH+ β-EP (0.1 [micro]g/h of each), or SAL. Cumulative weight gain and FI were significantly suppressed in the MSH group during the entire study. These suppressive effects were partly reversed by β-EP during the first 3d. At 3d, the SAL group gained 26.1± 2.2g, while MSH gained 5.6 ± 5.1g ($p=.001$); MSH+β-EP gained 16.7 ± 4.2g ($p=.06$ vs MSH) and was not different from SAL. Similarly, MSH+β-EP cumulative FI was not significantly different from SAL at 3d. On Days 4-7, β-EP no longer blocked the effects of MSH on FI or weight gain. Thus β-EP is initially able to antagonize the effects of MSH on FI and more profoundly, on body weight, yet these effects subside after 3 days, possibly because of tolerance to β-EP. These experiments illustrate how POMC-derived peptides can interact to regulate food intake and body weight. This study highlights the importance of understanding how the balance between α-MSH and β-EP is maintained and the potential role of differential POMC processing in regulating energy balance. Nothing to Disclose: RD, KM, SVD, SLW

Pub #	P2-294
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Role of Vaspin, Chemerin, and Omentin-1 in the Hypothalamic Control of Feeding
Author String	L Brunetti, C Di Nisio, L Recinella, G Orlando, A Chiavaroli, S Leone, C Ferrante, M Vacca G d'Annunzio University, Chieti, Italy
Body	**Context:** Adipose tissue-derived hormones (adipokines) play a key role in the regulation of energy homeostasis. Visceral adipose tissue-derived serpin (vaspin) improves glucose tolerance and insulin sensitivity in diet-induced obese mice (1). Chemerin may increase insulin sensitivity in adipose tissue and seems to be associated with several key aspects of metabolic syndrome (2). Decreased levels of omentin-1 are associated with increasing obesity and insulin resistance (3). **Objectives:** Our study aimed to investigate the role of vaspin, chemerin and omentin-1 on food intake and gene expression of hypothalamic peptides which play a key role in feeding regulation. **Design:** Male Wistar rats were injected into the arcuate nucleus (ARC) of the hypothalamus, at 0900 h, with either vehicle (saline; n=8), vaspin (1 [mu]g/kg; n=9), chemerin (8 [mu]g/kg; n=9), or omentin-1 (8 [mu]g/kg; n=9). Food intake in the following 24 hours was recorded, after which rats were sacrificed. Total RNA was extracted from hypothalami and reverse transcribed to evaluate gene expression of agouti-related peptide (AgRP), neuropeptide Y (NPY), orexin-A, cocaine and amphetamine-regulated transcript (CART), corticotrophin releasing hormone (CRH) and proopiomelanocortin (POMC) by real-time reverse transcription polymerase chain reaction (real-time RT PCR). Food intake data were analyzed by one-way analysis of variance (ANOVA), followed by Newman-Keul's multiple comparison test. Gene expression data, deriving from relative quantification, were analyzed by one-sample t test. The level of statistical significance was set at $P<0.05$. **Results:** Compared to vehicle, intrahypothalamic vaspin injection significantly decreased feeding (ANOVA, $P=0.0002$; post-hoc $P<0.01$ vs. vehicle), while chemerin and omentin-1 had no effect. Vaspin treatment was associated with a significant reduction of NPY ($P=0.0018$) and increase of POMC ($P=0.0042$) gene expression in the hypothalamus. Chemerin treatment led to a significant increase of both AgRP ($P=0.0002$) and POMC ($P=0.0001$) gene expression in the hypothalamus. On the other hand, omentin-1 treatment did not modify hypothalamic gene expression of AgRP, NPY, orexin-A, CART, CRH or POMC. **Conclusions:** Vaspin adds to leptin and adiponectin as an adipokine triggering anorectic pathways in the hypothalamus, where reduction of NPY and increase of POMC mRNA levels could mediate inhibition of feeding. On the other hand, chemerin and omentin-1 have no effects on feeding behavior. (1) Hida K,et al., Proc Natl Acad Sci USA 2005; 102:10610 (2) Takahashi M et al.,FEBS Lett 2008; 582:573 (3) de Souza Batista CM et al., Diabetes 2007; 56:1655 Sources of Research Support: MIUR grants. Nothing to Disclose: LB, CDN, LR, GO, AC, SL, CF, MV

Pub #	P2-295
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Effects of Visfatin (PBEF/NAMPT) on Feeding and Hypothalamic Peptide Gene Expression in the Rat
Author String	L Brunetti, R Lucia, C Di Nisio, A Chiavaroli, G Orlando, C Ferrante, S Leone, V Michele G d'Annunzio University, Chieti, Italy
Body	Visfatin, also known as pre-beta-cell colony-enhancing factor (PBEF) or nicotinamide phosphoribosyltransferase (NAMPT), is a cytokine that is produced by adipose tissue, skeletal muscle, liver and immune cells (1). Central administration of visfatin causes orexigenic effects in chicks (2) and circulating visfatin levels have been reported to be elevated in type 2 diabetes mellitus and obesity (3). In the present study, we aimed to investigate, in male adult Wistar rats fed a standard laboratory diet, the effects of visfatin on food intake and gene expression of hypothalamic peptides which play key roles in the central regulation of feeding, in particular agouti-related peptide (AgRP), neuropeptide Y (NPY), cocaine- an amphetamine-regulated transcript (CART), corticotropin-releasing hormone (CRH), proopiomelanocortin (POMC). Rats were injected into the arcuate nucleus (ARC) of the hypothalamus, at 09.00 a.m., with either vehicle (saline; n=16) or visfatin (12 microg/kg; n=16). Food intake was recorded at 2 and 24 hours following injection, after which rats were sacrificed. Total RNA was extracted from hypothalami and reverse transcribed. Gene expression of hypothalamic peptides was evaluated by quantitative real-time polymerase chain reaction (PCR) using beta-actin as the housekeeping gene, both 2 and 24 hours after the injection. Relative changes in individual gene expression were determined using the comparative $2^{-[Delta][Delta]Ct}$ method. Compared to vehicle, visfatin significantly increased food intake, at 2 and 24 hours post-injection. As regards to hypothalamic peptide gene expression, 24 hours after visfatin treatment, we observed a significant reduction of CRH mRNA and CART mRNA, respect to vehicle treated rats, while no effect was found at 2 hours following intrahypothalamic visfatin injection. We can conclude that visfatin plays an orexigenic role ir the rat hypothalamus which could be partially mediated by the acute reduction in CRH and CART gene expression, as detected 24 hours after the injection. 1) Samal B et al., Mol Cell Biol 1994; 14:1431 2) Cline MA et al., Behav Brain Res 2008; 186:293 3) Catalan V et al., Nutr Metab Cardiovasc Dis 2010; Jan 25. [Epub ahead of print] Sources of Research Support: MIUR grants. Nothing to Disclose: LB, RL, CDN, AC, GO, CF, SL, VM

Pub #	P2-296
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Isotonic or Hypertonic Extracellular Volume Expansion Activates Kiss1 Neurons in the Anteroventral Periventricular and Arcuate Nucleus
Author String	LO Margatho, CF Elias, LL Elias, J Antunes-Rodrigues School of Medicine of Ribeirao Preto, University of São Paulo (USP), Ribeirão Preto, Brazil; Division of Hypothalamic Research, Department of Internal Medicine, University of Texas Southwestern Medical Center Dallas, TX
Body	**OBJECTIVE:** We investigated the neuronal activation of the anteroventral periventricular nucleus (AVPV) and arcuate nucleus (Arc) induced by isotonic (iso) or hypertonic (hyper) extracellular volume expansion (EVE). **METHODS and RESULTS:** In adult male Kiss1-Cre transgenic mice (20-30 g) a catheter was inserted into the right external jugular vein and advanced to the right atrium. Through this procedure extracelular volume was expanded by injection of isotonic (NaCl 0.15M) or hypertonic saline (NaCl 0.3 M), 2 ml/100 g b.w., over 1 min. Ninety minutes after EVE the mice were anesthetized with ketamine (5 mg/100 g) and xylazine (1 mg/100 g) and perfused transcardially with normal saline followed by 4% paraformadehyde. The brains were removed and brain sections of the AVPV and Arc were processed were first processed for c-Fos immunoreactivity (c-For-ir) and then for co-localization with the green fluorescent protein immunoreactivity (GFP-ir, green cytoplasm). Briefly, the free-floating sections were submitted to standard peroxidase reaction for c-Fos-ir. The peroxidase label was detected using diaminobenzidine hydrochloride as chromogen that produces a blue-black nuclear reaction product. These series of sections were then incubated in primary anti-GFP antisera (1:5000, made in chicken, Aves Labs) and AlexaFluor 488-conjugated goat anti-chicken as secondary antisera. This reaction could be viewed by a cytoplasm green fluorescent protein. Dual labeled neurons co-expressing GFP-ir and c-Fos-ir were quantified. Quantification of dual labeled neurons was expressed by percentage of co-localization in the AVPV and in two levels of the Arc. Cells were counted in one side in each nucleus. In the AVPV, the percentage of Kiss1 neurons activated after iso or hyper EVE were approximately 6.5% and 6.7%, respectively. We observed that approximately 10.6% and 10.3% Kiss1 neurons were activated in the medial Arc after an iso or hyper EVE, respectively. In the posterior Arc, we found that Kiss1 neurons were also activated in response to iso (10.7%) or hyper (9.6%) EVE. **CONCLUSION:** Our findings reveal that Kiss1 neurons in the AVPV and Arc nucleus may exhibit small to moderate activation in response to changes in extracellular volume. These results will allow us to further elucidate the mechanisms by which these neurons are influenced by EVE-induced regulatory responses (neuroendocrine and behavioral) to achieve body fluid homeostasis. Sources of Research Support: Fapesp (Proc # 2010/50917-0), University of Texas Southwestern Medical Center at Dallas. Nothing to Disclose: LOM, CFE, LLE, JA-R

Pub #	P2-297
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Economic and Genetic Influences on Food Choice in the Tubby Obese Mice
Author String	DJ Good, GC Davis, JC Jacob Virginia Polytechnic Institute and State University, Blacksburg, VA; Virginia Polytechnic Institute and State University, Blacksburg, VA
Body	Mouse models have been used in numerous studies to investigate the relationship between genotypes, food intake and weight subject to changes in the cost of acquiring a single food. However, humans are subjected to an environment of food choice, and incur costs to choosing foods. Increasing food choices and decreasing food prices, especially in high fat foods, have been identified as important contributors to the current human obesity epidemic. We hypothesized that genotype-specific behavioral responses and phenotypic outcomes related to body weight could differ significantly between genotypes in a choice setting. Using normal mice (WT), and mice with a mutation in the Tubby gene (Tub-Mut), this research investigated behavioral and phenotypic response differences between genotypes in animals confronted with a choice of high fat and low fat foods at different prices. Previous work from our laboratory using Tub-Mut mice showed that these animals were hypophagic relative to WT animals, and that obesity was due to reduced physical activity. Our new studies show that for both genotypes, as the price of the high fat food falls, consumption of that food increases, but consumption of the low fat food does not decrease in a compensatory fashion. The Tub-MUT mice showed a significantly higher total consumption compared to WT mice over the course of the study, due mainly to the higher consumption of the lower priced high fat food with no compensatory reduction in the consumption of low fat food. For both genotypes, weight and body fat percentage increases with decreasing high fat food price, again likely due to the overall increase in total food intake. Interestingly, serum leptin and serum gherlin levels were not significantly different between genotypes or between price points, although there was a trend in both genotypes for higher leptin levels with lower priced high fat food. Glucose tolerance tests show a significant effect of price of high fat food in Tub Mutant mice, but not WT mice, suggestive of a pre-diabetic state. These results demonstrate that accounting for food choice in mouse food intake studies is critical to understanding the complex regulation of body weight and obesity. Incorporating economic conditions, namely, food choice and differential prices, into these studies allows researchers to begin to create conditions that more closely model human food environments. Nothing to Disclose: DJG, GCD, JCJ

Pub #	P2-298
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	The Effect of Glucose on Hypothalamic Neuropeptide Y (NPY) and Cocaine- and Amphetamine- Regulated Transcript (CART) Release Investigated Using Static Incubation of Hypothalamic Explants
Author String	SS Hussain, E Richardson, N Buckley, G Bewick, SR Bloom, JV Gardiner Hammersmith Hospital, Imperial College London, London, UK
Body	Glucose alters the activity of hypothalamic neurones involved in regulating appetite (1). Arcuate nucleus NPY releasing neurones stimulate feeding. The identification of glucose-sensitive NPY releasing hypothalamic neurones suggests a strong role for these neurones in mediating changes in appetite induced by alterations of glucose. CART is a neurotransmitter abundantly expressed in regions of the hypothalamus involved in energy homeostasis. Although its exact role in this regard is still under debate, our earlier work suggests it is an orexigenic peptide (2). To gain a better understanding of the mechanism involved in glucoprivic feeding and the role of CART in appetite, we investigated the changes in NPY and CART release by the hypothalamus at different concentrations of glucose, using static incubation of hypothalamic explants. Hypothalami from 20 male Wistar rats (mean weight 280.4±2.3g) were incubated in artificial CSF (aCSF) for a 2-h equilibration period. The hypothalamic explants were then incubated for three 45 min periods in 600 [micro]l aCSF containing 3, 8 (baseline) and 15mM glucose in randomised order. Finally, the viability of the tissue was verified by 45-min incubation in aCSF containing 56 mM KCl. At the end of each incubation period, supernatants were removed and assayed for NPY and CART release by radioimmunoassay. Only explants that showed a greater secretion of NPY or CART with 56 mM KCl as compared to baseline were considered viable. NPY release in aCSF containing 3, 8 (basal) and 15mM glucose was 285.2±92.8, 100±36.1 and 130.7±25.8 percent of basal NPY release, respectively. There was a significant increase in NPY release with aCSF containing 3mM glucose versus baseline glucose. CART release in aCSF containing 3, 8 (basal) and 15mM glucose was 95.1±10.6, 100±6.8 and 78.8±3.9 percent of basal CART release, respectively. There was a significant decrease in CART release with aCSF containing 15mM glucose versus baseline glucose. These findings are consistent with previous findings that suggest an important role for glucose-sensitive NPY releasing hypothalamic neurones in stimulating glucoprivic feeding and support CART being an orexigenic peptide. They raise the possibility that a subset of CART releasing hypothalamic neurones are glucose inhibited. This work also supports the use of static incubation of hypothalamic explants to study the effects of glucose on neuropeptides involved in regulating appetite and glucoprivic feeding. (1) Blouet C, et al., Behav Brain Res 2010; 209(1):1-12. (2) Smith KL, et al., Obesity 2008; 16(10): 2239-2244 Sources of Research Support: Wellcome Trust Clinical Research Fellowship awarded to SSH. Integrative Mammalian Biology Capacity Building Award, FP7-HEALTH-2009-241592 EurOCHIP grant, NIHR Biomedical Research Centre Funding Scheme. Nothing to Disclose: SSH, ER, NB, GB, SRB, JVG

Pub #	P2-299
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Effects of Peptide Hormones and Neurotransmitters on *In Vitro* Ghrelin Secretion by Ghrelin-Producing Cell Line, MGN3-1
Author String	H Iwakura, H Ariyasu, H Hosoda, K Hosoda, K Nakao, K Kangawa, T Akamizu Kyoto University Hospital, Kyoto, Japan; National Cerebral and Cardiovascular Center, Osaka, Japan; Kyoto University Graduate School of Medicine, Kyoto, Japan
Body	Ghrelin is a 28-amino acid peptide hormone mainly produced by the stomach with strong growth hormone-secreting and orexigenic activities. To understand the physiologic role of ghrelin, it is crucial to study both the actions of ghrelin and the regulation of ghrelin secretion. Although ghrelin actions has been extensively revealed, the direct factors regulating ghrelin secretion by ghrelin-producing cells (X/A-like cells), however, is not fully understood. Recently, we have developed a ghrelin-producing cell line MGN3-1 from a gastric ghrelinoma isolated from ghrelin promoter SV40-T antigen transgenic mice (1). The MGN3-1 cell is the first cell line derived from a gastric ghrelin-producing cell that preserves the ability to secrete of substantial amounts of ghrelin under physiological regulation. In the previous study, we have reported that insulin and somatostatin directly suppresses ghrelin secretion by MGN3-1 cells in vitro(1). In this study, we examined the effects of peptide hormones and neurotransmitters on in vitro ghrelin secretion by the MGN3-1 cells. Among peptide hormones, oxytocin and vasopressin significantly stimulated ghrelin secretion by MGN3-1 cells. As MGN3-1 cells express only oxytocin receptor mRNA, not vasopressin receptors mRNA, oxytocin is the likely regulator, with the effect of vasopressin mediated by a cross-reaction. We also discovered that dopamine stimulates ghrelin secretion from MGN3-1 cells in a similar manner to the previously-known ghrelin stimulators, epinephrine and norepinephrine. MGN3-1 cells expressed mRNA encoding dopamine receptors D1a and D2. The dopamine receptor D1 agonist fenoldopam stimulated ghrelin secretion, while the D2, 3 agonist bromocriptine did not, indicating that the stimulatory effect of dopamine on ghrelin secretion is mediated by the D1a receptor. In conclusion, we identified two direct regulators of ghrelin, oxytocin and dopamine. These findings will provide new direction for further studies seeking to further understand the regulation of ghrelin secretion, which will in turn lead to greater understanding of the physiologic role of ghrelin. (1) Iwakura et al., Endocrinology 2010; 151:2940 Nothing to Disclose: HI, HA, HH, KH, KN, KK, TA

Pub #	P2-300
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Fasting Co-Suppresses Nesfatin-1 and GPR12 in Mouse Hypothalamic Appetite Center: Implications for Energy Metabolism
Author String	D Osei-Hyiaman, L Sophie-Dreher, S Nishimura, J Encinas Kobe Pharma Research Institute, Nippon Boehringer-Ingelheim, Kobe, Japan
Body	Nesfatin-1 is a recently discovered endogenous satiety peptide that is cleaved from its precursor nucleobindin-2 (NUCB2) by prohormone convertase. Nesfatin-1 and its precursor, NUCB2 are expressed in the hypothalamus and in peripheral tissues and are both regulated by nutritional status. Circulating nesfatin-1 levels change with metabolic state, showing significant inverse correlations with BMI and body fat mass when sampled after fasting. There is currently no known cognate receptor for nesfatin-1, nor have the pharmacology or signaling mechanisms related to its role in satiety been established yet. Here we show that 24-hour fasting suppresses both nesfatin-1 and the orphan G-protein coupled receptor GPR12 immunoreactivity in mouse hypothalamus (PVN, LH, DMD, ArC, ME, PeVN, and VMH) and that these effects in both cases can be reversed by feeding. Although a direct interaction between the peptide and GPR12 has not been demonstrated our findings indicate that both GPR12 and endogenous nesfatin-1 may be regulated coordinately by changes in metabolic state. Further studies are underway to identify a possible relationship. Nothing to Disclose: DO-H, LS-D, SN, JE

Pub #	P2-301
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Obese Rats Show Prolonged Changes in Thermogenic Properties of Brown Fat in Response to an Acute Immune Challenge
Author String	MM Sheppard, J Pohl, GN Luheshi, BC Woodside Concordia University, Montreal, Canada; Douglas Mental Health Research Institute, McGill University, Montreal, Canada
Body	Diet-induced obesity exacerbates and prolongs the febrile and anorectic responses to acute immune challenge. Recent data from our laboratory indicate that recovery of body weight following the anorexia induced by an acute immune challenge is significantly delayed in obese rats compared to lean controls. In this study, we tested the hypothesis that UCP1 expression in brown adipose tissue is increased in obese rats as a result of immune challenge. Twenty adult male wistar rats were divided into two weight matched groups. The lean group received free access to standard laboratory chow, and the obese group to chow and a highly palatable liquid diet supplement. Testing began when lean rats weighed ~500 g, and when obese rats weighed ~ 600g. Food intake and body weight were measured daily. On day 4, rats received intraperitoneal injections of either lipopolysaccharide (100 [micro]g/kg), a component of the cell wall of gram negative bacteria commonly used to induce acute immune challenge, or physiological saline (1 mg/kg). On the 6th day post injection rats were perfused with autoclaved saline and the intrascapular brown fat, retroperitonal and epididymal white fat, soleus, plantaris, and gastrocnemius muscles were removed, weighed, and frozen until processing. RNA was extracted from brown fat and expression of UCP1 was determined by quantitative PCR. The retroperitoneal and epididymal fat pads of obese LPS-treated rats weighed less than those of saline-treated obese rats but the amount of brown fat did not differ between these groups. There was a main effect of diet on UCP1 expression in brown fat, however. Expression of UCP1 was significantly higher in obese rats than in lean rats. As predicted, LPS treatment significantly increased expression of UCP1 in brown fat for obese but not lean rats. These results suggest that there is a prolonged effect of acute immune challenge on the thermogenic capacity of brown adipose tissue in obese rats, perhaps leading to a change in the pattern of energy utilization. Further studies are underway to determine whether energy utilization in other metabolically active tissues is also altered in LPS-treated obese rats, and to investigate the mechanisms through which these changes might be triggered. Nothing to Disclose: MMS, JP, GNL, BCW

Pub #	P2-302
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	The Anorectic Cytokine CNTF Activates Hypothalamic Ghrelin- and Urocortin-Expressing Neurons Both *In Vitro* and *In Vivo*
Author String	MJ Purser, PS Dalvi, DD Belsham University of Toronto, Toronto, Canada; University of Toronto, Toronto, Canada; University of Toronto, Toronto, Canada
Body	Ciliary neurotrophic factor (CNTF), a potent cytokine and neuronal growth factor, reduces feeding behaviour and induces weight loss in both humans and mice. We have also demonstrated a role for CNTF in the immortalization of adult-derived neuronal cultures from mouse hypothalamus via GLP-1 mediated neurogenesis. However, the exact central mechanisms by which CNTF affects energy homeostasis or cell proliferation, and the extent to which CNTF influences other hypothalamic networks, has yet to be determined. Experiments of this nature have previously been challenging, in part due to the lack of representative hypothalamic cell models. To address this, we employed clonal, immortalized mouse hypothalamic cell lines generated in our lab. The mHypoE-20/2 line endogenously expresses the CNTF receptor and specific neuropeptides, such as ghrelin and urocortin. Both of these peptides have been linked to energy homeostasis, although their inherent mechanisms of action are largely unknown. Using real-time RT-PCR (qRT-PCR) we examined the effects of CNTF on the mRNA levels of ghrelin and urocortin in the mHypoE-20/2 cell model. We found that treatment of 10 ng/mL CNTF significantly increased ghrelin mRNA by 0.7-fold at 4 h, suppressed ghrelin mRNA by 43% at 48 h, and suppressed urocortin mRNA by 56% at 48 h, as compared to vehicle-treated controls. This suggests that ghrelin and urocortin may function as downstream mediators through direct CNTF receptor activation. Therefore, we set out to elucidate the effects of CNTF on ghrelin and urocortin neurons *in vivo* . We performed intracerebroventricular injections of 0.5 mg/mL CNTF into mice, and examined its effects on ghrelin and urocortin neurons at 2 h post-exposure. Through double-label immunohistochemistry using specific antibodies against c-Fos, a marker of neuronal activation, ghrelin, and urocortin-2, we showed that central CNTF administration significantly activated ghrelin neurons in the dorsal dorsomedial nucleus and periventricular area, urocortin-2 neurons in the ventromedial hypothalamus, and significantly activated both ghrelin and urocortin-2 neurons in the arcuate, ventral dorsomedial and paraventricular nuclei. In conjunction, both our *in vitro* and *in vivo* studies point to a potential role for CNTF in regulating hypothalamic ghrelin- and urocortin-expressing neurons to mediate its recognized effects on energy homeostasis, neuronal proliferation, and/or neurogenesis. Sources of Research Support: Canadian Institutes for Health Research (CIHR), Canadian Diabetes Association (CDA), Canada Foundation for Innovation (CFI), Banting and Best Diabetes Centre (BBDC), the Canada Research Chairs (CRC) Program and the Ontario Graduate Scholarship (OGS) Program. Nothing to Disclose: MJP, PSD, DDB

Pub #	P2-303
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Missense Mutations in the Basic Helix-Loop-Helix Transcription Factor Single-Minded 1 Result in Severe, Early-Onset Obesity
Author String	S Ramachandrappa, A Raimondo, J Keogh, E Henning, A Cali, S Brage, N Wareham, S O'Rahilly, I Barroso, M Whitelaw, IS Farooqi University of Cambridge, Cambridge, UK; University of Adelaide, Adelaide, Australia; University of Cambridge, Cambridge, UK; Hinxton, Cambridge, UK

Body

Single-minded 1 (SIM1) is a basic helix-loop-helix Per ARNT SIM transcription factor which is involved in the development and postnatal function of the paraventricular nucleus of the hypothalamus. We hypothesised that SIM1 insufficiency may contribute to human obesity based on the phenotype of SIM1 haploinsufficient mice which are obese and hyperphagic (1,2).

We sequenced the coding region of SIM1 in 1776 Caucasian patients with severe, early onset obesity recruited to the Genetics of Obesity Study and in 1690 ethnically matched population based controls from a large UK study, the Isle of Ely study. Non-synonymous mutations were significantly enriched in severely obese patients compared to controls ($p<0.002$). Thirteen different heterozygous missense mutations in SIM1 were identified in 22 unrelated patients. In extended family studies, SIM1 mutations co-segregated with overweight/obesity. Nine of the thirteen mutations significantly reduced the ability of SIM1 to activate a SIM1 responsive reporter gene in a luciferase reporter assay.

Our genetic and functional data provide direct evidence for a role for SIM1 in the control of energy homeostasis in humans. Metabolic phenotyping of SIM1 mutation carriers revealed an increased ad libitum food intake and normal basal metabolic rate. SIM1 mutation carriers had significantly lower systolic blood pressure measurements, showed attenuated rises in heart rate on waking and had increased respiratory quotients when compared to obese controls reflecting underlying autonomic dysfunction. These phenotypic characteristics are features which we have previously reported in melanocortin 4 receptor (MC4R) deficient patients (3,4). MC4R deficient patients have consistently been found to be taller than obese controls whereas SIM1 mutation carriers do not show this phenotype. Interestingly several patients also had neurobehavioural abnormalities with 13 of the 17 probands studied showing some degree of cognitive deficit.

We demonstrate that mutations in SIM1 represent a novel monogenic cause of human obesity. Although the target genes of SIM1 are currently unknown, the phenotypic similarity between patients with SIM1 and MC4R mutations suggests that SIM1 may regulate energy homeostasis by affecting melanocortin signalling. This is consistent with a body of evidence from murine models which suggest a role for SIM1 in central melanocortin signalling.

(1)Michaud, J.L., et al., Sim1 haploinsufficiency causes hyperphagia, obesity and reduction of the paraventricular nucleus of the hypothalamus. Hum Mol Genet, 2001. 10(14): p. 1465-73.
(2)Holder, J.L., Jr., et al., Sim1 gene dosage modulates the homeostatic feeding response to increased dietary fat in mice. Am J Physiol Endocrinol Metab, 2004. 287(1): p. E105-13.
(3)Farooqi, I.S., et al., Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. N Engl J Med, 2003. 348(12): p. 1085-95.
(4)Greenfield, J.R., et al., Modulation of blood pressure by central melanocortinergic pathways. N Engl J Med, 2009. 360(1): p. 44-52.

Nothing to Disclose: SR, AR, JK, EH, AC, SB, NW, SO, IB, MW, ISF

Pub #	P2-304
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Congenital Leptin Deficiency and Brain-Related Acute and Long-Term Effects of Leptin Therapy
Author String	S Frank, M Heni, A Moss, J von Schnurbein, A Fritsche, H-U Haring, S Farooqi, H Preissl, M Wabitsch University of Tübingen, Tübingen, Germany; International Max Planck Research School, Tübingen, Germany; Eberhard Karls University Tübingen, Tübingen, Germany; University of Ulm, Ulm, Germany; University of Cambridge, Cambridge, UK; University of Arkansas for Medical Sciences, Little Rock, AR
Body	Congenital leptin deficiency has a large impact on peripheral and central mechanisms. Patients with mutations in the leptin gene have undetectable leptin levels, are obese, hyperphagic and suffer from impaired satiety as well as from metabolic and immunological malfunctions. Treatment with recombinant human leptin can reverse these symptoms. There is growing evidence for brain related changes due to leptin therapy. We report the case of a 15 year old leptin deficient girl who has a homozygous transition in exon 3 of the LEP gene. Functional magnetic resonance imaging was performed before leptin therapy as well as shortly afterwards (3 days) and after 6 months. During the investigation the patient was stimulated with food (high and low caloric) as well as non food pictures. Results show acute and long term effects in the amygdala, the orbitofrontal cortex and in substantia nigra/ventral tegmental area for the comparison of food and non food pictures. For the comparison of high and low caloric pictures, acute effects in the ventral striatum and the orbitofrontal cortex could be observed as well as acute and long term effects in the hypothalamus. This study gives new insight in the influence of leptin therapy on brain functions in leptin deficiency. Nothing to Disclose: SF, MH, AM, JvS, AF, H-UH, SF, HP, MW

Pub #	P2-305
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Development of an Anti-Ghrelin Vaccine for Obesity Treatment
Author String	S Andrade, MC Carreira, A Ribeiro, L Teixeira, D Monteiro, M Lage, F Casanueva, MP Monteiro Instituto de Ci[ecirc]ncias BIomédicas Abel Salazar, Porto, Portugal; University of Santiago de Compostela, Santiago de Compostela, Spain; Instituto Salud Carlos III, Santiago de Compostela, Spain
Body	Ghrelin is a gut hormone that acts in the hypothalamus that stimulates appetite and decreases energy expenditure. Ghrelin plasma levels are generally low in obese subjects, but increase substantially with diet induced weight loss, which may contribute to the difficulty in maintaining a long-term weight loss. Some types of bariatric surgery are able to prevent weight loss induced rise in ghrelin levels that has been pointed as one on the reasons for the long-term success of the procedure. The purpose of this study was to develop a therapeutic anti-ghrelin vaccine to be used in the treatment of obesity, using "Virus Like Particles[rdquo] (VLP) as immunogenic substance to make possible the production of antibodies against endogenous molecules. We have developed an immunoconjugate of ghrelin chemically conjugated to the NS1 protein of the bluetongue virus (BTV) as immunogenic substance, which was inoculated in normal weight mice and mice with diet-induced obesity (DIO). The vaccinated normal weight mice developed increasing titres of anti-ghrelin antibodies, showed a statistically significant decrease in food intake (804,01 g immuneconjugate vs 838,91 g NS1 vs 823,09 g PBS $p<0.001$) and epididymal white adipose tissue (3.70±0.65 vs 2.24±0.31, $p=0.02$), together with a significant increase in energy expenditure (0.0146±0.001 immuneconjugate vs 0.0138±0.001 kcal/h/kg NS1 vs 0.0129±0.001 kcal/h/kg PBS,$p<0.05$) when compared to the control groups. In DIO mice, the vaccine also induced the production of increasing titres of anti-ghrelin antibodies, a decrease in food intake 24h after each immunization (66%, 82% and 50% for the vaccinated group for each immunization compared to the PBS control with $p<0.05$, $p<0.01$ and $p<0.05$, respectively). There was a statistically significant increase of energy expenditure for the vaccinated group (0.0207±0.001kcal/h/kg) when compared to the control groups (0.0159±0.002 kcal/h/kg for PBS and 0.0140±0.002 for NS1, $p<0.01$ e $p<0.05$). A decrease in NPY expression in the basal hypothalamus was found for vaccinated mice when compared control groups (vaccine 0.59±0.09; NS1 1.03±0.12; PBS 1.0±0.13; $p<0.05$).In summary, the anti-ghrelin vaccine progresses with the development of anti-ghrelin antibodies, decreases food intake, decreases hypothalamic orexigenic signals and increases energy expenditure. Therefore this therapeutic vaccine might become an alternative treatment tool to be used with diet and exercise for obesity. Sources of Research Support: UMIB is funded by grants from FCT (POCTI/FEDER), Portugal. Disclosures: ML: Study Investigator, Merck BV. Nothing to Disclose: SA, MCC, AR, LT, DM, FC, MPM

Pub #	P2-306
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Co-Administration of the Gut Hormones PYY and GLP-1 to Human Volunteers Reduces Food Intake and Brain Activation in Appetite Centers
Author String	V Salem, AD De Silva, CJ Long, AM Makwana, RD Newbould, EA Rabiner, MA Ghatei, SR Bloom, PM Matthews, JD Beaver, WS Dhillo Imperial College, London, UK; GlaxoSmithKline, London, UK
Body	The physiological post-prandial release of the gut hormones PYY and GLP-1 is implicated in triggering CNS mechanisms underlying satiety. However, the combined effects of PYY and GLP-1 on brain circuits underlying satiety in humans remain unknown. Objective: To determine changes in CNS neuronal activity following single and combined infusions of PYY and GLP-1, using blood oxygen level dependent functional magnetic resonance imaging (BOLD fMRI) in human volunteers. Methods: 15 normal weight adult volunteers underwent 5 treatment visits in a randomized, cross-over design. On each visit each subject received a 90 min intravenous infusion of one of the following: Saline following an 730kcal breakfast Saline following an overnight fast GLP-1 0.8 pmol/kg/min following an overnight fast PYY 0.3 pmol/kg/min following an overnight fast Combined GLP-1 & PYY at the above doses following an overnight fast Twenty minutes following the start of each infusion, fMRI data were acquired while participants viewed images of appetising and bland foods. An *ad libitum* buffet lunch was served to estimate caloric intake following the completion of each infusion. Results: On the visits when saline was infused, caloric intake at the buffet lunch was reduced by 25% when the participants had eaten breakfast. Gut hormone infusion reduced caloric intake in the fasted state compared to saline infusion (17%, 12% and 30% reductions for GLP-1, PYY and GLP-1+PYY respectively). On the fasted saline visit, appetising food images evoked greater BOLD signal relative to bland foods, in the putamen and insula. Single administration of GLP-1 or PYY alone attenuated the difference in BOLD response between appetising and bland foods in the fasted state. This was comparable with that observed in fed subjects receiving saline. Greater attenuation was observed in fasted subjects receiving co-administration of GLP-1 & PYY ($P<0.05$ cf saline). Conclusions: These data show for the first time in humans, that changes in CNS neuronal activity following co-administration of GLP-1 and PYY are similar to those observed physiologically after a meal. Sources of Research Support: Medical Research Council Clinical Training Fellowship awarded to VS; Wellcome Trust/GSK Translational Medicine Training Fellowship awarded to ADS; NIHR Career Development Fellowship awarded to WSD. Nothing to Disclose: VS, ADDS, CJL, AMM, RDN, EAR, MAG, SRB, PMM, JDB, WSD

Pub #	P2-307
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Functional Neuroimaging in Craniopharyngioma: Useful Tool To Better Understand Hypothalamic Obesity?
Author String	CL Roth, E Aylward, O Liang, G Pauley, N Kleinhans, E Schur University of Washington, Seattle, WA; University of Washington, Seattle, WA
Body	One of the most striking examples of childhood obesity is observed in children with craniopharyngioma (CP). Several factors leading to hypothalamic obesity (HO) in CP patients have been identified, but important unanswered questions remain, such as whether damage to homeostatic centers also alters reward-mediated eating behavior and whether central processing of satiety is affected. Energy homeostasis and appetite are critically regulated by hypothalamic nuclei, as well as by non-homeostatic processes (including cognition, emotion, and reward processing) that are directed primarily by corticolimbic and higher cortical brain regions We utilized functional magnetic resonance imaging (fMRI), a method of *in vivo* brain observation, to examine food-related activity in brain centers that control appetite. We hypothesized that hypothalamic damage due to CP and its treatment results in enhanced perception of food reward. Pre- and post-meal neuronal activity in brain regions of interest (ROI; bilateral N. accumbens, insula, striatum, and medial orbitofrontal cortex) was assessed by blood oxygen level dependent (BOLD) response to visual food cues in four CP patients vs. four age-matched controls. Participants viewed blocked photographs of food classified into fattening and non-fattening groups, alternating with non-food object blocks. Mean z-scores for ROIs were calculated for the contrast of fattening > non-fattening food. Subjects underwent the first fMRI scan, received a high-calorie test meal to suppress appetite, and had a second fMRI scan 30 min after the test meal. Before and after the scans, hunger ratings were performed and blood samples for insulin, peptide YY, and ghrelin were taken. Following the test meal, controls showed suppression of activation in ROIs while CP patients showed trends towards higher activation in ROIs. Post-meal changes of ghrelin correlated significantly with changes in activation in the N. accumbens and the striatum. These very preliminary data support our hypothesis that perception of food cues may be altered in HO, especially after eating. The fMRI approach may be applicable for performing future mechanistic studies of the brains' response to food in patients with HO. Nothing to Disclose: CLR, EA, OL, GP, NK, ES

Pub #	P2-308
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Inhibition of Acyl-Ghrelin Release in Healthy Young Adults during Euglycemic Hyperinsulinemic Clamp
Author String	R Nass, J Liu, S Pezzoli, L Farhi, B Gaylinn, M Thorner University of Virginia, Charlottesville, VA
Body	Ghrelin is a 28-amino acid peptide which has orexigenic and growth hormone (GH) releasing effects and is thought to play a role in energy homeostasis. Ghrelin is found in the circulation in two forms: acyl- and des-acyl. The orexigenic and GH releasing effects of ghrelin are thought to be mediated by acyl-ghrelin. We have previously reported that acute administration of insulin decreases circulating ghrelin levels in healthy older adults age >60 years (1). The aim of this study was to examine whether insulin has an inhibitory effect on acyl-ghrelin release in a group of healthy young adults and whether there is a dose dependent effect. Ten men age (mean ± SD) 23.1 ± 2.6 yr; BMI 24.5 ± 3.8 kg/m2 were studied in a single-blind, placebo-controlled study during three overnight admissions on the General Clinical Research Center of the University of Virginia. During a euglycemic hyperinsulinemic clamp, the volunteers received a 3-h insulin infusion (1 mU/kg/min or 0.4 mU/kg/min) or a 3-h saline infusion. Using an in-house two-site sandwich assay, acyl-ghrelin was measured in plasma every 10 min for 390 min (90 min before the start of the infusion, 180 min during the infusion and 120 min after the infusion was stopped). Serum insulin levels were measured every 20 min on an Immulite 2000. Results: Under euglycemic conditions, acyl-ghrelin concentrations were suppressed during both insulin infusions ($p<0.05$, repeated measure analysis). Mean (±SEM) insulin levels (uU/mL) during infusions were 2.7 ± 0.11 (saline), 20.52 ± 2.01 (0.4 mU/kg/min insulin) and 48.19 ± 4.99 (1 mU/kg/min infusion).Mean (±SEM) acyl-ghrelin levels (pg/mL) during infusions were 23.6 ± 1.2 (saline), 14.2 ± 0.48 (0.4 mU/kg/min insulin) and 12.4 ± 0.55 (1 mU/kg/min infusion). After the insulin infusion was stopped, acyl ghrelin levels started to recover within 30 min, independent of the insulin infusion rate. Conclusion: Insulin acutely inhibits the release of acyl-ghrelin in healthy young men. Recovery of acyl-ghrelin within 30 minutes suggests that there is an accumulation of acyl-ghrelin in a readily releasable pool during inhibition of release by insulin. Based on our previous data, the inhibitory effect of insulin is unrelated to the age of the study volunteers (1). (1) Nass R et al., Abstr. 2159, 90th Endocrine Society Meeting, SF,2008 Sources of Research Support: K23 RR018770 (to R.N.), M01 RR 00847 (to the General Clinical Research Center at the University of Virginia). Nothing to Disclose: RN, JL, SP, LF, BG, MT

Pub #	P2-309
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Circulating Vaspin and Visfatin Are Not Affected by Acute or Chronic Energy Deficiency or Leptin Administration in Humans
Author String	ES Kang, F Magkos, E Sienkiewicz, CS Mantzoros Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; Yonsei University College of Medicine, Seoul, Korea; Boston VA Healthcare System, Boston, MA; Harvard School of Public Health, Boston, MA

Body

OBJECTIVE: Animal studies indicate that prolonged fasting decreases serum vaspin concentration; leptin administration reserves this change, and has also been shown to stimulate the production and secretion of visfatin from adipose tissue in vitro. We thus examined whether circulating vaspin and visfatin are affected by short- and long-term energy deprivation and whether leptin administration in physiological and pharmacological doses affects their concentrations in human.
METHODS: We measured circulating levels of vaspin and visfatin 1) before and after 72-h of total starvation (inducing severe hypoleptinemia) with concomitant administration of either placebo or physiological replacement doses of recombinant methionyl human leptin (metreleptin 0.04-0.1 mg/kg/day for men, 0.08-0.2 mg/kg/day for women) in normal-weight men (n=6) and women (n=7), 2) before and after 72-h of total starvation (inducing severe hypoleptinemia) with concomitant administration of high doses of metreleptin (0.3 mg/kg/day) in fasting healthy subjects (n=13), 3) during chronic energy deficiency in 8 women with exercise induced energy deficiency and hypothalamic amenorrhea treated with metreleptin in physiological replacement doses for 3 months, and 4) during chronic energy deficiency in women with hypothalamic amenorrhea treated with either placebo or metreleptin in physiological replacement doses for 3 months.
RESULTS: Fasting for 72 h decreased leptin concentrations (to 21% of baseline, P=0.002) but had no significant effect on vaspin and visfatin levels (P=0.110 & P=0.071, respectively). Nor did normalization of leptin levels affect the concentrations of these adipokines (P=0.929 and P=0.999, respectively). Metreleptin administration in replacement doses to women with exercise induced chronic energy deficiency and hypothalamic amenorrhea did not significantly alter vaspin and visfatin concentrations, whether relative to baseline (P=0.262 & P=0.162, respectively) or relative to placebo administration (P=0.952 & P=0.921, respectively). Pharmacological doses of leptin in healthy lean and obese men and women did not affect circulating vaspin and visfatin levels (P=0.981 & P=0.907, respectively).
CONCLUSIONS: Circulating vaspin and visfatin are not affected by acute or chronic energy deficiency leading to hypoleptinemia and are not regulated by leptin in human subjects, indicating that these adipocyte-secreted hormonal regulators of metabolism are independently regulated in humans.

Sources of Research Support: Grant Number UL1 RR025758 and M01-RR-01032- Harvard Clinical and Translational Science Center, from the National Center for Research Resources. Funding was received from the National Institute of Diabetes and Digestive and Kidney Diseases grants DK58785, DK79929 and DK81913, AG032030, and the National Research Foundation of Korea Grant funded by the Korean Government MEST, Basic Research Promotion Fund) (NRF-2010-013-E0008).

Nothing to Disclose: ESK, FM, ES, CSM

Pub #	P2-310
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Binge Eating Behaviors Are Related to Insulin Sensitivity
Author String	JP Dunn, P Marks-Shulman, I Feurer, BW Patterson, J Kaiser, NN Abumrad Vanderbilt University School of Medicine, Nashville, TN; Vanderbilt University School of Medicine, Nashville, TN; Washington University School of Medicine, St Louis, TN
Body	Binge eating behaviors are common in obesity yet the reasons for this are uncertain. We hypothesized that binge eating behavior is related to insulin resistance. We studied 8 lean (59±7kg, 22±3 kg/m^2) and12 obese (108±17 kg, 41±6 kg/m^2) females with similar ages (41±9 vs 40±8yrs, p=0.651); Persons with depression, known psychiatric diagnoses, or taking medications that alter metabolism or appetite were excluded. Subjects completed the 16-item Binge Eating Scale (BES) and a 5-hour oral glucose tolerance test (OGTT). Higher BES scores indicate more binge eating behavior. Insulin sensitivity was calculated by OGTT minimal model technique. T -tests were used for between-group comparisons of means. Linear regression was used to determine relationship of BES scores to insulin sensitivity in all subjects and in each weight group. Multiple linear regression was used to determine relationship of BES scores to insulin sensitivity after adjusting for body mass index (BMI). Summary data are presented as mean± SD in lean vs obese, respectively. BES scores trended towards being lower in the lean (6±6 vs 11±6, p=0.077) while ranges were similar in both groups (1-17 vs- 3-20). Insulin sensitivity was higher in the lean subjects (11.2± 4.1 vs 3.9± 2.4, 10^{-4} min^{-1}/[micro] U/ml, p<0.001). BES was associated with insulin sensitivity in all subjects (r^2=0.413, b= -0.489, p=0.002, Fig.1), and in the lean (r^2=0.557, b= -0.550 p=0.034 vs r^2=0.199, b= -0.174, p=0.146 in the obese). After adjusting for BMI, BES was independently associated with insulin sensitivity in all subjects (model R^2=0.661 p<0.001; b(BES) = -0.298, p=0.024; b(BMI) = -0.262, p=0.003). BES, a measure of binge eating behavior, is negatively associated with insulin sensitivity even after adjusting for BMI. Sources of Research Support: NIEHS K12 ESO15855 (J.P.D.), NIDDK RO1-DK070860, (N.N.A.), VU CTSA (1 UL1 RR024975), VU DRTC (DK20593), WU Nutrition and Obesity Research Center (P30 DK56341). Nothing to Disclose: JPD, PM-S, IF, BWP, JK, NNA

Pub #	P2-311
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Association of Polymorphisms in the 3[prime] Untranslated Region (3[prime]UTR) of the Type 1 Histamine Receptor (*Hrh1*) with BMI and Fat Mass in Children and Adults
Author String	YD Salinas, M Sorouri, EA Stern, AH Ali, JA Yanovski National Institutes of Health, Bethesda, MD

Body

Background: Animal research suggests that central nervous system histaminergic tone plays an important role in energy homeostasis and body weight regulation. In rodents, disruption of HRH1 signaling by pharmacologically decreasing the CNS histamine pool leads to increased food intake and body fat.[1] Accordingly, *HRH1* knockout mice develop mature-onset obesity characterized by hyperphagia.[2] Therefore, we hypothesized that single nucleotide polymorphisms (SNPs) in the *HRH1* locus would be associated with changes in body mass index (BMI) and body fat mass.
Methods: The coding sequence, intron/exon borders, and UTRs of *HRH1* were sequenced in 51 lean and 50 obese children. For the current analysis, 590 children (age 1-17y; 12.1±3.2y; 54.5% female; 38.9% African American, 61.1% White) and 288 adults (40.3±10.9y; 74.7% female; 31.9% African American, 68.1% White) were genotyped for the identified SNPs in the 3'UTR. ANCOVAs compared subjects grouped by genotype for differences in BMI (adjusted for age, sex, and race) and fat mass (adjusted for age, sex, race and height).
Results: 3 previously identified SNPs--rs346070 (C/T), rs3732941 (T/C) and rs73123314 (G/A)--were found in the 3'UTR of *HRH1*. Adults with two minor alleles for rs3732941 (T/C) had significantly higher BMI and fat mass [BMI 41.34±2.93 for CC vs. 33.05±5.92 for TT+TC, p=0.005 and 44.80±4.70 for CC vs. 31.36±1.00 kg fat for TT+TC, p=0.005]. ANCOVA models, however, revealed no significant association (all p-values > 0.05) between any of the 3 SNPs and BMI-z scores or fat mass in children. Children with at least one minor allele for rs3732941 had similar BMI-z and fat mass to those with only the major allele [BMI-z 1.25±0.09 for TC+CC vs. 1.10±0.09 for TT, p=0.168 and 21.41±1.24 for TC+CC vs. 20.65±1.33 kg fat for TT, p=0.616].
Conclusion: rs346070 and rs73123314 were not associated with adiposity in children or adults and rs373294 was associated with BMI and fat mass only in an adult cohort. Since *HRH1* knockout mice develop mature-onset obesity, our data in adults are consistent with the possibility that rs3732941 may be associated with decreased *HRH1* expression in humans that might slowly affect food intake and thus body weight only in adulthood. As these effects are not paralleled in children, confirmatory studies in different and larger cohorts are needed to test this hypothesis.

1. Fukagawa, K., et al., Neuronal histamine modulates feeding behavior through H1-receptor in rat hypothalamus. Am J Physiol, 1989. 256(3 Pt 2): p. R605-11.
2. Masaki, T., et al., Involvement of hypothalamic histamine H1 receptor in the regulation of feeding rhythm and obesity. Diabetes, 2004. 53(9): p. 2250-60.

Sources of Research Support: The intramural program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, the Office on Research in Women's Health, the Office of Behavioral and Social Sciences Research, and the National Institute on Minority Health and Health Disparities.

Nothing to Disclose: YDS, MS, EAS, AHA, JAY

Pub #	P2-312
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Hypothalamic Signaling in Feeding Behavior & Metabolic Regulation (1:30 PM-3:30 PM)
Title	Liver Transplantation Normalizes Altered Circulating Ghrelin in Basal Conditions and during Glucose Tolerance Test in Liver Failure Patients
Author String	F Pita, MT Diz-Lois, F Suarez, J Garcia-Buela, S Sangiao-Alvarellos, T Martinez, O Vidal, F Cordido University Hospital A Coru[ntilde]a, A Coru[ntilde]a, Spain; University Hospital A Coru[ntilde]a, A Coru [ntilde]a, Spain; University Hospital A Coru[ntilde]a, A Coru[ntilde]a, Spain; University Hospital A Coru [ntilde]a, A Coru[ntilde]a, Spain; University of A Coru[ntilde]a, A Coru[ntilde]a, Spain
Body	Context: Ghrelin has important actions on feeding and weight homeostasis. Anorexia is a problem of paramount importance in patients with advanced cirrhosis, contributing to malnutrition. Experimental data exist, which suggest that ghrelin could protect hepatic tissue. Both fasting and post-oral glucose tolerance test (OGTT) ghrelin concentrations are controversial in liver cirrhosis and are unknown after liver transplantation. We have previously found significantly decreased fasting and post-OGTT plasma ghrelin levels in patients with liver failure who were candidates for transplantation when compared with control subjects. Objective: Our aim was to study fasting ghrelin concentrations and their response to an OGTT in liver failure patients before and after liver transplantation. Patients and methods: 21 patients (14 males and 7 females) with severe liver failure studied before (pretransplantation, PreT) and 6 months after liver transplantation (posttransplantation, PostT) were included, and 10 age- and body mass index-matched healthy or overweight subjects as the control group (Cont). After an overnight fast, 75 g of oral glucose were administered; glucose, insulin, and ghrelin were obtained at baseline and at times 30, 60, 90, and 120 min. Insulin and serum GH were measured by Immulite and total ghrelin was measured by RIA. The area under the curve (AUC) was calculated by a trapezoidal method. Intragroup comparisons were based on Wilcoxon test and comparisons between patients and controls were based on Mann-Whitney U test. Results: Fasting ghrelin (median and range, pg/ml) levels were lower in PreT: 539 (309-1262) than in Cont: 643 (523-2163), P=0.045. Fasting ghrelin levels increased after liver transplantation, 539 (309-1262) vs 910 (426-3305), for PreT and PostT respectively, P=0.001. The AUC of ghrelin (pg/ml min) was lower in PreT: 63 900 (37 260-148 410) than in Cont: 76 560 (56 160-206 385), P=0.027. The AUC of ghrelin increased in PostT, 63 900 (37 260-148 410) vs 107 595 (59 535-357 465), for PreT and PostT respectively, P=0.001. Fasting levels and the AUC of ghrelin were similar in PosT and Cont. Conclusion: Decreased fasting and post-OGTT ghrelin levels in liver failure patients were normalized after liver transplantation. The presence of decreased fasting and post-OGTT ghrelin levels could contribute towards anorexia or other complications, in patients with cirrhosis. Nothing to Disclose: FP, MTD-L, FS, JG-B, SS-A, TM, OV, FC

Pub #	P2-313
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Prolactin Promotes Liver Regeneration after Partial Hepatectomy
Author String	B Moreno-Carranza, C Vega, F Lopez-Barrera, G Nava, H Cruces-Solis, A Quintanar-Stephano, N Binart, G Martinez de la Escalera, C Clapp Instituto de Neurobiolgía, Campus UNAM- Juriquilla, Querétaro, Mexico; Centro de Ciencias Básicas, Autónoma de Aguascalientes, Aguascalientes, Mexico; INSERM - Faculté de Médecine Paris-Sud, Le Kremlin-Bic[ecirc]tre, France
Body	Liver regeneration after injury depends on the coordinated proliferation of hepatocytes and blood vessels. After partial hepatectomy (PH), the capillary blood vessels of the liver, or sinusoids, proliferate allowing nutrients and growth factors to reach the newly replicating hepatocytes. Prolactin (PRL) is a potent liver mitogen and a proangiogenic factor in different organs. PRL stimulates blood vessel growth either directly by promoting the proliferation of endothelial cells or indirectly by stimulating the production of proangiogenic factors like vascular endothelial growth factor (VEGF). Here, we studied the effects of PRL on liver growth and VEGF expression after 70% PH. Male Wistar rats were implanted with two anterior pituitary glands (AP) under the kidney capsule to increase circulating PRL levels, and after fifteen days they were subjected to 70% PH. AP-implanted rats showed a 10-fold increase in serum PRL levels compared to the non-grafted controls. Injection of grafted rats with the dopamine D2 receptor agonist bromocriptine, an inhibitor of PRL secretion, blocked the increase of serum PRL. On day two after PH, the high PRL levels in AP-implanted rats correlated with a significant increase in the ratio of liver to body weight as compared to sham and 70% PH controls. This increase was prevented by bromocriptine, indicating that it was due to the hyperprolactinemia-induced stimulation of liver growth. Moreover, real-time RT-PCR showed higher VEGF expression in the liver of AP-implanted rats, supporting the notion that PRL stimulation of liver growth involves angiogenesis. To characterize the role of endogenous PRL on liver growth, we performed the 70% PH in PRL receptor-deficient (PRLR-/-) and wild-type (PRLR+/+) mice. In agreement with the supporting role of PRL in liver regeneration, PRLR-/- mice showed smaller livers and reduced liver regeneration after PH compared to PRLR+/+ counterparts. In conclusion, PRL promotes liver regeneration, and this action may involve VEGF-induced angiogenesis. These findings indicate the potential of prolactin as an effective addition to therapies for improving hepatic function after liver resection and transplantation. Sources of Research Support: National Council of Science and Technology (CONACYT) Grant 127496. Nothing to Disclose: BM-C, CV, FL-B, GN, HC-S, AQ-S, NB, GMdlE, CC

Pub #	P2-314
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Prolactin Opposes the Effects of FoxO1 on Islet DNA Synthesis and Gene Expression
Author String	R Arumugam, D Fleenor, D Lu, E Horowitz, M Freemark Duke University Medical Center, Durham, NC; Duke University Medical Center, Durham, NC; Duke University Medical Center, Durham, NC
Body	The forkhead protein 1 (FoxO1) reduces islet beta cell replication and increases beta cell apoptosis. Down-regulation of FoxO1 is essential for beta cell compensation to insulin resistance; conversely, FoxO1 is over-expressed in the islets of diabetic adults. In recent studies we showed that prolactin (PRL), a beta cell mitogen reduces FoxO1 mRNA levels in isolated rat islets and insulinoma cells and decreases nuclear (active) FoxO1 protein levels after serum-starvation. We therefore hypothesized that PRL would oppose the effects of nuclear over-expression of FoxO1 and reverse its anti-mitotic and pro-apoptotic effects. To that end, we over-expressed (10-15x) a constitutively active nuclear form of FoxO1 in rat islets and examined its effects on [3H]-thymidine incorporation and cell cycle gene expression in the presence or absence of PRL. Over-expression of FoxO1 reduced [3H]-thymidine incorporation by 62% ($p<0.05$). Conversely, PRL increased thymidine incorporation by 84% ($p<0.05$) and blocked the effects of Fox. FoxO1 up-regulated the cyclin-dependant kinase inhibitor p27 (+33%; $p<0.01$) as well as BCL-2 (+58%; $p<0.001$) and BCL-6 (+256%; $p<0.001$) and down-regulated p21, cyclins B1 and E1, PDX1, and NKX6.1. In contrast PRL upregulated (+40-420%) cyclins A2, B1, B2, D2, cyclin-dependent kinase 1 (CDK1), IRS2, and PTTG1 (securin) and down-regulated (-20%) p27 and menin. PRL attenuated the effects of FoxO1 on p27, BCl-2 and BCL-6 but did not reverse the FoxO1-dependent decrease in PDX1. FoxO1 attenuated the effects of PRL on A and B cyclins, cyclin D2, and CDK1. The findings suggest a novel paradigm by which the lactogenic hormones (a) promote beta cell replication through induction of D, A, and B cyclins and CDK1; and (b) increase beta cell survival and prevent stress-dependent beta cell dysfunction through induction of PTTG1 and attenuation of FoxO1-dependent (p27 and BCL-6) gene expression. These findings may have implications for the pathogenesis and treatment of diabetes mellitus in pregnancy and other insulin resistant states. Sources of Research Support: NICHD (HD024192), American Diabetes Association (7-08-RA-46), and Pfizer Corporation (to MF) and the Duke Children's Miracle Network (to RA). Nothing to Disclose: RA, DF, DL, EH, MF

Pub #	P2-315
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Functional Impact of Manipulation in Relative Orientation of Human Prolactin Receptor Domains
Author String	W Liu, CL Brooks The Ohio State University, Columbus, OH; The Ohio State University, Columbus, OH

Body

Hormone binding creates active receptor dimers for class 1 cytokine receptors but the detailed molecular mechanism by which these receptors are activated by their ligands is not well characterized and it is unknown if these receptors share common mechanisms. A rotation model has been proposed for the activation of human erythropoietin receptor (hEPOr) (1) and human growth hormone receptor (hGHr) (2) and is supported by evidence showing that alanine additions at the junction of the transmembrane (TM) and intracellular (IC) domains and/or within the TM domain influenced receptor activities. This evidence suggests that alanine additions changed the relative orientations of the IC domains and their subsequent activation. We wished to determine if a similar mechanism was at play with human prolactin receptor (hPRLr). Up to four alanines were added between the TM and either the IC or extracellular (EC) domains to extend the TM helix and to rotate the IC or EC domains. Also, up to four glycines were placed between the TM and IC domains to provide increased flexibility between these two domains. Wild-type long-form hPRLr or various mutant receptors were expressed in human embryonic kidney 293T cells that express endogenous Janus kinase 2. In the absence of human prolactin (hPRL) none of the alanine or glycine additions increased receptor phosphorylation above that of wild-type hPRLr. In the presence of hPRL both wild-type hPRLr and each of the mutant receptors were successfully phosphorylated. We conclude that hPRLr is not activated by a similar rotation mechanism as hGHr and hEPOr. Neither is hPRLr activated by increased freedom of the IC domain provided by glycine additions. In a second set of experiments both wild-type hPRLr and either alanine or glycine extended receptors were co-expressed in 293T cells. In the absence of hPRL there was no detectable ligand-independent phosphorylation of hPRLr. This suggests that a piston movement between the hPRLr pair is not involved in their activation. These results also prompted us to re-evaluate the significance of ligand-independent hPRLr dimers.

(1) Constantinescu SN et al., Mol Cell 2001; 7:377
(2) Brown RJ et al., Nat Struct Mol Biol 2005; 12:814

Nothing to Disclose: WL, CLB

Pub #	P2-316
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Significance of Intermolecular Disulfide Linkage in Human Prolactin Receptor Ligand-Independent Dimerization and Ligand-Induced Activation
Author String	W Liu, CL Brooks The Ohio State University, Columbus, OH; The Ohio State University, Columbus, OH
Body	Receptor dimers in the absence of ligand have been demonstrated for human prolactin receptor (hPRLr) (1, 2) but the role of these ligand-free dimers is unclear. A population of the ligand-free hPRLr dimers was determined to be redox-sensitive by western blotting under reducing and non-reducing conditions (1). In this study, the role of inter-receptor disulfide linkage in the process of ligand-free dimerization of the long-form o the hPRLr was investigated. 12 cysteine residues in different domains of hPRLr were mutated to serine residues except for the four N-terminal extracellular cysteine residues critical for protein folding. In the absence of hPRL, the intracelluar (IC) cysteine residues, particularly C242, were shown to be the primary participants in intermolecular disulfide linkage in redox-sensitive hPRLr dimers. The removal of C184 and C225, the two unpaired cysteine residues close to and within the transmembrane domain, enhanced the formation of redox-sensitive hPRLr dimers, indicating inhibition of dimer formation by these two residues. Interestingly, C184 and C225 displayed a secondary role in the formation of intermolecular disulfide linkage when all IC cysteine residues were mutated to serine residues. However, this secondary disulfide linkage by C184 and C225 requires the presence of the box 1 portion of the IC domain. Finally, the removal of all 12 cysteine residues abolished the formation of ligand-free redox-sensitive hPRLr dimers. Given the complexity of the formation of intermolecular disulfide linkage in ligand-free hPRLr dimers, we next examined the role o these ligand-free redox-sensitive hPRLr dimers in ligand-induced activation. The hPRLr mutant where the formation of redox-sensitive ligand-free dimers was abolished was successfully activated by hPRL treatment, indicating that these redox-sensitive hPRLr dimers are not required for ligand-induced activation. Furthermore, wild-type redox-sensitive hPRLr dimers were not phosphorylated following hPRL activation, as evidenced on western blotting against phosphotyrosine under non-reducing condition. We conclude that ligand-free redox-sensitive hPRLr dimers do not participate in hPRL activation. (1) Quazi AM et al., Mol Endocrinol 2006; 20:1912 (2) Gadd SL et al., Mol Endocrinol 2006; 20:2734 Nothing to Disclose: WL, CLB

Pub #	P2-317
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Role of Endogenous Prolactin in Anterior Pituitary Cell Renewal
Author String	J Ferraris, F Boutillon, B Marie, S Adriana, G Vincent, P Daniel Instituto De Investigaciones En Reproducción, Ciudad Autonoma De Buenos Aires, Argentina; Inserm U845 -Research Center Growth and Signaling- PRL/GH Pathophysiology, Paris, France

Body

The anterior pituitary (AP) gland is under a constant cell turnover. In female rats, this process appears to be coordinated by circulating levels of gonadal steroids (1). During proestrus, when AP apoptosis rate is the highest, a peak in circulating levels of prolactin (PRL) occurs. PRL receptor (PRLR) KO mice show an increase in pituitary weight and it was observed that PRL decreases lactotroph proliferation in vitro (2). Our hypothesis is that PRL participates in AP cell turnover, regulating proliferation and/or apoptosis rate. The aim of this study was to investigate the effect of endogenous PRL on AP proliferation and apoptosis. Wild type (WT) and transgenic mice (TG) expressing the pure PRLR antagonist del1-9-G129R-hPRL (3) were injected with bromodeoxyuridine (BrdU). Pituitary were obtained, weighed and processed to evaluate proliferation (BrdU incorporation by immunohistochemical detection) and apoptosis (TUNEL assay). In females, there was no difference of pituitary weight between both genotypes. In contrast, pituitary weight of male TG mice was higher than those from WT mice (WT 1.89 mg ±0.06, TG 2.23 mg ±0.16, $p<0.05$ Mann Whitney test). AP proliferation index was higher in male TG mice than in WT males (WT 0.083%, TG 0.19%, $p<0.01$, [chi][sup2]), whereas in female TG mice the percentage of BrdU positive cells decreased with respect to WT females (WT 1.28% TG 0.57%, $p<0.01$ [chi][sup2]). The apoptotic index did not differ between WT and TG male or WT and TG female mice. PRL activity is mediated by different PRLR subtypes, and their expression could be modulated by gonadal steroids. We investigated expression of the various PRLR isoform in AP. Also, we studied the effect of PRLR antagonist on PRLR expression (long and short isoforms S1, S2, S3). Quantitative PCR was performed using specific primers for each PRLR isoform (4). The main isoform expressed in male and female AP is the long isoform, in contrast to the liver where the short isoform predominates. The presence of the PRLR antagonist modified the expression of PRLR isoforms in AP of both genders. In females, we also observed that the ratio of long versus short isoforms fluctuated along the estrous cycle.

In summary, the chronic blockade of PRLR induces changes in AP cell proliferation indicating that PRL is involved in AP cell renewal. Additionally, changes in AP PRLR expression observed in mice expressing the PRLR antagonist del1-9-G129R-hPRL, suggest a regulatory role of PRL on its own activity in the AP.

1. Zárate S, Zaldivar V, Jaita G, Magri L, Radl D, Pisera D, Seilicovich A. Front Horm Res. 2010;38:25-31.
2. Schuff KG, Hentges ST, Kelly MA, Binart N, Kelly PA, Iuvone PM, Asa SL, Low MJ., J Clin Invest. 2002 110:973-81
3. Rouet V, Bogorad RL, Kayser C, Kessal K, Genestie C, Bardier A, Grattan D, Kelder B, Kopchick JJ, Kelly PA, Goffin V., PNAS 107(34):15199-204
4. Ben-Jonathan N, LaPensee CR, LaPensee EW., Endocr Rev. 2008, 29(1):1-41.

Sources of Research Support: PICT 092 Agencia Nacional de promoción de ciencia y técnica (ANPCYT) (PICT 092),Universidad de Buenos Aires UBACYT (M057), INSERM-CONICET project cojoint de cooperation international 2009-2010, Association for International Cancer Research (AICR grant #05-0603), the Institut National de la Santé et de la Recherche Médicale and University Paris Descartes.

Nothing to Disclose: JF, FB, BM, SA, GV, PD

Pub #	P2-318
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	The Effect of Cleaved Prolactin on Involution of the Mouse Mammary Gland
Author String	M Maehara, M Suzuki, M Ishida, T Harigaya Meiji University, Kawasaki, Japan
Body	Prolactin (PRL) is a peptide hormone that mainly secreted from PRL cells of the anterior pituitary gland. Furthermore, PRL has also been found in variety of non-pituitary tissues including mammary gland (MG). It is well known to the important role of PRL in growth and differentiation of MG during pregnancy and lactation. However, the effect of PRL on the postlactating MG involution is not well established. On the other hand, cleaved PRL, one of PRL variants, has been well known to be an antiangiogenic factor in cancer, and induce apoptosis-mediated vascular endothelial involution. Speculating on the role of cleaved PRL in normal mouse MG in various physiological stages, we postulated that 23kDa PRL in mammary epithelial cells could be cleaved, and this cleaved PRL might induce apoptosis and involution of MG after weaning. In the present study, we performed several experiments including western blotting and immunofluorescent staining to determine the presence of cleaved PRL and its possible protease Cathpsin D (CathD) in postweaning MG. Following results were obtained; (1) CathD cleaved 23kDa PRL into 16kDa PRL at pH3.5, and the cleavage site was revealed between 147 and 148 residue by N-terminal amino acid sequence analysis of cleaved fragments. (2) 23kDa PRL was observed in basement membrane of acini. (3) 23kDa PRL was cleaved by incubation with microsomal fraction of postweaning mouse MG. (4) Cleaved PRL existed in Golg apparatus, and cleaved PRL and CathD were co-localized in basement membrane of mammary epithelial cells (5) Cleaved PRL was detected in culture medium after incubation of day 14 lactating mouse MG tissues with the presence of 23kDa PRL. (6) Mammary epithelial apoptotic cells were increased and cleaved PRL was detected in these cells of day 3 postweaning mouse MG. In the mouse MG, it was revealed that 23kDa PRL was cleaved by CathD. The cleaved PRL was confirmed ir Golgi apparatus, and also existed in extracellular fluid after incubation with 23kDa PRL and MG. Therefore, our results could not describe clearly whether 23kDa PRL was cleaved in intracellular or extracellular fluid in postweaning mouse MG. On the other hand, these results suggest the possibility that 23kDa PRL was cleaved by CathD in postweaning mouse MG, and the cleaved PRL seemed to involve in process of apoptosis-mediated MG involution. Nothing to Disclose: MM, MS, MI, TH

Pub #	P2-319
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Analysis of Cleaved Prolactin in Heart and Cardiomyocyte
Author String	R Nakajima, H Nakamura, K Yanagiya, M Suzuki, M Ishida, T Harigaya Meiji University, Kawasaki, Japan
Body	Cleaved prolactin (PRL), one of PRL variants, has been known to be an antiangiogenic and proapoptotic factor. Recent study reported that cleaved PRL might involve peripartum cardiomyopathy (PPCM). The PPCM is characterized by systolic heart failure in late stage of pregnancy up to five months postpartum, but its etiology is unknown yet. An experimental specific STAT3 deletion in cardiomyocyte induced PPCM in female mice. The activity of protease to cleave native PRL was enhanced in this mouse. Furthermore, the presence of cleaved PRL in serum was shown in PPCM patients. However, it is unclear whether cleaved PRL involves on the pathogenic mechanism of PPCM. In this study, we investigated to determine cleaved PRL in hearts of female mice during peripartum, and its effect on H9c2 cell line of rat caridiomyocyte. The hearts were obtained from virgin (VG), postpartum (PP), and 10 days after postpartum (L10) of female mice. Heart weights were enlarged in PP and L10 mice. The analysis of PRL and PRL-receptor (PRL-R) mRNA expressions by Real Time RT-PCR revealed that PRL mRNA was not detected in either sample. However, PRL-R mRNA was detected and decreased to L10. PRL and cleaved PRL were detected in all samples by western blotting. These levels were increased in PP and L10 To investigate the localization of cleaved PRL in heart, we performed immunohistochemical procedures with lectin staining which specifically indicated capillary endothelial cells. The cleaved PRL localization was shown in capillary endothelium in all groups. We also examined mRNA expressions of PRL and PRL-R in H9c2 cells and virgin rat hearts by RT-PCR. PRL expression was not detected in either sample. PRL-R expression was detected in rat heart, but not in H9c2 cells. In evaluation of cleaved PRL on H9c2 in culture by determination of mitochondrial activity, there was no difference in its value between cleaved PRL and control. In present result, PRL gene is not expressed in heart, but PRL-R exists in cells except cardiomyocyte. However, cleaved PRL is present in normal heart, and localized in capillary endothelium. Therefore, cleaved PRL might involve the capillary degradation, but not direct damage to cardiomyocyte. Sources of Research Support: Grant from the Ministry of Health, Labour and Welfare, Japan. Nothing to Disclose: RN, HN, KY, MS, MI, TH

Pub #	P2-320
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Cux2 Is a Transcriptional Regulator of Growth Hormone-Dependent, Sex-Specific Genes in Female Mouse Liver: Adenoviral Expression and ChIP-Seq Analysis
Author String	T Peters, Y Zhang, DJ Waxman Boston University, Boston, MA
Body	Adenoviral vectors have a strong tropism for hepatocytes, and can be used to infect liver cells in vivo. We have found that the efficiency of infection can be greatly improved using scid immunodeficient mice. Using this approach we investigated the role of Cux2, a growth hormone-regulated, female-specific transcription factor with repressor activity [1], in sex-specific gene expression in adult mouse liver. Male mice, which are deficient in Cux2, were injected with adenovirus expressing Cux2 (Ad-Cux2) and killed 5 d later. Genome-wide transcriptional profiling revealed that Ad-Cux2 down regulated 211 male-specific genes and up regulated 141 female-specific genes, i.e., ~30% of all sex-specific genes. In contrast, only 4 male-specific genes were up regulated and 11 female-specific genes down regulated by Ad-Cux2. In other studies, ChIP-Se analysis identified 472 Cux2 binding sites in untreated female mouse liver. These sites were absent in male liver, and 86% overlapped with previously identified [2] regions of DNase hypersensitivity (open chromatin). De novo motif discovery using the Cux2-bound sequences identified a Cux2 binding motif, consensus sequence A/GATCAAT, that is most closely related to the motif for HNF6 (E value < 4 x 10-9) and more distantly related to PBX1 and Cux1/CDP motifs (E value = 10-5 ~10-6). ChIP-qPCR verified Cux2 binding ir adult female but not male liver for several genomic regions identified by ChIP-Seq (sites nearby Meis1, Fh1, Cmah, and Cml5), consistent with the absence of Cux2 in adult males. In immature mice, where Cux2 is expressed in both sexes, Cux2 was bound to these sites in both female and male liver. 65 of the 472 Cux2 binding sites showed greater DNase hypersensitivity in male than female liver (4.93-fold enrichment) but only 2 sites showed female-enriched DNase hypersensitivity. Cux2 may thus enforce liver sex-specificity by binding to male-enriched hypersensitivity sites in female liver leading to repression of the associated male-specific genes. Supporting this proposal, Gene Set Enrichment Analysis revealed that genes nearby Cux2 binding sites are significantly enriched in transcripts repressed by Ad-Cux2 (enrichment score = 2.6, p = 0). Furthermore, the binding of Cux2 in prepubertal male mouse liver suggests that Cux2 contributes to the prepubertal repression that is a common characteristic of many of the genes showing male-specific, growth hormone-regulated expression in adult liver. [1] Laz EV, Holloway MG, Chen CS, Waxman DJ. Characterization of Three Growth Hormone-Responsive Transcription Factors Preferentially Expressed in Adult Female Liver. (2007) Endocrinology 148: 3327-3337. [2] Ling G, Sugathan A, Mazor T, Fraenkel E, Waxman DJ. Unbiased, genome-wide in vivo mapping of transcriptional regulatory elements reveals sex differences in chromatin structure associated with sex-specific liver gene expression. Mol. Cell Biol. 2010 Dec; 30(23):5531-44 Sources of Research Support: In part by NIH grant DK33765 (to DJW). Nothing to Disclose: TP, YZ, DJW

Pub #	P2-321
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Somatotropes Lacking Leptin Receptors Secrete Less Growth Hormone (GH) and Cannot Be Restored or Rescued, *In Vitro*, by Growth Hormone-Releasing Hormone (GHRH)
Author String	MM Syed, C Crane, AC Haney, MA Cozart, AK Odle, N Akhter, GV Childs University of Arkansas for Medical Sciences, College of Medicine, Little Rock, AR
Body	Pituitary somatotropes are 30-40% of adult rodent pituitary cell populations. Their percentages can be reduced to 15% by overnight food deprivation, and then restored, in vitro by 1 h exposure to 100-pg/ml leptin. This suggests that leptin is important in the maintenance of somatotropes in the adult (1). Similarly, when we deleted leptin receptor, exon 17 in somatotropes, with Cre-loxP technology (2), mice became growth hormone deficient and the % of somatotropes declined to 10-15% of the population. This report describes studies of cultured pituitary cells from these deletion mutant mice, designed to determine if somatotrope numbers or secretion could be restored, in vitro. Freshly dissociated pituitary cells from 3-6 month old male deletion mutants and littermate controls (n=3 mice/group X 4 experiments) were plated overnight and then stimulated for 3 h with 0, 0.3, 3, 10, or 30 nM growth hormone releasing hormone (GHRH) or 1-10 nM leptin. Media were collected for GH immunoassay, with multiplex kits and the Luminex system (Millipore). The cells were fixed and immunolabeled for GH with 1:225,000 anti-rGH (A Parlowe, NIDDK Hormone Distribution Office). Counts of GH cells from control mice showed that 0.3 nM GHRH stimulated an increase in percentages of immunolabeled somatotropes from 26±2% (vehicle control) to 38±3% ($p<0.02$). There was a further dose-dependent increase to 40-45% in cultures exposed to 3, 10 or 30 nM GHRH ($p<0.01$). Control mice cultures stimulated with 1-10 nM leptin showed an increase in GH cells to 35-38% ($p<0.05$). Control cultures secreted 1.5-3X more GH in response to GHRH ($p<0.01$), but not to leptin. In contrast, cultured cells from deletion mutant mice lacking leptin receptors in somatotropes contained 15±1.5% immunolabeled somatotropes, which correlated well with the 50% lower basal GH secreted by this population ($p<0.01$). As expected from their lack of leptin receptors, they did not respond to leptin. Concentrations of 0.3-10 nM GHRH did not restore either numbers of somatotropes or their secretion of GH. However, 30 nM GHRH stimulated GH secretion 2--fold to levels that were 110% of those secreted by control cultures ($p<0.05$). These findings correlate with our in vivo evidence for low serum GH in these deletion mutants (2). The results demonstrate leptin's importance to somatotropes. Because GHRH cannot rescue or restore the deletion mutant GH cell population, in vitro, leptin must be a partner in maintaining GH cells. (1) Crane C et al., J Histochem Cytochem 2007 55: 1059-1073 (2) Childs GV et al., Endocrinology 2011; 152: 69-81 Sources of Research Support: NIH 1R01HD059056 awarded to GVC, NA; NIH NCRR P20 RR020146 Project IV awarded to NA, MS, GVC mentor; NIH P30 NS047546 (core); NIH R03 HD059066 awarded to GVC. Nothing to Disclose: MMS, CC, ACH, MAC, AKO, NA, GVC

Pub #	P2-322
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	The Discovery of Esterase 1 as a Novel Transcriptional Repressor of Growth Hormone Receptor Gene Expression: A Unique Non-Catalytic Role for a Carboxyesterase Protein
Author String	AK Pasupulati, J Sun, P Goel, T Maures, C Lu, R Menon University of Michigan, Ann Arbor, MI; University of Michgan, Ann Arbor, MI
Body	In addition to being essential for postnatal growth, pituitary growth hormone (GH) also exerts major roles in the metabolism of fat, protein, and carbohydrates in mammals. These pleiotropic actions of GH result from engagement with the GH receptor (GH-R). To identify novel factors that transduce free fatty-acid's (FFA) effects on the GH-R promoter, a cDNA phage expression library was screened to identify Esterase 1 (ES1) as a protein binding to the FFA-response element, L2-D2, in the murine GH-R L2 promoter. ES1 (*Ces1c*; *Ces-N Es1*; BC028907; NM_007954; MGI 95420) belongs to the ubiquitously expressed mammalian carboxyesterase multigene superfamily of α,β-hydrolase fold proteins. Ectopically expressed ES1 inhibited GH-R promoter activity. Catalytically inactive ES1 protein retained inhibitory activity on the GH-R promoter thus excluding the possibility that the effect on the GH-R promoter was via an indirect effect of ES1 on the intracellular metabolism of FFA. Ectopic expression of ES1 in 3T3-F442A pre-adipocytes inhibited the expression of endogenous GH-R mRNA and protein. Cell fractionation experiments and confocal microscopy established that ES1 localizes both to the cytoplasm and the nucleus. Experiments demonstrated CRM-1 dependent export of ES1 from the nucleus and the presence of a nuclear export signal in ES1. The domain of ES1 responsible for the effect on the GH-R promoter was localized to the C-terminal portion of the ES1 protein. Chromatin immunoprecipitation experiments demonstrated specific binding of ES1 to the FFA-response element in the GHR promoter. The in vivo significance of ES1's effect on GH-R expression was suggested by the finding that, in high-fat diet vs normal chow fed mice, the increase in steady state abundance of liver ES1 mRNA correlated with decreased liver GH-R mRNA expression. We conclude that ES1 is a novel trancriptional repressor of GH-R gene expression. Furthermore, our results establish a unique non-catalytic role for a carboxyesterase, and thus expand the potential biological roles of this protein superfamily. Nothing to Disclose: AKP, JS, PG, TM, CL, RM

Pub #	P2-323
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Identification of the GH Signaling Protein SH2B1β as a Focal Adhesion Protein That Regulates Focal Adhesion Size and Number
Author String	NJ Lanning, H-W Su, LS Argetsinger, C Cater-Su University of Michigan Medical School, Ann Arbor, MI; University of Michigan Medical School, Ann Arbor MI
Body	The adaptor protein SH2B1ß is a GH signaling molecule that is recruited to JAK2 in response to GH, leptin and prolactin. We have shown that SH2B1ß modulates processes involving regulation of the cytoskeleton, including cellular motility and differentiation. To describe more fully how SH2B1ß regulates cell motility, we assessed the subcellular localization of GFP tagged WT and mutated SH2B1ß using confocal microscopy of living cells. SH2B1ß, but not SH2B1ß(R555E) lacking a functional SH2 domain, localized to focal adhesions Consistent with the presence of SH2B1ß in focal adhesions, SH2B1ß coprecipitated with the focal adhesion protein talin. Focal adhesions contain multiple protein kinase C (PKC) isoforms that regulate focal adhesion formation and cell motility. SH2B1ß has been shown to be phosphorylated in response to phorbol 12-myristate 13-acetate (PMA)-induced PKC activation. We therefore investigated whether PKC regulates SH2B1ß in focal adhesions. We found PMA to induce a rapid redistribution of SH2B1ß out of focal adhesions. Serines 161 and 165 in SH2B1ß lie within consensus PKC substrate motifs. Mutating serines 161 and 165 to alanines abrogated PMA-induced redistribution of SH2B1ß out of focal adhesions and significantly decreased the dynamic cycling of SH2B1ß into and out of focal adhesions, the latter assessed using FRAP (fluorescence recovery after photobleaching). Mutating Ser 161 and 165 to alanine also increased the size of focal adhesions. GH stimulation increased the dynamic cycling of SH2B1ß WT into and out of focal adhesions, an effect that was lost when serines 161 and 165 were mutated to alanines. In contrast to the Ser to Ala mutations, mutating Ser165 to glutamate enhanced the dynamic cycling of SH2B1ß; GH was unable to further enhance the cycling. Mutating Ser165 to glutamate also decreased the amount of SH2B1ß in focal adhesions and significantly increased the number of focal adhesions per cell. Consistent with the above changes in focal adhesions, mutating serines 161 and 165 to alanines impaired the ability of SH2B1ß to enhance GH-mediated macrophage migration. Together, these results suggest that SH2B1ß is a novel focal adhesion protein whose cycling into and out of focal adhesions regulates focal adhesion number and size. The dynamics of SH2B1ß-dependent cycling are regulated by both GH and PKC, and appear to be critical for the ability of SH2B1ß to enhance GH-dependent cell motility. Sources of Research Support: NIH RO1-DK54222, T32-HD007505, and a University of Michigan Rackham Regents Fellowship. Nothing to Disclose: NJL, H-WS, LSA, CC-S

Pub #	P2-324
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Targeted Abrogation of Growth Hormone (GH) Action in Macrophages Impairs Insulin Sensitivity and Decreases $CD4^+$ $FoxP3^+$ T (Treg) Cells in Adipose Tissue
Author String	C Lu, AK Pasupulati, C Lumeng, Y Fan, MA Sperling, RK Menon University of Michigan, Ann Arbor, MI; University of Pittsburgh, Pittsburgh, PA

Body

Recent studies reveal that diet induced obesity (DIO) is characterized by decreased numbers of anti-inflammatory $CD4^+$ $FoxP3^+$ T (Treg) cells and increased proinflammatory macrophages (M1) in adipose tissue (AT), changes that correlate with DIO associated insulin resistance (IR). In this context, it is posited that AT macrophages (ATM) are key regulators of AT T cell (ATT). However, there is paucity of knowledge of factors that regulate the interaction between ATM and ATT. We previously reported on GH-dependent regulation of ATM function including the expression and secretion of ATM cytokines & chemokines (Endocrinology 2010: 151; 2189). Based on these prior results we hypothesized that GH action on ATM modulates the ATM-ATT interaction. To test this hypothesis, we analyzed ATT in a macrophage-specific GH receptor KO (MacGHR KO) model. At 24 wk of age, MacGHR KO & control (Cntrl) mice fed normal diet (ND) had similar intraperitoneal glucose tolerance test (ipGTT) profiles. In contrast at 60 wk of age, MacGHR KO on ND exhibited impaired ipGTT and insulin tolerance test (ITT) profiles. Impaired GTT & ITT in the MacGHR KO was accompanied by a decreased number of AT $CD4^+$ $FoxP3^+$ Treg cells compared to Cntrl mice(24±2.9% vs 13±2.6% [mean±SEM], Cntrl vs MacGHR KO; $p<0.05$). Similarly, following 26 wk on high fat diet (HFD), MacGHR KO showed significant decrement in $CD4^+$ $FoxP3^+$ Treg (23.5± 2.8% vs10±0.9% [mean±SEM], Cntrl vs MacGHR KO; $p<0.05$) and impaired glucose tolerance. The decrement in $CD4^+$ $FoxP3^+$ Treg cells was tissue-specific since the splenic content of these cells remained similar in MacGHR KO vs Cntrl. Targeted PCR arrays revealed that CCL3/MIP-1α was expressed at higher level in MacGHR KO mice on HFD. Likewise, CXCL9 was expressed at a higher level in 60 wk old MacGHR KO mice on ND. Since both these cytokines are produced by proinflammatory M1 macrophages, our cumulative results, including the decreased number of anti-inflammatory Treg cells, suggest that in the absence of GH action on the ATM, inflammation in the AT compartment is increased. We conclude that GH regulates the cytokine profile of ATM, and thereby modulates interaction(s) between ATM and ATT. These results in mice provide novel insights into the non-growth promoting roles of GH in the maintenance of metabolic homeostasis in the human adult and the elderly. We propose that administration of GH could have salutary effects on the chronic inflammation and IR associated with DIO.

Nothing to Disclose: CL, AKP, CL, YF, MAS, RKM

Pub #	P2-325
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Growth Hormone-Induced JAK2 Signaling and GH Receptor Downregulation: Role of GH Receptor Intracellular Domain Tyrosine Residues
Author String	L Deng, J Jiang, SJ Frank University of Alabama at Birmingham, Birmingham, AL; University of Alabama at Birmingham, Birmingham, AL; Birmingham VA Medical Center, Birminhgam, AL
Body	Growth hormone receptor (GHR) is a transmembrane glycoprotein of the cytokine receptor superfamily. GH binding to GHR at the cell surface activates the receptor-associated cytoplasmic tyrosine kinase, JAK2, and downstream ERK, PI3 kinase, and STAT5 signaling pathways to promote pleiotropic metabolic and somatogenic effects. GHR has six conserved tyrosine residues in its intracellular domain (ICD); phosphorylation of several of these tyrosines is required for GH-induced STAT5 activation, but not for JAK2 activation. We previously demonstrated by reconstitution of a GHR-deficient human fibrosarcoma cell that GH-dependent downregulation of a porcine GHR with all ICD tyrosines mutated to phenylalanine (GHR-MYFc8) is markedly reduced compared to wild-type porcine GHR (WT GHR) (1). We now address further the consequences of selective abrogation of GHR tyrosine phosphorylation on the kinetics of GH signaling and the GHR's cellular itinerary by comparing cells stably expressing GHR-MYFc8 vs. WT GHR. Stability of cell surface (mature) GHR in the absence of ligand stimulation was assessed by anti-GHR immunoblotting of cells treated with cycloheximide to block new GHR synthesis. The half-life of GHR-MYFc8 did not differ from WT GHR under these conditions, indicating similar stability of GHRs that reach the cell surface. Similarly, GHR-MYFc8 and WT GHR were equally susceptible to phorbol ester-induced metalloproteolysis, indicating that the conformation of the proximal extracellular domain (ECD) was unchanged by the ICD tyrosine mutations. GH-induced GHR disulfide linkage, which occurs via an ECD cysteine residue near the membrane was also unchanged, indicating GH engages the mutant GHR similarly to WT GHR. Notably, however, GH-induced JAK2 tyrosine phosphorylation was markedly prolonged in cells expressing GHR-MYFc8 compared to WT GHR. Thus, reduced GHR downregulation apparently potentiates continued JAK2 activation. Furthermore, GHR internalization was assessed with a surface biotinylation-based assay and acute (< 15 min) GH-induced GHR-MYFc8 internalization was significantly impaired compared to WT GHR. These data indicate that mutation of all ICD tyrosines to phenylalanine does not alter the receptor's ECD conformation, GH engagement, or acute GHR triggering, but severely impairs GH-induced GHR downregulation at the early internalization stage, thereby prolonging activation of JAK2, presumably at the cell surface. (1) Deng L, et al., Mol Endocrinol 2007; 21:1537 Sources of Research Support: NIH R01 DK58259 (SJF) and VA Merit Review (SJF). Nothing to Disclose: LD, JJ, SJF

Pub #	P2-326
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Use of Phosphoproteomic Analysis To Identify Novel Growth Hormone Signaling Proteins in 3T3-F442A Preadipocytes
Author String	BN Ray, HK Kweon, LS Argetsinger, PC Andrews, C Carter-Su University of Michigan Medical School, Ann Arbor, MI; University of Michigan Medical School, Ann Arbor MI
Body	To further our understanding of GH signaling networks and identify novel GH signaling pathways and responses, we applied mass spectrometry-based phosphoproteomics to identify proteins phosphorylated in response to GH. Proteins in 3T3-F442A preadipocytes were labeled by stable isotope labeling with amino acids in cell culture (SILAC). Following GH treatment (0 and 5 or 15 min), Tyr-phosphorylated tryptic peptides were isolated by immunoaffinity purification (IAP). Peptides in the IAP flow-through were fractionated using a strong cationic exchange (SCX) column and then enriched for Ser and Thr phosphopeptides using a ZrO2 column. Fractions were analyzed using a nanoLC LTQ-Orbitrap mass spectrometer (Thermo). After 5 min GH treatment, we identified 2127 phosphorylation sites. Of these, 168 were GH responsive (>20% increase or decrease; $p<0.05$). In a separate experiment with 15 min GH treatment, we identified 428 phosphorylation sites. Of these, 45 were significantly changed by GH. Several of these were known to be GH-dependent phosphorylation sites, but the vast majority were novel GH-regulated sites. Known sites include the activating Thr/Tyr in Erks 1/2 and the activating pTyr in Stats 5a and 5b. Immunoblotting confirmed the GH sensitivity for five of the novel GH-dependent sites for which phosphospecific antibodies are available. These include: 1) Tyr423 in Shc1 implicated in binding grb2 and initiating the Ras/Erk1/2 pathway; 2) Ser863 of regulatory associated protein of mTOR (raptor) whose phosphorylation acts as a switch to enhance raptor phosphorylation; 3) Thr246 of Proline Rich Akt Substrate, 40kD (PRAS40) whose phosphorylation enhances mTORC1 activation; 4) Ser455 of ATP-citrate lyase (ACLY) whose phosphorylation activates ACLY synthesis of cytosolic acetyl-CoA; and 5) Ser707 of Na^+/H^+ exchanger-1 (NHE1), which increases NHE1 activity when phosphorylated by Rsk90. Motif analysis of the GH-dependent phosphorylation sites revealed that after 5 min with GH, the most commonly phosphorylated motif was a PKA/Akt consensus site (RXTXXS/T). Consistent with this analysis, ACLY and PRAS40 are substrates of Akt. Raptor is also downstream of Akt although not a direct substrate. By 15 min, the most commonly detected phosphorylation sites were WW GroupIV binding sites (pS/pTP). This study provides evidence that SILAC-based phosphoproteomic analysis can detect changes in phosphorylation as low as 20% to provide significant insight into GH signaling. Sources of Research Support: NIH RO1-DK34171; P41-RR18627. Nothing to Disclose: BNR, HKK, LSA, PCA, CC-S

Pub #	P2-327
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Growth Hormone Signaling Facilitates Muscle-Fat Cross-Talk to Mediate Insulin Resistance
Author String	A Vijayakumar, Y Wu, H Sun, C Liu, S Yakar, D LeRoith Mount Sinai School of Medicine, New York, NY; National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD
Body	Growth hormone (GH) modulates insulin sensitivity in a tissue specific manner. Liver-specific knockout of the growth hormone receptor (GHR) in mice resulted in increased hepatic triglyceride (TG) content, hepatic steatosis, impaired TG secretion and insulin resistance[1]. We generated a skeletal muscle-specific GHR knockout mouse model (mGHRKO) that displayed enhanced insulin sensitivity when fed regular chow and resistance to high-fat diet (HFD) induced insulin resistance. Hyperinsulinemic - euglycemic clamps revealed that the improvement in insulin sensitivity in the mGHRKO mice was due to increased insulin-stimulated glucose disposal (96% more than the controls) as well as a greater extent of suppression of hepatic glucose production (37% more than the controls). Additionally, while control mice developed hepatic steatosis when fed HFD, mGHRKO mice did not. Interestingly, mGHRKO mice also demonstrated significantly reduced fat mass, and the cross-sectional area of adipocytes from mGHRKO mice was significantly smaller than that of controls. Food intake did not differ between mGHRKO and control mice on regular or HFD feeding. Likewise, insulin signaling, in liver, skeletal muscle or adipose tissue, as measured by the phosphorylation of the insulin receptor, Akt and Erk, did not differ between the groups under basal conditions. Our data reveal important roles for GH in mediating the cross-talk between muscle and adipose tissue during diet-induced obesity. 1 Fan Y, Menon RK, Cohen P, Hwang D, Clemens T, DiGirolamo DJ, Kopchick JJ, Le Roith D, Trucco M, Sperling MA. Liver-specific deletion of the growth hormone receptor reveals essential role of growth hormone signaling in hepatic lipid metabolism. J Biol Chem. 2009 Jul 24;284(30):19937-44. Nothing to Disclose: AV, YW, HS, CL, SY, DL

Pub #	P2-328
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Extension of Maximum Lifespan in Growth Hormone-Releasing Hormone Knockout Mice
Author String	A Spong, M Masternak, O Arum, R Salvatori, A Bartke Southern Illinois University School of Medicine, Springfield, IL; Johns Hopkins Hospital, Baltimore, MD
Body	Mice with a targeted disruption of the Growth Hormone (GH)-Releasing Hormone gene (GHRHKO mice) exhibit isolated GH deficiency (IGHD) and a dwarf phenotype. An ongoing longevity study in our lab indicates that GHRHKO mice have an extension of both median and maximum lifespan compared to their homozygous normal controls. The median lifespan of female and male GHRHKO mice is extended by 289 and 310 days, respectively (n=50 mice per group). This effect on longevity is consistent with the marked life-extension observed in a variety of mouse mutants with inhibited somatotropic signaling, including the hypopituitary *Ames* and *Snell* dwarf mice, the GH Receptor Knockout (GHRKO) mouse, and the *little* mouse, which has a mutation in the GHRH receptor. The GHRHKO mouse shares important phenotypic characteristics with these long-lived mutants, including reduced body size, reduced serum IGF-1, and increased insulin sensitivity. To investigate the relationship of the mechanism of life-extension in GHRHKO mice with the life-extending effects of a calorie restriction (CR) diet, we imposed a 40% CR diet in GHRHKO mice starting at 2-4 months of age. CR increased the median lifespan of female +/+ normal mice by 102 days and, interestingly, also increased median lifespan in female knockout mice by 214 days, relative to *ad libitum* fed knockouts (from 953 days in the *ad lib* group to 1167 days on CR). By contrast, CR increased median lifespan in +/+ normal males (by 180 days) but had no effect on median lifespan in male GHRHKO mice. At the time of abstract submission, the effect of CR on maximum lifespan in GHRHKO mice cannot yet be determined. In parallel with the gender-specific effects of CR on median lifespan in the GHRHKO animals, CR resulted in a significant decrease in plasma insulin levels in female, but not male, GHRHKO mice. Plasma levels of the insulin-sensitizing adipokine adiponectin were higher in male knockout mice than in +/+ normal mice, and were further increased by CR. These findings implicate the GHRHKO mouse as a promising model organism in the study of the role of GH and insulin signaling in calorie restriction and aging. The observed sex difference in the effect of CR on insulin also adds new evidence for the association of reduced insulin level with extended longevity. Sources of Research Support: NIA. Nothing to Disclose: AS, MM, OA, RS, AB

Pub #	P2-329
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Evaluation of Genome-Wide Bcl6 and Stat5 Occupancy by ChIP-Sequencing: Bcl6 and Stat5 Mediate Reciprocal Regulation of GH Target Genes
Author String	G Lin, C LaPensee, Z Qin, J Schwartz University of Michigan, Ann Arbor, MI; University of Michigan, Ann Arbor, MI; Emory University, Atlanta, GA
Body	Growth Hormone (GH) regulates diverse physiological processes such as growth and metabolism through regulation of specific target genes. Signal Transducer and Activator of Transcription (Stat) 5 is a key activator of GH responses. In contrast, mechanisms by which GH regulates transcriptional repression are poorly understood. The transcriptional repressor Bcl6 (B-cell lymphoma 6) was previously identified as a novel GH-responsive molecule. Expression of SOCS2 (Suppressor Of Cytokine Signaling 2) was strongly inhibited by Bcl6, while Stat5 induced SOCS2 expression with GH treatment. Chromatin immunoprecipitation (ChIP) showed reciprocal occupancy of endogenous Bcl6 and Stat5 at the SOCS2 promoter in response to GH (1). To examine the relationship between Bcl6 and Stat5 as reciprocal regulators of transcription, we evaluated the occupancy of Bcl6 and Stat5 at a genome-wide level using high-throughput ChIP-Sequencing. 3T3-F442A adipocytes were treated with or without GH (48 h) and subjected to ChIP for Bcl6 or Stat5. ChIP-Sequencing libraries prepared from the samples were sequenced and the results analyzed with the H-Peak algorithm. H-Peak identified 1000-9000 regions of occupancy for Bcl6 or Stat5 on each of the genomic profiles. A Bcl6-enriched signal peak was observed near the GH-regulated sequence of SOCS2, consistent with previous ChIP results. Motif analysis of ChIP-Sequencing results showed that sequences occupied by Bcl6 matched strongly with predicted Bcl6 motifs, and sequences occupied by Stat5 matched strongly with predicted Stat motifs. Preliminary gene ontology analysis of genes located near the strongest signal peaks shows occupancy of Bcl6 and/or Stat5 near or on genes involved in transcription, RNA binding, metabolism, signal transduction, development, and membrane trafficking, among predicted functions. Analysis of the ChIP-Sequencing profiles identified several gene candidates showing reciprocal occupancy by Bcl6 and Stat5 in response to GH, in addition to SOCS2. The roles of coregulators as components of a mechanism by which Bcl6 and Stat5 mediate reciprocal regulation of gene expression are under evaluation. This work provides insight into novel mechanisms of transcriptional regulation in response to GH involving reciprocal relationships between activating and repressing factors in a major growth regulatory pathway. (1) Chen Y et al., Endocrinology 2009; 150(8):3645-3654. Nothing to Disclose: GL, CL, ZQ, JS

Pub # P2-330

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)

Title Role of Histone-3 Lysine 27 Trimethylation (K27-me3) in Repression of Female-Specific, Growth Hormone-Regulated Genes in Male Mouse Liver

Author String A Sugathan, EV Laz, A Rampersaud, DJ Waxman
Boston University, Boston, MA

Body

Growth hormone regulates the expression of more than 1,000 genes in mouse liver in a sex-dependent manner by transcriptional mechanisms, with large sex-differences (>100-fold) characterizing some of these genes. Here we used chromatin immunoprecipitation combined with high throughput sequencing to investigate whether K27-me3, which characterizes a stable, inactive chromatin structure and repression of transcription, is associated with the silencing of male-specific genes in female liver, or of female-specific genes in male liver. We used SICER, an algorithm designed to identify broad enriched domains ('islands') from genomic sequencing data (1), to identify chromosomal regions enriched in K27-me3 marks. We found K27-me3 enriched on the X-chromosome in female compared to male liver, consistent with its role in X-chromosome inactivation (2). On autosomes, K27-me3 was enriched in male mouse liver across the gene body and in flanking regions of a subset of female-specific genes, including *Cyp2a4*, *Cyp2c39*, *A1bg*, *Fmo3*, *Hao3* and *Sult3a1*, where results were validated by qPCR. The female-specific genes preferentially marked with K27-me3 in male liver showed a significantly higher sex difference in expression than those that were not so marked, and the sex-difference in K27-me3 sequence read density was inversely correlated with the magnitude of the sex-difference in gene expression ($r = -0.5$, $p < 10^{-3}$). Male-specific genes preferentially marked with K27-me3 islands in female liver were also identified, but showed a lower sex-difference in sequence reads, and no correlation with the magnitude of sex-difference in gene expression was observed. These findings support the proposal that K27-me3 plays a specific role in the silencing of female-specific genes in male liver. The pituitary/GH-dependence of the female-specific genes thus marked indicates that K27-me3 is a constitutive repressive mark that requires the female GH profile to relieve repression in female liver.

(1) Zang C et al., Bioinformatics 2009; 25:1952
(2) Chow J and Heard E., Curr Opin Cell Biol 2009; 21:359

Sources of Research Support: In part by NIH grant DK33765 (to DJW).

Nothing to Disclose: AS, EVL, AR, DJW

Pub #	P2-331
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Proteomic and Physiological Differences in Growth Hormone Receptor Null Mice with Advancing Age
Author String	J Ding, A Jara, DE Berryman, JJ Kopchick Ohio University, Athens, OH; Ohio University, Athens, OH; Ohio University, Athens, OH; Ohio University, Athens, OH
Body	Growth hormone receptor null (GHR-/-) mice are dwarf, insulin sensitive and long-lived despite having an obese phenotype. In order to identify characteristics associated with their increased longevity, we studied age-related physiological and plasma proteomic changes in GHR-/- mice. We observed a less dramatic age-related weight loss at 24 months in GHR-/- mice compared to wild type (WT) controls. We also observed a fat depot-specific change dependent on genotype and age; that is, only the subcutaneous fat was significantly increased throughout life in GHR-/- mice whereas other fat pads were either not significantly different or decreased as compared to WT controls despite overall obesity. Fasting glucose levels were lower in GHR-/- mice at 9 months but the same for WT at 24 months. Fasting insulin levels remained lower in GHR-/- mice than WT for both ages. In addition to these physiological changes, we identified genotype and gender differences in plasma proteins by two-dimensional gel electrophoresis at ages 8, 16 and 24 months. GHR-/- mice had increased apolipoprotein A-4 (APOA4) and retinol-binding protein-4 (RBP-4) and decreased APOE, α-2 macroglobulin isoform 1, haptoglobin (HP) and mannose-binding protein-C. These changes were observed throughout the ages examined in both genders. Gender differences were found in specific isoforms of APOE, RBP-4, HP and hemoglobin beta regardless of genotype. Females also exhibited differential aging profiles in that specific isoforms of APOA4 and APOA1 increased and RBP-4 decreased during aging but remained relatively constant during aging in males. Lastly, significant interaction between genotype and gender was found in specific isoforms for APOE, RBP-4 and HP. For example, two isoforms of HP were decreased in WT females compared to WT males but remained the same in GHR-/- male and females. These results suggest that GHR-/- mice have a beneficial lipid profile and a reduced inflammatory state. Further data will be presented on levels of plasma lipids and inflammatory markers in these mice. In conclusion, we found changes in GHR-/- mice that favor a longer lifespan in terms of weight, fat distribution, insulin levels and plasma proteome. Additionally, notable gender differences exist in proteomic profiles. Sources of Research Support: NIA (AG19899 and AG031736), NIDDK (DK075436), the State of Ohio's Eminent Scholar Program that includes a gift from Milton and Lawrence Goll, and a grant from DiAthegen LLC. DEB is supported in part by funds from NIDDK (DK064905) and NIA (AG031736). All work was further supported by the Diabetes Research Initiative at Ohio University and by a grant from AMVETS. Nothing to Disclose: JD, AJ, DEB, JJK

Pub #	P2-332
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Targeted, Dose-Dependent Inhibition of Growth Hormone Secretion by the Botulinum Neurotoxin-Based TSI SXN101000 and SXN101742
Author String	JD Leggett, EJ Waite, PMH Marks, A Martinez, SL Lightman University of Bristol, Bristol, UK; Abingdon, Oxford, UK
Body	Acromegaly is an endocrine disease caused by excess secretion of growth hormone (GH) typically from a pituitary adenoma. Current treatments are not effective in all cases and can be associated with undesirable side-effects. Botulinum neurotoxin-based molecules offer a novel mechanism for suppressing hormone releas via the cleavage of SNARE proteins essential for membrane-vesicle fusion. We have characterised the novel botulinum-neurotoxin-based Targeted Secretion Inhibitors (TSI) SXN101000 and SXN101742 engineered by Syntaxin to specifically target GH secreting cells, cleave SNARE proteins therein suppress GH release. To assess the effect of treatment on pulsatile GH secretion we used an automated system to take serial 10 minute blood samples via an implanted i.v. cannula from male Sprague Dawley rats (250-275g) over a 24 hour period. The effect of a single i.v. dose (0.3mg/kg) of SXN101000 given 4 days prior to sampling was assessed against vehicle treated controls and a 12 hour infusion of the somatostatin analogue Octreotide (10ug/kg/hour i.v.). We further examined the effect of different doses of the related TSI SXN101742 and the effect of a control (SXN101884) in which the catalytic domain was mutated to delete the SNARE endopeptidase activity. Plasma GH was measured by RIA and hormone secretion parameters analysed using PulseXP. Treatment with SXN101000 significantly suppressed GH secretion compared to the control group reducing the mean number of GH pulses from 9.83 ±0.79 to 1.00 ±0.82 ($p< 0.001$), the mean pulse mass by >95% (57.1ng ±8.95 to 3.72ng ±2.47, $p< 0.001$) and the mean total amount of GH secreted (including basal secretion) by >40% (2691 ±387.5 to 1605 ±112.3, $p< 0.05$). There was no significant difference between the effects of Octreotide and SXN101000 during the period of infusion. SXN101742 suppressed GH secretion in a dose-dependent manner significantly reducing both the mean pulse mass (0.1mg/kg: $p< 0.001$; 0.3mg/kg: $p< 0.001$; 1.0mg/kg: $p< 0.001$) and the total amount of GH secreted (0.1mg/kg: $p< 0.001$; 0.3mg/kg: $p< 0.001$; 1.0mg/kg: $p< 0.05$). The control SXN101884 had no significant effect on GH secretion when compared to the vehicle controls. SXN101000 and SXN101742 exert a powerful inhibitory effect on GH secretion 4-5 days post treatment, significantly suppressing pulsatile release and total hormone secreted over 24 hours. The data provide support for the development of these TSI for use as a novel treatment for acromegaly. Sources of Research Support: Syntaxin Ltd. Disclosures: PMHM: Employee, Syntaxin. AM: Employee, Syntaxin. Nothing to Disclose: JDL, EJW, SLL

Pub #	P2-333
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Effect of Endogenous Growth Hormone Dynamics on Overnight Free Fatty Acid Concentrations in Healthy Men
Author String	TL Stanley, H Makimura, H Lee, CY Chen, SK Grinspoon Massachusetts General Hospital, Boston, MA; Massachusetts General Hospital, Boston, MA
Body	Elevated free fatty acids (FFA) have been implicated in hyperglycemia and insulin resistance, yet the determinants of nocturnal, post-absorptive FFA levels in humans are not well-characterized. Growth hormone (GH) is lipolytic, and exogenous GH treatment increases FFA. GH pulses largely at night, yet the relationship between endogenous GH and FFA is not known. To characterize effects of endogenous GH secretion on nocturnal FFA, 13 healthy, non-diabetic men (age 45±12y; BMI 27.3±4.5kg/m^2) underwent overnight frequent sampling for GH and FFA. Samples were available every 10min for 11 men and every 20min for 2 men. The computerized deconvolution algorithm *AutoDecon* was used to analyze pulse characteristics. Body composition was measured by dual energy x-ray absorptiometry (DEXA), and visceral and subcutaneous adipose tissue (VAT and SAT) were measured by CT at L4. Insulin sensitivity was assessed using euglycemic-hyperinsulinemic clamp. Mean overnight FFA in the cohort were 396±164 [mu]M, and mean overnight GH level was 0.7±0.8 mcg/L. FFA levels appeared to increase through the course of the night, peaking at approximately 1am. In univariate analysis, mean overnight FFA were positively associated with mean overnight GH (r=0.57, p=0.04) and GH nadir (r=0.65, p=0.02), whereas there were no significant associations between mean FFA and number of GH secretion events or mean GH peak area. In cross-correlation analysis between GH and FFA, GH was most strongly associated with FFA at a lag of 150min (r=0.19 [95% CI 0.13-0.25]) such that serum GH concentrations at any point were significantly associated with FFA levels 150 minutes later. There were no significant univariate associations between mean FFA and age, BMI, body fat or lean, VAT or SAT, fasting glucose, or insulin-stimulated glucose uptake. In multivariate modeling, mean overnight GH (β-estimate±SE 200±45 per mcg/L, p=0.002) and percent body fat by DEXA (β-estimate±SE 18±5 per kg, p=0.006) were positively associated with mean overnight FFA, whereas lean mass by DEXA (β-estimate±SE -12±3 per kg, p=0.009) was negatively associated with mean FFA (R^2=0.75, p=0.004 for model). Neither fasting glucose nor insulin sensitivity as assessed by clamp was significantly associated with mean overnight FFA in multivariate modeling. These data demonstrate a novel relationship between overnight endogenous GH secretion and FFA and help to elucidate factors that contribute to FFA concentrations in the post-absorptive state. Sources of Research Support: National Institutes of Health grant R01DK063639 and K24DK064545 to SKG. NIH funding also provided through K23DK087857 to HM and K23DK089910 to TLS. Grant M01RR01066 and UL1RR025758, Harvard Clinical and Translational Science Center, from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health. Disclosures: SKG: Investigator, Theratechnologies; Consultant, Serono, Theratechnologies. Nothing to Disclose: TLS, HM, HL, CYC

Pub #	P2-334
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Glucocorticoids Stimulate Human Growth Hormone Production and Somatotroph Number and Facilitate Responsiveness to Exogenous Growth Hormone-Releasing Hormone in Pituitary Cell Culture
Author String	H Vakili, Y Jin, JI Nagy, PA Cattini University of Manitoba, Winnipeg, Canada
Body	Non-primate and primate growth hormone (GH) can be structurally and, as a result, functionally distinct. Differences also extend beyond the coding region to include promoter and regulatory sequences, and thus it is possible that the signals/mechanisms under which production of rodent and primate GH are subjected and controlled also vary. Transgenic (TG) mice were generated containing all five members of the human (h) GH gene family including pituitary GH (hGH-N), placental GH variant (hGH-V), and chorionic somatomammotropin (hCS-A, hCS-B and hCS-L), as well as the hGH locus control region in a 171 kb fragment of chromosome 17; the locus control region ensures appropriate expression of hGH-N in the pituitary in vivo. Primary pituitary cells isolated from 171hGH/CS-TG mice provide a model system to study hGH production in vitro. The pituitary-specific transcription factor Pit-1 is essential for development of GH-producing somatotrophs, and adrenal glucocorticoids are required for functional maturation of these cells during fetal development. However, regulation of hGH-N synthesis by glucocorticoids in 'normal' postnatal pituitary cells has not been reported. Treatment of primary 171hGH/CS-TG mouse pituitary cells with 200 nM dexamethasone resulted in a significant 4.5 and 2-fold stimulation of hGH RNA and protein levels, respectively, and levels of hGH in the culture medium increased 1.8-fold in 48 hours. There was also a significant 1.7-fold increase in hGH-positive cells in response to DEX treatment, based on their immunocytochemical detection as a proportion of total cell number (DAPI-stained nuclei). In addition, the effect of DEX treatment on growth hormone releasing hormone (GHRH) receptor was assessed, and RNA levels increased ~20-fold in 24 hours. In light of this response, the effect of 1 [mu]M GHRH for 30 min on hGH secretion from primary pituitary cells with and without pre-treatment with 200 nM DEX for 24 hours was examined. A significant 1.6-fold increase in hGH secretion was observed with DEX pre-treatment. These data suggest that acute DEX treatment can significantly increase the proportion of hGH-positive cells and hGH production, and raise the possibility that glucocorticoids facilitate hGH secretion in response to exogenous GHRH by increasing GHRH receptor levels. Sources of Research Support: Canadian Institutes of Health Research (MT-10853). Nothing to Disclose: HV, YJ, JIN, PAC

Pub #	P2-335
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	The Growth Hormone-Responsive Transcriptional Repressor Bcl6 Regulates Genes Associated with Lipid Metabolism and Adipogenesis
Author String	CR LaPensee, S Li, JD Lin, AL Dent, J Schwartz University of Michigan, Ann Arbor, MI; University of Michigan, Ann Arbor, MI; Indiana University School of Medicine, Indianapolis, IN
Body	Growth Hormone (GH) regulates normal growth and metabolism. We recently reported that the transcriptiona repressor B-cell Lymphoma 6 (Bcl6) is present in adipocytes and regulated by GH, suggesting a novel mechanism of transcriptional regulation by GH. Bcl6 KO mice were found to exhibit a striking reduction in adipose tissue mass compared to wild-type (WT) mice, suggesting a link between Bcl6 and fat mass. We investigated whether alterations in lipid metabolism and/or adipogenesis contribute to this relationship. Lowe hepatic triglycerides in male Bcl6 KO mice suggested that Bcl6 might contribute to the regulation of lipid metabolism. Since Bcl6 inhibits and GH increases expression of Suppressor of Cytokine Signaling (Socs2), we examined other GH-induced genes associated with lipid metabolism which we found contained predicted Bcl6 binding sites in their promoters. Among these, expression of fatty acid/[Delta]5 desaturase (Fads1) and acyl CoA synthetase 5 (Acsl5), as well as Socs2, was elevated in liver of Bcl6-KO mice, suggesting similar patterns of regulation of some genes in lipid metabolic pathways mediated by Bcl6. During adipogenesis, we found that Bcl6 mRNA expression increased in 3T3-F442A adipocytes. In addition, expression of mRNA for adipogenic transcription factors C/ebpα, Ppparγ, and the adipocyte-specific marker aP2, were much lower than WT in the limited amount of adipose tissue obtained from Bcl6 KO mice. The anti-adipogenic genes Pref-1 and Gata3 were higher in Bcl6 KO adipose tissue, also consistent with a role of Bcl6 in adipogenesis and with reduced adipose tissue in Bcl6 deficiency. Together, these studies indicate that Bcl6 contributes to regulation of genes associated with lipid metabolism and adipogenesis, and suggest that this transcriptional repressor may play a role in GH-regulated lipid metabolism. Sources of Research Support: ADA 7-09-BS-168 awarded to JS. Nothing to Disclose: CRL, SL, JDL, ALD, JS

Pub #	P2-336
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	The Cryptic Peptides Prepro-Thyrotropin-Releasing Hormone 186-199 and 194-199 Suppress Anterior Pituitary Prolactin Secretion *In Vivo* and *In Vitro*
Author String	E Shortridge, C Foradori, A Quihuis, MF Robert, TJ Wu, RJ Handa University of Arizona, Phoenix, AZ; San Diego State University, San Diego, CA; Uniformed Services University of the Health Sciences, Bethesda, MD
Body	Prepro-thyrotropin releasing hormone (ppTRH)-178-199 is one of several peptide fragments cleaved during TRH synthesis and has been implicated as a regulator of neuroendocrine function. PpTRH 178-199 has been shown to acutely inhibit the stress-induced rise in ACTH, corticosterone (CORT), and prolactin (PRL) in the rat. The purpose of this study was to characterize the active domain of ppTRH 176-199 in the regulation of PRL secretion using *in vivo* and *in vitro* approaches. The ppTRH fragments 186-199, 186-191 and 194-199 were administered to adult male Sprague-Dawley rats 15 min. prior to restraint stress to determine the peptide's active moiety in regulating PRL secretion. Animals were euthanized after 20 min of stress and plasma was assayed for circulating PRL using EIA. PpTRH 186-199 significantly attenuated the stress-induced PRL rise in male rats in a dose-dependent fashion. This effect was mimicked by ppTRH 194-199 but not by ppTRH 186-191. At the highest dose (10 mg/kg BW), ppTRH 194-199 also reduced the stress-induced rise in plasma CORT. *In vitro* studies were performed using the rat GH/PRL-secreting MMQ cell line. MMQ cells were treated with ppTRH 186-191 or 194-199 and media was assayed for PRL. Cells were examined for changes in PRL mRNA. There was a significant decrease in media levels of PRL 30 minutes after treatment o MMQ cells with ppTRH 194-199 versus vehicle. Furthermore, MMQ cells primed with 10nM estradiol for 48 hours increased PRL secretion, which was also reduced following ppTRH 194-199 treatment for 30 min or 4 hrs, but not after 24 hrs of treatment. No difference was seen in PRL mRNA in cells exposed to ppTRH 186-199 or 194-199. These data indicate that the carboxy terminal fragment of preproTRH 178-199 (aa194-199) contains all the activity of this ppTRH cryptic peptide for regulation of PRL and CORT secretion. This suggests a potential moiety responsible for interaction with the peptide's receptor. The inhibitory effect of ppTRH 194-199 on peptide secretion and not on mRNA synthesis implicates it as an effector of hormone secretion rather than protein synthesis and the short-lived duration of its effects supports a role as an acute effector of PRL secretion. The target receptor of the ppTRH 178-199 fragment remains unknown, however, the use of ppTRH 194-199 as a peptide bait may prove useful in identifying the receptor. Nothing to Disclose: ES, CF, AQ, MFR, TJW, RJH

Pub #	P2-337
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	The Effects of Increased GH/IGF-I Axis Action in a Murine Model of Chronic Asthma
Author String	D Cruz-Topete, A Basu, A Shaw, R Malgor, EO List, JJ Kopchick Ohio University, Athens, OH; Ohio University, Athens, OH; Ohio University, Athens, OH

Body

Allergic asthma is characterized by chronic inflammation of the airway walls associated with eosinophil infiltration, bronchoconstriction and airway remodeling. Controlling these pathological events is critical for asthma medical management. The GH/IGF axis has been proposed to play a role in airway inflammation and remodeling in asthma (1, 2). Some studies suggest that expression of IGF-I and IGFBP-3 is associated with increased airway hyperresponsiveness and remodeling (1, 3). Interestingly, a recent study indicated that IGFBP-3 inhibits airway inflammation by attenuating the expression of proinflammatory molecules and the migratory response of eosinophils (4). Therefore, the precise role of the GH/IGF axis in asthma remains elusive. In order to further investigate the role of GH/IGF-I in asthma pathology, we have initiated studies in bovine GH transgenic (bGH) mice. bGH mice are giant and possess increased serum levels of GH, IGF-I, IGFBP-3, and insulin. Thus, the use these mice will allow us to investigate the impact of elevated GH/IGF-I action on airway inflammation in chronic asthma. To accomplish this objective, wild-type (WT) and bGH mice were immunized with 10 [mu]g of ovalbumin (OVA) adsorbed onto $Al(OH)_3$ on days 0 and 14. At day 21, mice were intranasally challenged with OVA (50 [mu]g) or PBS (vehicle control) 3 days/week for 2 weeks. Lung tissues and bronchoalveolar lavage fluid (BALF) were collected for all groups 24 hours after the final OVA administration. Total cell numbers were quantified in BALF. No significant differences in the numbers of neutrophils, macrophages and lymphocytes were found between mouse genotypes. However, eosinophil counts in BALF were diminished in OVA-challenged bGH mice compared to WT mice. Interestingly, the levels of T helper type 2 (Th2) cytokines, IL-4 and IL-5, and eosinophil-attracting chemokines, eotaxin and RANTES, were also lower in bGH mice. The decrease of these chemokines provides a possible explanation of why eosinophil numbers are reduced in BALF of bGH mice. Histological analysis o lung tissues are currently being performed. Our results suggest that the GH/IGF-I axis may play a role more complex than previously anticipated in the inflammatory events associated to chronic asthma. If our findings are confirmed, GH may serve as a potential therapeutic to attenuate some of the events associated with airway inflammation in chronic asthma.

1. Yamashita N et al.,Cell Immunol 2005;235:85-91
2.Veraldi KL et al., Am J Respir Crit Care Med 2009;180:611-7
3.Kawaguchi M et al., Clin Exp Allergy 2010;40:1036-43
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Sources of Research Support: In part by the State of Ohio s Eminent Scholar Program that includes a gift from Milton and Lawrence Goll, by a grant from the Diabetes Research Initiative at Ohio University, by a grant from the AMVETS organization, and by NIH grants AG19899 & AG031736.

Nothing to Disclose: DC-T, AB, AS, RM, EOL, JJK

Pub #	P2-338
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Effect of CBP Phosphorylation on Growth Hormone Signaling in the Somatotroph
Author String	ED Pine-Twaddell, RS Miller, C Romero, D Avtanski, S Radovick Johns Hopkins University, Baltimore, MD
Body	**Background**: CREB-Binding Protein (CBP) is a nuclear protein that acts as a co-activator of Pit1 (aka POU1F1) mediated growth hormone (GH) gene expression (1). Phosphorylation of CBP at serine 436, a highly conserved region, via the PKA phosphorylation pathway is required for Pit1 dependent promoter activation. Substitution of alanine in this region (S436A) prevents phosphorylation as demonstrated in the CBP-KI mouse (2). **Hypothesis:** The regulation of growth hormone synthesis/secretion is dependent on signaling pathways involving CBP phosphorylation, and disruption of this pathway causes GH axis dysregulation. **Results:** Baseline average fasting serum growth hormone levels were significantly higher in male S436A CBP-KI (CBP-KI) mice than WT (3.1 ng/mL vs. 0.34 ng/mL, p[le]0.005). Random measurements at various points in the light/dark and fed/fasting cycle showed a trend towards higher serum GH levels in CBP-KI mice (14.9 ng/mL) vs. WT mice (8.9 ng/mL). Growth releasing factor (20 [mu]g/mouse) resulted in lower stimulated GH levels in CBP-KI (1.97 ng/mL) than wild-type mice (4.28 ng/mL). On a high fat diet (HFD) both groups had lower GH levels, with CBP-KI remaining higher than WT (0.67 ng/mL vs 0.21 ng/mL). Male CBP-KI mice have lower weights from 4-30 weeks of age, reaching significance at peripubertal weeks 5-7; no significant differences in length compared to WT littermates were observed. Body composition also differs between the groups; CBP-KI mice have significantly less body fat (p[le]0.01) and similar levels of lean body mass. When the mice were placed on a HFD, all mice showed weight gain and increase body fat percentage; ultimately, there were no differences in length, weight, or body composition between the CBP-KI and WT mice. **Conclusions:** As has been shown previously in vitro, the inability to phosphorylate at the S436A site does not allow dissociation of CBP from Pit-1, hence GH transcription may be constitutively active, driving higher basal GH levels in the mouse model. A mildly increased basal level of GH would explain the differences seen in body composition with leaner mice with similar length. The response of CBP-KI mice to GHRH is diminished compared to WT, indicating there may be a difference in the PKA mediated secretory response to GHRH. Thus, phosphorylation of CBP at position 436 is necessary for normal basal GH secretion and response to GHRH. (1) Cohen, LE et al. J Clin Invest. 1999 Oct;104(8):1123-30. (2) Zhou, XY et al. Nat Med. 2004 Jun;10(6):633-7. Nothing to Disclose: EDP-T, RSM, CR, DA, SR

Pub #	P2-339
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Cortistatin Is Not a Somatostatin Analog but Stimulates Prolactin Release and Its Deficit Causes Plasma Insulin Decrease and Male-Selective Glucose Impairment: Role of Ghrelin
Author String	J Cordoba-Chacon, MD Gahete, AI Pozo-Salas, AJ Martinez-Fuentes, F Gracia-Navarro, L De Lecea, RD Kineman, JP Castano, RM Luque University of Cordoba, Instituto Maimónides Investigación Biomédica de Córdoba (IMIBIC) and CIBER Fisiopatologia Obesidad y Nutricion, Cordoba, Spain; University of Illinois at Chicago, Chicago, IL; Jesse Brown Veterans Affairs Medical Center, Chicago, IL; Stanford University School of Medicine, Palo Alto, CA
Body	Cortistatin (Cort) and somatostatin (SST) are two neuropeptides that share remarkable structural, pharmacological, and functional similarities. Although Cort was considered a natural SST analogue acting through their shared receptors (sst1-5), emerging evidence indicates that these peptides may in fact exert unique roles via selective receptors (e.g. Cort, not SST, binds ghrelin-receptor GHS-R1a). Here, we investigated the role of Cort using a Cort knockout (KO) mouse model and primary pituitary cell cultures of male/female mice and female primates (baboons). Specifically, we present the first thorough endocrine-metabolic characterization of male/female Cort-KO mice at the hypothalamic, pituitary and systemic (pancreas-stomach-adrenal-liver) levels. These unveiled unique, unpredicted regulatory actions of Cort on the pituitary-metabolic axis, distinct from those of SST. Specifically, Cort exerts an unexpected stimulatory role on prolactin (PRL) secretion, which is in striking contrast with the direct inhibitory effect exerted by SST on PRL secretion. Interestingly, use of a specific antagonist for GHSR1a fully blocked Cort-stimulated PRL release in baboon pituitary cells, thereby indicating that Cort acts via the GHSR1a to induce its stimulatory effect on PRL secretion. The physiological relevance of this novel PRL-stimulatory action of Cort is as yet unknown however, in support for such relevance is our finding that the percentage of Cort-KO females that successfully cared for their first litter was significantly lower compared to female controls (Cort+/+). In addition, Cort played important gender-dependent inhibitory actions on somatotrope-(GH) and corticotrope-(ACTH) axes, which are elevated in Cort-KO mice and are inhibited by cortistatin in vitro, being females more sensitive to cortistatin actions. However, gonadotrope or thyrotrope function was not altered in Cort-KO. Furthermore, Cort deficit uncovered a major, gender-dependent role of this peptide in the regulation of glucose-insulin homeostasis, as it caused an overall impairment of insulin-mediated glucose clearance (GTT/ITT) in male Cort-KO vs. Cort+/+, not share by female Cort-KO or male/female SST-KO. The fact that these actions are not mimicked by SST and are strongly gender-dependent offers new grounds to investigate the hitherto underestimated physiological relevance of Cort in the regulation of endocrine and metabolic process. Sources of Research Support: FI06/00804 (to JCC), FPU-AP20052473 (to MDG); R01DK030677 and Veterans Affairs Merit Award (to RDK), BFU2010-19300 and CTS-5051 (to JPC) and RYC-2007-00186, BFU2008-01136/BFI (to RML). Nothing to Disclose: JC-C, MDG, AIP-S, AJM-F, FG-N, LDL, RDK, JPC, RML

Pub #	P2-340
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Control of β-Cell DNA Synthesis and Gene Expression: Effects of Prolactin Receptor Knockdown
Author String	DE Fleenor, R Arumugam, TC Becker, MS Freemark Duke University Medical Center, Durham, NC; Duke University Medical Center, Durham, NC
Body	The striking increases in beta cell mass and insulin production in humans during the perinatal period and pregnancy coincide with a surge in the levels of placental lactogen (PL) and prolactin (PRL), which stimulate beta cell replication and glucose-stimulated insulin secretion in human and rodent islets and insulinoma cells. The molecular mechanisms by which the lactogens promote beta cell expansion remain poorly understood. In recent investigations we showed that PRL treatment of isolated male rat islets increases expression of cyclins A2, B1, B2, and D2 and CDK1 and attenuates the rise in FoxO1, p27, p57 and, variably, menin after serum starvation. An adenoviral siRNA specific for cyclin D2 attenuated markedly the effect of PRL on islet DNA synthesis. Conversely, the effects of PRL on islet ^{3}H-thymidine incorporation and cell cycle gene expression were potentiated by glucose. The biological actions of PL and PRL are mediated through binding to the PRL receptor (PRLR). Here we examined the effects of PRLR knockdown on beta cell DNA synthesis and cell cycle gene expression. To that end, we treated INS-1 832-13 rat insulinoma cells with an adenoviral siRNA specific for the rat PRL receptor (PRLR); control cells were treated with a scrambled adenoviral siRNA. The cells were incubated in 10% fetal calf serum, which contains placental lactogen, PRL, GH and other beta cell mitogens. Under these conditions, the PRLR siRNA reduced PRLR mRNA levels 75-80%. ^{3}H-thymidine incorporation and gene expression were assessed after a 72-hr incubation. The PRLR siRNA reduced ^{3}H-thymidine incorporation by 22% ($p<0.01$). There were small but significant reductions (~15-25%, $p<0.05$) in the levels of cyclins B1, B2, CDK1, FoxO1 and p27; however, the expression of cyclin D2, , and Tph1 decreased by 52% ($p<0.001$), 28%, and 23%, respectively (all $p<0.01$), while the expression of cyclin D1 and p21 increased by 24% ($p<0.01$) and 43% ($p<0.001$), respectively. These findings suggest that PRLR signaling is required for normal DNA synthesis, maintenance of cyclin D2 and IRS-2 expression, and suppression of p21 in rat beta cells. Since cyclin D2 and IRS-2 are required for beta cell replication in the postnatal period and for the beta cell compensatory response to insulin resistance, our observations suggest novel roles for the lactogens in the establishment and maintenance of beta cell reserve and the pathogenesis of beta cell hyperplasia in obesity and other insulin-resistant states. Nothing to Disclose: DEF, RA, TCB, MSF

Pub #	P2-341
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Lack of Sleep and Activity May Contribute to Adult-Onset Obesity in Deletion Mutant Mice Lacking Leptin Receptors in Pituitary Somatotropes
Author String	N Akhter, AK Odle, MA Cozart, MM Syed, AC Haney, GV Childs University of Arkansas for Medical Sciences, Little Rock, AR
Body	Leptin signals are important to the maintenance of somatotrope functions. When exon 17 of the leptin recepto gene is deleted selectively in somatotropes by Cre-loxP technology (1), mice express lower serum and pituitary levels of growth hormone (GH) and develop adult onset obesity by 6 months of age (1). In order to determine factors causing the obesity, 3-5 month old deletion mutant mice and littermate controls were studied in an Oxymax Complete Lab Animal Monitoring System (CLAMS, Columbus Instruments). The automated system detected no differences in the amount of food consumed. Deletion mutant females expended less energy than controls, showing significantly lower ($p<0.01$) heat production; Controls had an average of 0.53±0.04 kcal/h (n=13) and mutants had 0.47±0.05 kcal/h (n=11). This correlated well with significantly lower ($p=0.02$) activity levels (detected by counts of infrared beam breaks in the X-Y axis); [790.6±38 (controls) and 633.5±47 (mutants)]. Mutants showed less rearing or jumping activity (# beam breaks, Z axis); [628±29 (controls) and 353±12 (mutants)]. When activity counts were used to define sleep epochs, deletion mutant and control females were not different. In contrast, deletion mutant males showed significantly lower ($p=0.017$) average percent of time sleeping. Control males (n=11) spent an average of 30±3% of their time sleeping compared with deletion mutants (n=8), which slept 16.5±1.5% of the time. Analysis of the activity levels during the 14 h sleep phase (light) showed that mutant males were not as active as controls exhibiting 468±65 ambulatory and 129±26 rearing counts compared with 552±73 ambulatory and 168±32 rearing counts in the controls ($p<0.05$). During the dark phase, the average activity counts from mutant and control males were similar. These studies show sex differences in behaviors that may contribute to the obesity seen in these deletion mutants by 6 months of age. Food intake is normal, which correlates with their normal serum leptin and hypothalamic leptin receptors (1). Mutant females expended less energy and showed reduced ambulatory or rearing activity during both light and dark cycles. Males spent less time sleeping, but were less active during the light phase. These studies show the importance of leptin signaling to promote normal levels of GH, which may help to establish normal sleep patterns (in males) and activity levels in both sexes. All of these factors are known to prevent obesity. (1) Childs GV et al., Endocrinology 2011; 152: 69-81 Sources of Research Support: NIH 1R01HD059056 awarded to GVC, NA; NIH NCRR P20 RR020146 Project IV awarded to NA, MS, GVC mentor; NIH P30 NS047546 (core); NIH R03 HD059066 awarded to GVC. Nothing to Disclose: NA, AKO, MAC, MMS, ACH, GVC

Pub #	P2-342
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	O-Linked Glycosylation of Growth Hormone Enhances Its Plasma Half-Life with Full Retention of Bioactivity
Author String	S Okada, J Xu, S Sankaran, MJ Kieliszewski, JJ Kopchick Ohio University, Athens, OH; Ohio University, Athens, OH; Ohio University, Athens, OH; Ohio University, Athens, OH

Body

Growth hormone (GH) is a potent regulator of growth and metabolism. Recombinant human (rh)GH is approved for GH deficiency (GHD) as well as conditions associated with growth impairment in the absence o GHD. hGH has a short plasma half-life (~20 min) mainly due to rapid renal clearance because of its small molecular mass (~22 kDa). Chemical derivatization with polyethylene glycol or expression as a fusion protein with polypeptides or albumin prolongs its plasma half-life but results in decreased bioactivity (1-3).

We have generated a biologically active rhGH with increased plasma half-life when synthesized as an arabinogalactan-protein (AGP) in tobacco BY-2 cells (4). This rhGH analog is expressed with 10 repeats of the AGP glycomodule Ser-Hyp (SO) at the C-terminus (rhGH-$(SO)_{10}$). SO repeats direct Hyp-O-glycosylation and increase the molecular mass of rhGH from 22 kDa to ~50 kDa (~2.5 kDa per glycomodule) rhGH-$(SO)_{10}$ exhibits the same binding affinity to cell surface GH receptors (R) as wild type hGH, indicating the bulky glyco units on the C-terminus do not interfere with its receptor binding abilities. Also, rhGH-$(SO)_{10}$ stimulates GH intracellular signaling in that tyrosine of Stat5 is phosphorylated to the same extent as wild type hGH in mouse fibroblasts expressing the mGHR. Furthermore, daily injections of rhGH-$(SO)_{10}$ at pharmacological doses into mice yielded both an increase in serum IGF-1 and enhancement of whole body growth similar to that observed with rhGH injections.

Here, we tested various numbers of Ser-Hyp (SO) in an attempt to find minimum effective number of glycosylation to achieve prolonged plasma half-life while retaining bioactivity. Addition of 2 glycomodules did not increase plasma half-life and was cleared at the same rate as wild type hGH. Addition of 5, 10, or 20 glycomodules all enhanced plasma half-life. Although the addition of 20 glycomodules improved plasma half life by more than 100-fold, bioactivity was significantly reduced. We observed that addition of 5 or 10 glycomodules improved plasma half-life by more than 10-fold without losing any bioactivity.

These results demonstrate the feasibility of Hyp-O-glycosylation for producing long-acting, biologically active rhGH and other therapeutic proteins.

(1) Clark R et al.,J Biol Chem 1996:21969
(2) Osborn BL et al.,Eur J Pharmacol 2002 456:149
(3) Schellenberger V et al.,Nat Biotechnol 2009 27:1186
(4) Xu J et al., Transgenic Res 2010 19:849

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Nothing to Disclose: SO, JX, SS, MJK, JJK

Pub #	P2-343
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	SOCS2 Is the Key Regulator of GH-Induced STAT Activation in Chondrocytes
Author String	C Pass, V MacRae, F Ahmed, C Farquharson University of Edinburgh, Edinburgh, UK; University of Glasgow, Glasgow, UK
Body	The precise signaling mechanisms within the growth plate by which GH regulates bone growth remain elusive. It is likely, however, to involve Suppressor of Cytokine Signaling-2 (SOCS2) which is a negative regulator of GH signaling via inhibition of the JAK/STAT pathway. This has been classically demonstrated by the overgrowth phenotype of $SOCS2^{-/-}$ mice which present with increased bone length. The aims of this study were to understand more fully the SOCS2 regulated mechanisms mediating GH action on bone growth. Western blotting indicated that in cultured wild-type (Wt) murine chondrocytes, SOCS2 expression was increased in response to GH (500ng/ml), but not IGF-1 (50 ng/ml) whereas neither SOCS1 nor 3 responded to either ligand. STATs1, 3 and 5 phosphorylation increased in response to GH, peaking at 30 min and declining thereafter in Wt chondrocytes. In contrast, STATs 1,3 and 5 activation was increased and prolonged in $SOCS2^{-/-}$ chondrocytes. No STAT activation by GH was observed in chondrocytes overexpressing SOCS2. Microarray analysis of 1-day-old uncultured Wt and $SOCS2^{-/-}$ chondrocytes suggested a varied gene expression profile including up-regulation of IGF-I expression in the $SOCS2^{-/-}$ cells. This was confirmed by RT-qPCR, which showed a 10-fold increase in IGF-1 expression in $SOCS2^{-/-}$ chondrocytes compared to Wt. This suggests that elevated GH signaling acts indirectly on chondrocytes, via IGF-I and this was confirmed in cultured Wt and $SOCS2^{-/-}$ metatarsals where the growth response to GH, which was only noted in $SOCS2^{-/-}$ metatarsals, was inhibited by the PI-3K inhibitor LY294002. As expected, IGF-I induced the growth of Wt and $SOCS2^{-/-}$ metatarsals and this was inhibited by LY294002 treatment. Underlying growth plate changes to explain the stimulatory effects on bone growth of increased GH signalling in $SOCS2^{-/-}$ mice were investigated in tibiae from 6 week-old Wt and $SOCS2^{-/-}$ mice. Bone growth rate (um/day) was increased in $SOCS2^{-/-}$ mice (95.2±5.3 vs 75.0±6.9; $P<0.05$). This increased growth was reflected by wider (um) growth plates (234.3±11.7 vs 190.4±6.1; $P<0.05$), hypertrophic zones (88.7±5.2 vs 70.1±4.3; $P<0.05$) and proliferating zones (137.7±7.2 vs 114.1±4.7; $P<0.05$), and increased chondrocyte proliferation (BrdU +ve cells/mm) (57.0±1.5 vs 45.0±2.0; $P<0.001$). These data confirm that SOCS2 is a key regulator of GH action on chondrocyte function and long bone growth and may represent an important target for growth modulation. Sources of Research Support: Biotechnology and Bioscience Research Council. Nothing to Disclose: CP, VM, FA, CF

Pub #	P2-344
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Assessment of the Growth Hormone System *In Vivo* in a Teleost Fish: A Unique Model of Growth Hormone Resistance in Skeletal Muscle
Author String	EN Fuentes, BT Bjornsson, IE Einarsdottir, JA Valdes, AI Molina Andres Bello University, Santiago, Chile; University of Gothenburg, Gothenburg, Sweden
Body	The growth hormone (GH) system is a key regulator of growth in vertebrates. This system has been conserved during the evolution of vertebrates, maintaining similar features and components through time. The role of this system in the skeletal muscle of mammals is not fully understood and very little is known for lower vertebrates. Fish skeletal muscle growth occurs throughout the entire life cycle through both hyperplasia and hypertrophy, setting fish apart from all other vertebrate classes. Moreover, fish species naturally experience prolonged periods of catabolic-anabolic nutritional conditions, triggering skeletal muscle wasting as part of a natural process, thus the regulation of muscle mass shows some differences compared to mammals. Therefore fish also represent an important and interesting model for studying fundamental muscle growth-regulatory mechanisms in vertebrates. In this context, we evaluated the role of the GH system in the skeletal muscle of a flatfish species. Inherent growth hormone resistance in muscle was found in this species due to high levels of plasma GH, low levels of insulin-like growth factor -1 (IGF-1), large amounts of truncated growth hormone receptors (tGHR), and low amounts of full-length growth hormone receptor (flGHR); concomitantly with slight activation of the JAK-STAT signaling pathway. Subsequently, in order to assess the dynamic of these molecules and their effects in skeletal muscle, fish were subjected to a long catabolic period (3 weeks fasting) and afterward were exposed to a long anabolic period (4 week refeeding). Plasma GH levels increase during fasting and are reestablished during refeeding. The amounts of flGHR are withdrawn completely during fasting and the tGHR shifts concomitantly with the impairment of the activation of the JAK-STAT signaling, ultimately decreasing IGF-1 production. During refeeding these molecules were reestablished. No significant changes were found for the growth hormone binding proteins (GHBP) during both periods. The present study describes for the first time the dynamic of the GH system in lower vertebrates, finding a unique, inherent GH resistance in muscle which is accentuated during long catabolic periods. This study contributes to the understanding of the nutrient-regulation of the GH system and its signaling pathways in skeletal muscle growth in non-mammalian species, hence providing insight concerning the events controlling somatic growth in vertebrates. Sources of Research Support: FONDECYT Grant 1090416; FORMAS Grant 2008-1258. Nothing to Disclose: ENF, BTB, IEE, JAV, AIM

Pub #	P2-345
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Expression of Liver Prolactin and Growth Hormone Receptors, and Sexually Dimorphic GH-Dependent Genes in Three Mouse Models with Different Levels of GH and PRL
Author String	MC Ramirez, MI Perez-Millan, AM Ornstein, D Becu-Villalobos IBYME-CONICET, Capital Federal, Argentina
Body	Pituitary GH secretion is sexually differentiated in many species and sexually dimorphic plasma GH profiles regulate the sex-dependent expression of a large number of liver-expressed genes, including many receptors, signaling molecules, and enzymes of steroid and drug metabolism, especially cytocrome P450s (CYPs). On the other hand, prolactin may impact on liver gene expression; therefore we sought to establish the expression of liver sexually dimorphic genes in three mouse models with different GH and prolactin profiles. Dopaminergic D2 receptor (D2R) knockout mice Drd2-/- have an altered GHRH-GH axis and are growth restricted; besides they have chronic hyperprolactinemia. We also worked with the pituitary conditioned Drd2-/- knockout mice with D2R disruption in lactotropes (lacDrd2-/-) obtained in our laboratory by Cre/LoxP technology; these mice have a conserved GH axis and chronic hyperprolactinemia. Finally, we analysed neonatally androgenized female mice (TP females), which have increased GH and lower prolactin pituitary content compared to control females. We analysed by real-time PCR the mRNA expression of the prolactin receptor (PrlR), GH receptor (GhR), Cyp2b9, Cyp2a4, Cyp2d9 and Mup 1/2/6/8 in the livers of the three mouse models. We found that in both knockout models PrlR mRNA expression was higher in females compared to males, and that it was increased in Drd2-/- and lacDrd2-/- females compared with wild-type females. There was a significant linear correlation between plasma prolactin levels and liver PrlR mRNA expression ($p=0.00094$). Conversely, in TP females PrlR mRNA levels were lower than in control females. Male specific expression was confirmed for Cyp2d9 and Mup 1/2/6/8 mRNAs (male/female ratios: 6.4 and 2.4, respectively), while Cyp2a4 and Cyp2b9 mRNAs were both expressed predominantly in female mice livers (female/male ratios: 7.7 and 11.6, respectively). These genes were not influenced by genotype. On the other hand, Mup 1/2/6/8 mRNA levels were lower in Drd2-/- mice but not in lacDrd2-/- mice. The expression of GhR mRNA was sex-independent and not altered in any of the three models. We conclude that elevated serum prolactin induces the liver expression of its receptor, irrespective of GH levels. On the other hand Mup 1/2/6/8 mRNA expression was more susceptible to GH alterations than the rest of sexually dimorphic GH dependent liver genes in our models. Sources of Research Support: CONICET PIP 640; ANPCYT PICT 2006 N 207, Argentina, AWARDED TO DBV. Nothing to Disclose: MCR, MIP-M, AMO, DB-V

Pub #	P2-346
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Susceptibility of GH Receptor Antagonist Mice to Diet-Induced Obesity
Author String	DE Berryman, T Yang, EO List, E Lubbers, K Troike, C Vesel, H Zhang, JJ Kopchick Ohio University, Athens, OH; Ohio University, Athens, OH; Ohio University, Athens, OH
Body	Mice deficient in or insensitive to GH (GH) exhibit increase in lifespan and improvements in insulin signaling. In contrast, mice transgenic for the GH receptor antagonist (GHA), which have a decrease but not absence of GH signaling, are dwarf and obese, yet display normal glucose homeostasis and normal lifespan. Several studies have reported the impact of high fat (HF) feeding in male mice deficient or insensitive to GH; however, the impact of HF feeding in GHA mice and the influence of gender has not been previously assessed. Thus, the purpose of this study was to test the susceptibility of male and female GHA mice to diet induced obesity as compared to wild-type (WT) controls. Mice were fed either a high fat (HF, 40% of calories) or low fat (LF, 10% of calories) diet over an 11-week feeding study, which is similar to a previous feeding study with GH insensitive mice (1). Body weight, body composition, food intake, glucose homeostasis, adipokine levels and tissue weights were assessed. Adipocyte size and liver triglyceride content were determined on dissected tissues. The results show that male GHA and WT mice had dramatic increases in body weight and fat mass on the HF versus the LF diet, in part due to hyperphagia. Body weight increases on the HF diet were due solely to gains in fat mass. Notably, both GHA and WT females had only marginal increases in body weight and fat mass with HF feeding as compared to male mice, suggesting a gender difference in susceptibility to diet induced obesity. GHA mice maintained better glucose homeostasis even on the HF diet. As has been reported previously, GHA mice had a disproportionally enlarged subcutaneous adipose depot on the LF diet although the weight of all adipose depots increased with HF feeding. Male and female GHA mice had high levels of leptin, which increased with HF feeding. Liver triglycerides were only elevated in male GHA mice relative to controls and were higher on both the LF and HF diet. Overall, GHA male mice were more sensitive to diet-induced obesity than WT littermates and were also more susceptible than previously reported for GH insensitive mice. However, the increase in obesity with a HF diet did not cause the same degree of impairment in glucose homeostasis as seen in WT controls. In addition, the resistance to weight gain with HF feeding in female mice, regardless of genotype, suggests a significant gender difference in response to diet manipulation. (1) Berryman DE et al, Endocrinology 2006; 147:2801-8 Sources of Research Support: In part by the State of Ohio's Eminent Scholar Program that includes a gift from Milton and Lawrence Goll, by the AMVETS, by the Diabetes Research Initiative at Ohio University, and by NIH grants DK083729, AG019899, AG031736. Nothing to Disclose: DEB, TY, EOL, EL, KT, CV, HZ, JJK

Pub #	P2-347
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Circadian and Nutritional Regulation of Hepatic Growth Hormone Receptor Abundance in Mice
Author String	Y Zhang, J Jiang, PA Berry, J Kim, ME Young, SJ Frank University of Alabama at Birmingham, Birmingham, AL; University of Alabama at Birmingham, Birmingham, AL; University of Alabama at Birmingham, Birmingham, AL; Birmingham VA Medical Center Birmingham, AL
Body	Growth hormone (GH), in addition to its somatogenic effects, profoundly impacts fuel metabolism and homeostasis. Liver is a major GH metabolic target organ. Pituitary GH is secreted in pulses and diurnally; in humans, GH pulse amplitude is greater during (non-active) nighttime hours. In addition to circulating levels, cellular GH sensitivity is in part determined by abundance of GH receptor (GHR), which binds GH in its extracellular domain and signals via intracellular domain interaction with the JAK2 tyrosine kinase and other proteins. GHR abundance is modulated at several levels, including transcriptional regulation, availability of JAK2 (which chaperones newly synthesized GHR to the cell surface and stabilizes it), GH-induced GHR downregulation, and GH-independent metalloprotease-mediated GHR cleavage (1). GHR metalloproteolysis is catalyzed by TACE (tumor necrosis factor-α cleaving enzyme) and negatively regulated by TIMP3 (tissue inhibitor of metalloproteinase 3), an endogenous TACE inhibitor (2,3). Realizing hepatic GHR's importance in physiology and pathology, we began studies in (nocturnal) mice to examine: 1) if hepatic GHR abundance, like GH levels, varies diurnally; and 2) if high fat diet (HFD) affects hepatic GHR abundance. For diurnal studies, male 22 wk old FVB mice on a 12 h light/12h dark cycle were sacrificed at 6 h intervals (lights on, mid-light, lights off, mid-dark) and livers were analyzed by immunoblotting and real-time RT/PCR. GHR and TIMP3 protein abundance both varied coordinately by roughly 2-fold throughout the 24h cycle, both peaking at the mid-light/lights off periods. Notably, TIMP3 mRNA oscillated 2.8-fold with its peak at the lights off period, but neither GHR nor TACE mRNA oscillated significantly. Thus, circadian variation in liver GHR protein abundance appears governed at a post-transcriptional level, potentially via TIMP3-mediated regulation of TACE activity. For HFD studies, 6 wk old male C57B6 mice were given chow vs. HFD (55% fat) for 10 wk and sacrificed in the mid-morning in the fasted state. Compared to chow-fed mice, hepatic GHR protein rose 3.5-fold and TIMP3 protein fell 2.4-fold in HFD mice. In contrast to the diurnal studies, GHR mRNA rose (~2-fold) in HFD in concert with increased protein abundance, suggesting upregulation of hepatic GHR by HFD at a transcriptional level. The significance of these findings in terms of effects on GH signaling and susceptibility to GHR proteolysis are under investigation. (1) Wang, X., et al., Mol Endocrinol 2008; 22:1427 (2) Zhang, Y., et al., Endocrinol 2000; 141:4342 (3) Zhang, Y., et al Abstract P3-108, 91st Annual Endocrine Society Meeting, June, 2009 Sources of Research Support: VA Merit Review (SJF). Nothing to Disclose: YZ, JJ, PAB, JK, MEY, SJF

Pub #	P2-348
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Growth Hormone (GH)-Dependent Tyrosine Nitration of Glomerular Podocyte Proteins
Author String	AK Pasupulati, A Vivekanandan-Giri, S Pennathur, RK Menon University of Michigan, Ann Arbor, MI; University of Michigan, Ann Arbor, MI; University of Michigan, Ann Arbor, MI
Body	**Background:** Glomerular podocytes (GP) play a critical role in the pathogenesis of diabetic nephropathy (DN). Overactivity of the GH/GH-receptor (GHR) axis is implicated in the pathogenesis of DN. We previously reported that GH increases reactive oxidants in the GP. Reactive nitrogen species (RNS), a reactive oxidant, can nitrate tyrosine residues and thus alter protein function. **Objective:** We investigated GH-dependent increase in nitrosative stress in GP and identified the protein targets of such RNS induced damage by mass spectrometry (MS). **Methods:** MPC-5 GP were treated with or without GH (500 ng/ml) in normo (5 mM glucose) or hyper (20 mM glucose)-glycemic milieu for 24 h. Isotope dilution LC/MS was used to quantify 3-nitrotyrosine (3-NT), a sensitive and specific marker for RNS in GP lysates. In parallel, we employed a targeted proteomic strategy to identify nitrated proteins. GP lysates were immunoprecipitated using anti-NT antibody. The enriched protein fraction was digested with trypsin prior to nano LC/MS. The resultant MS/MS spectra were analyzed using Proteome Discoverer software with peptide mass tolerance of 1.5 Da and a parent ion tolerance of 1.4 Da. The results were validated using PeptideProphet and ProteinProphet (Scaffold) using an adjusted probability of >0.9 for peptides and >0.95 for proteins against the mouse International Protein Index database. To confirm *in vivo* relevance of these findings, glomerular protein fractions were isolated from mice injected with GH (1.5 [mu]g/g IP x 4 doses) and analyzed as described above. **Results:** Levels of protein-bound 3-NT were markedly elevated in cell lysates of GP exposed to GH and glucose, consistent with RNS generation. An osmotic effect of glucose was excluded by experiments using L-glucose. The proteomic studies revealed a novel set of nitrated proteins. There was a significant overlap between the proteins thus identified in the cell culture studies and in glomerular extracts from mice treated with GH. The majority of the proteins identified were cytoskeletal proteins, such as actin, nebulin, and titin. **Conclusions:** This study provides direct evidence that GH increases tyrosine nitration of specific GP proteins both *in vitro* and *in vivo*. GP cytoskeletal proteins are particular targets for GH-dependent tyrosine nitration. We postulate that GH-dependent nitration of GP cytoskeletal proteins is detrimental to GP function and contributes to the pathogenesis of DN. Nothing to Disclose: AKP, AV-G, SP, RKM

Pub #	P2-349
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Investigating the Influence of Lysine Residues, Ubiquitination, and Elongin B/C Interactions on Cytokine-Inducible SH-2 Domain Protein Stability
Author String	PJ Jensik, LA Arbogast Southern Illinois University School of Medicine, Carbondale, IL
Body	The cytokine inducible SH-2 domain protein (CIS) inhibits prolactin receptor signaling and has been implicated in the prolactin insensitive state of hypothalamic dopamine neurons during lactation. CIS proteins are ubiquitinated and often rapidly degraded. They are involved in an E3 ubiquitin ligase complex with Elongin B/C proteins. The aims of this study were to: 1) identify lysine residues responsible for CIS ubiquitination and 2) determine the influence of lysine residues and the Elongin B/C interaction domain on CIS protein stability. CIS contains six conserved lysine residues at positions 59, 72, 98, 121, 188 and 208. Site directed mutagenesis of a HA-CIS expression construct was used to generate constructs with single lysine to arginine (K1R), five lysine residues to arginine (K5R), all six lysine residues to arginine (K6R) or Elongin B/C interaction domain (B/C) mutations. To determine CIS ubiquitination sites, catecholaminergic CAD cells were co-transfected with FLAG-ubiquitin and K1R or K6R mutants followed by FLAG immunoprecipitation and Western blot analysis of HA-CIS. All K1R mutations showed ubiquitin attachment, whereas the K6R mutation showed none. To identify specific lysine residues responsible for CIS degradation, HEK 293T cells were transfected with wtCIS, K1R, K5R or K6R constructs followed by analysis of HA-CIS. The K6R mutation increased CIS levels by 3.4 fold compared to wtCIS. The K1R mutations at 59, 98 and 121 increased CIS levels by 40%, whereas mutations at 72, 188 and 208 decreased or had no effect on CIS levels. The K5R mutants with lysines at 59, 98 and 121 had 1.5 fold higher levels than wtCIS, while K5R mutants with lysines at 72, 188 and 208 had 2.0 to 2.5 fold higher levels. To understand the influence of ubiquitination and Elongin B/C interactions on CIS degradation, HEK 293T cells were transfected with constructs for wtCIS, K6R, B/C or K6R with a B/C mutation. Cells were treated with cycloheximide for 0, 2 or 4 hours and CIS levels evaluated. The wtCIS and B/C mutated proteins showed similar degradation profiles. The K6R mutation showed reduced degradation compared to wtCIS. The combination K6R with B/C mutation further decreased CIS degradation rate compared to K6R mutation alone. In conclusion, these results indicate that multiple lysines contribute to the CIS ubiquitination state and CIS protein stability. Elongin B/C interaction may contribute to CIS degradation in the absence of ubiquitination. Sources of Research Support: NIH grants HD045805 and HD048925. Nothing to Disclose: PJJ, LAA

Pub #	P2-350
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Expression of Growth Hormone (GH)-Regulated, Sex-Dependent Genes in Mouse Liver through Postnatal Development
Author String	T Peters, DJ Waxman Boston University, Boston, MA

Body

We investigated changes in the expression of sex-specific genes during postnatal development to gain insight into the relationship to sex-dependent alterations in plasma GH patters previously implicated in liver sex differences. qPCR analysis of five male-specific genes (Hsd3b5, Cyp2d9, C6, Cyp2u1 and GST[Pi]) revealed low expression in male liver at 2, 3, and 4 wk of age, when plasma GH levels are low, followed by induced expression by 8 wk. In contrast, four female-specific genes (Cyp3a16, Cyp2b9, Acot3 and Cux2) were expressed at a high level in both male and female liver at 2 and 3 wk of age, after which expression was suppressed in males by 4 or 8 wk. Next, we used global transcriptional profiling to investigate whether these patterns are common to other sex-biased genes. Only 7 genes showed female specificity, and only one non-Y chromosome gene showed male-specificity in common at both 3 wk and 4 wk of age, whereas at 8 wk, 485 male-specific genes and 359 female-specific genes were detected. 394 of the 485 male-specific genes were induced in male liver from 3 wk to 8 wk, indicating that gene induction at puberty is the major mechanism regulating the expression of the male-specific genes. 175 of the 359 female-specific genes identified at 8 wk were repressed in male liver from 3 wk to 8 wk. In contrast, in female liver, a majority sex-specific did not show significant changes in expression from 3 wk to 8 wk. 76% of the male-specific genes induced at 8 wk that were characterized in an earlier study [1] were down regulated by hypophysectomy in male liver, consistent with their dependence on the adult male GH pattern, whereas 15% were up regulated (derepressed) in hypophysectomized female liver. The male-specific genes induced in male liver by 8 wk showed a 1.76-fold enrichment for genes down regulated by an adenovirus expressing Cux2, a known female-specific repressor expressed in prepubertal but not adult male liver, consistent with the loss of Cux2 expression in pubertal males contributing to the observed derepression of many male-specific genes. Together, these results show that adult sex differences in mouse liver gene expression primarily result from the changes in pituitary hormone profiles that occur in males at puberty, leading to induction or depression of many male-specific genes and repression of many female-specific genes in the developing male liver.

[1] Wauthier V, Sugathan A, Meyer RD, Dombkowski AA, Waxman DJ. Intrinsic Sex Differences in the Early Growth Hormone Responsiveness of Sex-Specific Genes in Mouse Liver. Mol Endocrinol, March 2010,24(3):667-678.

Sources of Research Support: In part by NIH grant DK33765 (to DJW).

Nothing to Disclose: TP, DJW

Pub #	P2-351
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	White Adipose Tissue Depots in GHR-/- Mice: Age-Related Changes in Comparison to Wild-Type Controls
Author String	L Sackmann Sala, CB Vesel, ER Lubbers, RD Munn, KM Troike, S Partee, DE Berryman, JJ Kopchick Ohio University, Athens, OH; Ohio University, Athens, OH; Ohio University, Athens, OH; Ohio University, Athens, OH; Ohio University, Athens, OH
Body	Growth hormone receptor gene-disrupted (GHR-/-) mice are dwarf and display enhanced insulin sensitivity and prolonged longevity in spite of increased fat accumulation. However, only some white adipose tissue (WAT) depots are disproportionally enlarged in GHR-/- mice. Therefore, these mice are a valuable tool to study WAT depot-specific effects on insulin responsiveness and lifespan. Protein profiles of WAT depots from 12 and 24-month-old GHR-/- mice were resolved by two-dimensional gel electrophoresis followed by mass spectrometry and were compared to corresponding profiles of wild-type mice. Plasma levels of insulin, leptin and adiponectin (total and high molecular weight) and adipocyte sizes were also determined. As expected, insulin levels were lower and adiponectin levels were higher in GHR-/- than wild-type mice, although no difference in leptin levels was detected. Cell sizes showed differences between genotypes, depots and age groups. Overall, the proteomic analysis showed numerous proteins that behaved similarly in individual WAT depots and age groups of wild-type and GHR-/- mice. Still, the intensity of 12 protein spots showed significant effects of genotype (EH domain-containing protein 2, S100-A10, α2-macroglobulin, and transthyretin) or significant interactions of genotype [times] age (β-hemoglobin), genotype [times] depot (annexin A5 and actin) and/or genotype [times] depot [times] age (annexin A5, actin and apolipoprotein A1). Some of these proteins are involved in membrane protein recycling and senescence-related processes. Correlations between the intensities of these spots and hormone levels or cell sizes were specific to a particular genotype, age group and/or WAT depot. The changes in the levels of these proteins might be responsible for the increased insulin sensitivity and extended lifespan found in GHR-/- mice. Sources of Research Support: In part by the State of Ohio's Eminent Scholar Program that includes a gift from Milton and Lawrence Goll; by NIH Grants DK075436-01, AG019899-06, and 1P01AG031736-01A1; by the Diabetes Research Initiative and the BioMolecular Innovation and Technology Partnership at Ohio University; and by AMVETS. Nothing to Disclose: LSS, CBV, ERL, RDM, KMT, SP, DEB, JJK

Pub #	P2-352
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Growth Hormone (GH) Regulates Interaction between Monocyte and Endothelial Cells Via MAPK Pathway
Author String	M Ishikawa, K Kuboki, T Morita, G Yoshino Toho University, Tokyo, Japan; Toho University, Tokyo, Japan
Body	Acromegaly is associated with a 2 to 3 fold increase in morbidity and mortality due to cardiovascular diseases Excess GH in plasma causes diabetes mellitus, insulin resistance, or hypertension, contributing to the development of atherosclerosis. However, the direct action of GH in development of atherosclerosis has still not been clear. In this study, molecular expressions, which are related to atherosclerosis, were investigated with GH stimulation on human umbilical cord vein cell (HUVEC) and THP-1 monocyte. HUVEC or THP-1 was stimulated by 10^{-9} M or 10^{-8} M human GH (hGH) with or without pretreatment of JAK2 or MEK1/2 inhibitor. The RNA was extracted, and the expression of VCAM-1, E-selectin, MCP-1, IL-6, and IL-8 were investigated by RT-PCR. For quantitative adhesion assay, THP-1 cells were fluorescently labeled with BCECF/AM. HUVEC cultured in 4-well cover slides were treated with or without hGH, and then co-incubated with BCECF-labeled THP-1 on a rotating platform. An hour later, the number of adherent BCECF labeled THP-1 on HUVEC was investigated. Human GH stimulated the mRNA levels of VCAM-1 (1.7-fold increase by 10^{-9} M hGH compared with control) and E-selectin (2.5-fold increase by 10^{-9} M hGH compared with control) on HUVEC. The effects were suppressed by MEK1/2 inhibitor pretreatment, but its were not suppressed by JAK2 inhibitor pretreatment. Human GH also stimulated the mRNA levels of MCP-1 and IL-6 on HUVEC, and the mRNA levels of MCP-1 and CCR2 on THP-1, but the increases were a small amount. And thereafter, the adhesion of BCECF-labeled THP-1 on HUVEC was increased by hGH treatment. In summary, hGH stimulated VCAM-1 and E-selectin expression via MAPK pathway, resulting in augmented adhesion of THP-1 on HUVEC. These data suggest that GH stimulates development of atherosclerosis directly. Nothing to Disclose: MI, KK, TM, GY

Pub #	P2-353
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Protein Tyrosine Phosphatase Activity and Functional Collaboration between IGF-I Receptor and GH Signal Transduction in Primary Osteoblasts
Author String	Y Gan, Y Zhang, J Jiang, SJ Frank University of Alabama at Birmingham, Birmingham, AL; University of Alabama at Birmingham, Birmingham, AL; Birmingham VA Medical Center, Birmingham, AL
Body	GH receptor (GHR) binds GH in its extracellular domain (ECD) to activate the GHR-associated cytoplasmic tyrosine kinase, JAK2. IGF-1 binds IGF-1R, a disulfide-linked heterotetramer with tyrosine kinase activity in its intracellular domain (ICD). Classically, IGF-1 is a GH effector in a [ldquo]linear[rdquo] GH4GHR4IGF-14IGF-1R pathway. Our recent studies suggest IGF-1R also subserves GH signaling in several novel ways: 1) GH induces a GHR-JAK2-IGF-1R complex, whose formation is independent of tyrosine phosphorylation of any of the partners; 2) Cotreatment with IGF-1 augments acute GH signaling; 3) deletion of IGF-1R in primary osteoblasts or human prostate cancer cells blunts acute GH signaling (1-3). In IGF-1R-deficient primary osteoblasts, adenovirally-driven re-expression of wild-type IGF-1R normalizes GH-induced STAT5 phosphorylation, but re-expression of a truncated IGF-1R that lacks most of its ICD (including its kinase domain) partially rescues GH-induced STAT5 activation and IGF-1 gene expression (2). Thus, IGF-1R ICD and kinase are dispensable for some of the IGF-1R functional collaboration with acute GH signaling. We now examine how protein tyrosine phosphatase (PTP) activity influences GHR-IGF-1R collaboration. Calvarial osteoblasts from IGF-1R-floxed mice were treated in vitro with a control adenovirus encoding GFP (Ad-GFP) or Ad-Cre to drive Cre expression and excise IGF-1R. As expected, GH (250 ng/ml; 15 min)-induced STAT5 tyrosine phosphorylation was ~60% less in Ad-Cre-infected cells vs. Ad-GFP-infected cells. Pretreatment with orthovanadate, a general PTP inhibitor (200 [mu]M; 1h) did not affect basal or GH-induced STAT5 phosphorylation in Ad-GFP-infected cells. However, GH-induced STAT5 phosphorylation was partially rescued in orthovanadate-treated Ad-Cre-infected cells (90% of orthovanadate-treated, Ad-GFP-infected cells). Pretreatment with the SHP1/2-specific PTP inhibitor, NSC-87877 (50 [mu]M; 1h), in contrast, failed to rescue GH signaling. However, a PTP-1B-specific inhibitor (10 [mu]M; 1h) mimicked the effect of orthovanadate. These data suggest deletion of IGF-1R impairs GH-dependent STAT5 signaling by allowing a PTP (perhaps PTP-1B) to more negatively regulate GHR activation than in the presence of IGF-1R. Further, IGF-1R ECD and ICD may augment GH signaling in differently; ECD affects GHR signaling conformation, while ICD impacts association of GHR-JAK2 with negative regulatory molecules. (1) Huang, Y., et al., Mol Endocrinol 2004; 18:1471 (2) Gan, Y., et al., Mol Endocrinol 2010; 24:644 (3) Zhang et al Abstract P3-233, 92nd Annual Endocrine Society Meeting, June, 2010. Sources of Research Support: NIH R01 DK46395 (to SJF). Nothing to Disclose: YG, YZ, JJ, SJF

Pub #	P2-354
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Acute Hepatic Growth Hormone Resistance Following Injury
Author String	RM Corrick, L Li, JL Messina University of Alabama Birmingham, Birmingham, AL
Body	Acute growth hormone (GH) resistance is frequently observed following severe injury. Because GH action is important for wound healing and maintenance of lean body mas, GH resistance may complicate recovery fron injury. In order to study the effects of injury on GH signaling, we subjected 12-week old male mice to soft-tissue trauma combined with hemorrhage. All procedures were conducted under continuous isoflurane anesthesia. Hemorrhage was accomplished by withdrawal of sufficient blood through femoral artery catheters to reduce mean arterial pressure to 35-40 mmHg. GH or vehicle was administered intravenously after 30, 60 or 90 min of hemorrhage, and livers were collected 10 min later. Additional mice were anesthetized, subjectec to soft-tissue trauma and arterial catheterization, and injected with GH or vehicle to control for the effects of surgical procedures. These "trauma alone" controls exhibited modest reductions in hepatic GH signaling compared to uninjured mice. However, severe decreases in GH signaling were measured after 30, 60 or 90 min of hemorrhage compared to controls. SDS-PAGE and Western analysis indicated a ~10 kDa decrease in molecular weight of the growth hormone receptor (GHR) following hemorrhage. We are unsure whether this decrease is due to a proteolytic event or impaired post-translational processing of GHR. However, such low molecular weight GHR was detected as early as 30 min following onset of hemorrhage, corresponding with severely impaired GH signaling, and persisted even after fluid resuscitation and 60 min of recovery. In contrast to full-length GHR, low molecular weight GHR was not tyrosine-phosphorylated in response to GH stimulation. These results suggest that the combination of injury and hemorrhage results in the rapid development of hepatic GH resistance, which may be due to changes to the GHR. Nothing to Disclose: RMC, LL, JLM

Pub #	P2-355
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	LAPS-HGH, Novel Long-Acting HGH (HM10560A) for Weekly or Less Frequent Administration
Author String	SY Jung, JS Lee, JS Kim, DJ Kim, IY Choi, CG Lim, YE Woo, YA Park, KM Park, SC Kwon Hanmi Pharm Co, Ltd, Seoul, Republic of Korea
Body	Recombinant human growth hormone (rhGH) is widely used for decades to treat growth hormone deficiency. Daily administration of rhGH is associated with patient compliance and there is need to develop long-acting rhGH which allows less frequent dosing to improve patient compliance. LAPS-hGH (HM10560A) is being developed by conjugating the rhGH and constant region of human immunoglobulin (LAPS-carrier) via non-peptidyl linker. In the weight gain assay on hypophysectomized rats, weekly administration of LAPS-hGH showed potent weight gain compared with that of daily administered rhGH. Dose dependent induction of IGF-1 was confirmed in normal beagle dogs and cynomolgus monkeys. Pharmacokinetic studies of subcutaneously injected LAPS-hGH in beagle dogs and cynomolgus monkeys showed significantly extended terminal half lives compared with rhGH. The safety profiles of LAPS-hGH were evaluated in several studies including single and repeat dose studies ir rats and monkeys, and the results showed that no LAPS-hGH specific toxicities were observed. Single dose escalation phase I study showed that LAPS-hGH was well tolerated in healthy volunteer and PK/PD profiles in human indicated that weekly or less frequent dosing of LAPS-hGH is possible. Nothing to Disclose: SYJ, JSL, JSK, DJK, IYC, CGL, YEW, YAP, KMP, SCK

Pub #	P2-356
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Characterization of the Putative Protein Encoded by a Novel Isoform of Sec24D mRNA Expressed in Dwarf Growth Hormone Receptor Gene-Disrupted Mice
Author String	E Gosney, B Kelder, M Grier, N Anderson, J Kopchick Ohio University, Athens, OH; Ohio University, Athens, OH; Ohio University, Athens, OH
Body	Growth hormone receptor/binding protein gene disrupted mice (GHR-/-) have a complete absence of GHR signaling. These mice are dwarf with low levels of IGF-1 and insulin. Despite subcutaneous obesity, they exhibit extended longevity. To elucidate potential mechanisms behind the insulin sensitivity and increase in lifespan of these animals, we have performed gene expression studies to discovery new gene transcripts that are differentially expressed in the dwarf animals. As reported previously, one of the novel transcripts identified, 5-9-4, is expressed in the liver, kidney and adipose tissue of GHR -/- animals but not in wildtype controls (WT), bovine GH transgenic or GH receptor antagonist transgenic mice. The full length cDNA for this transcript has been isolated and sequenced. This transcript is expressed from the Sec24D gene, located on chromosome 3 proximal to the gene for methyltransferase-like 14. The full-length transcript for 5-9-4 is 2,203 nucleotides in length with an open reading frame that encodes a putative protein of 487 amino acids. This putative protein has a theoretical pI of 6.79 and molecular weight of 54,351 kDa. The cDNA for 5-9-4 has been cloned into the pBK CMV eukaryotic expression vector. A poly histidine tag (6x) has been appended to the 3' end of the coding region for the putative protein using an In-Fusion cloning kit (Clontech). Expression and characterization of this protein will be analyzed in the human liver cell line HepG2-C3A. We hope to determine the function of this Sec24D isoform, particularly as it may relate to energy balance, metabolism and longevity. Sources of Research Support: In part by the State of Ohio's Eminent Scholar Program that includes a gift from Milton and Lawrence Goll, by grants from the Diabetes Research Initiative at Ohio University, the AMVETS organization, and NIH grants DK083729, AG19899 and AG031736. Nothing to Disclose: EG, BK, MG, NA, JK

Pub #	P2-357
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Involvement of 16kDa Prolactin in Spermatogenic Cell Apoptosis in Mice with Experimental Cryptorchidism
Author String	Y Yanagisawa, M Shimada, K Sobue, M Ishida, T Harigaya Meiji University, Kawasaki, Japan
Body	Prolactin (PRL) is primarily secreted from the anterior pituitary gland as 23kDa protein. On the other hand, N-terminal cleaved 16kDa PRL, one of PRL variants, has been well known to have an antagonistic function to intact 23kDa PRL. In our precise study, 16kDa PRL existed in the adult mouse spermatid and sperm tail. However, its function is not clear yet. In the present study, we focused on apoptotic action that is one of functions of 16kDa PRL. In order to investigate the effect of 16kDa PRL on spermatogenic cell apoptosis and to detect the protease for 23kDa PRL, experimental cryptorchidism in mice were performed to induce an apoptosis on testicular germ cells. Mice were sacrificed at 1 or 2 week after surgery. Testes were removed and used for immunohistochemistry (IHC), western blotting (WB) and RT-PCR analysis. Apoptotic cell was determined by TUNEL staining. IHC was performed to check the localization of 16kDa PRL and Cathepsin D (CathD) which was reported as protease for 23kDa PRL. RT-PCR was performed to investigate PRL, PRL receptor (PRLR) and CathD gene expressions in mouse cryptorchid testis. In addition, we attempted WB to determine quantitative changes of 16kDa PRL and CathD during apoptotic progression. As a result, 16kDa PRL was detected in sperm tail at one week and in apoptotic cell at two weeks after operation. On the other hand, most CathD signals showed co-localization with 23kDa PRL, but not 16kDa PRL. RT-PCR analysis revealed expressions of PRL, PRLR and CathD in cryptorchid testis. Although CathD increased with progression of spermatogenic cell apoptosis, 16kDa PRL in two weeks cryptorchid testis was less than that of one week cryptorchid testis. It is unclear, however, whether this decrease of 16kDa PRL is due to the apoptosis of sperm. These results seem to suggest that 16kDa PRL may be associated with germ cell apoptosis in mouse cryptorchid testis and CathD would be cleaved 23kDa PRL into 16kDa PRL in mouse cryptorchid testis. Further studies are necessary to determine how 16kDa PRL participates in germ cell apoptotic pathways. Nothing to Disclose: YY, MS, KS, MI, TH

Pub #	P2-358
Session Information	POSTER SESSION: BASIC/TRANSLATIONAL - Growth Hormone & Prolactin (1:30 PM-3:30 PM)
Title	Evidence for a Direct Effect of Growth Hormone on the Mammary Epithelium during Lactation
Author String	LT Strand, LP McDonnell, DG Peterson California Polytechnic State University - San Luis Obispo, San Luis Obispo, CA
Body	The indirect influence of growth hormone (GH) on lactation, mediated by IGF-I, has been well characterized, though recent studies have demonstrated a direct effect of GH on expression of milk protein and other genes in mammary epithelial cell lines. Due to the inherent complexities of a whole animal system, and the questionable biological relevance of an immortalized cell line, we selected primary cell culture models to validate the previously reported response to GH elicited by mammary epithelial cell lines such as the bovine MAC-T. Primary mammary epithelial cells (MEC) were isolated from two breeds of lactating dairy cows (Jersey and Holstein), as well as pregnant and lactating mice by density gradient centrifugation following enzymatic digestion. All cells were grown to near confluence and then induced to a lactating phenotype in a classic lactation medium containing dexamethasone, insulin, and prolactin and supplemented with either 0 or 10ng/ml GH. The epithelial nature of the cells isolated was verified by identification of cytokeratin expressior through immunocytochemistry (bovine) and flow cytometry (murine). Primary MEC isolated from pregnant mice were not affected by GH. Primary MEC isolated from all lactating animals exhibited similar increases in milk protein mRNA expression in response to GH treatment to that observed in MAC-T cells, although to a lesser magnitude. Abundance of GH receptor mRNA expression was increased in response to GH in primary MEC treated for 1-2 d but declined to levels lower than the control with longer duration of treatment. Insulin-like growth factor I (IGF-I) mRNA was detected in all cell preparations, including the MAC-T, though the effects of GH on milk protein mRNA did not correlate to changes in IGF-I mRNA, indicating that the effects of GH are not mediated through IGF-I. The expression of insulin-like growth factor binding protein-3 (IGFBP3) mRNA was significantly increased in response to GH in MAC-T cells; Jersey MEC showed similar patterns, but to a lesser magnitude. This IGFBP3 increase was not observed in Holstein MEC, pregnant or lactating murine MEC. The results observed in primary cells isolated from lactating animals support the results observed in MAC-T and indicate that GH may influence mammary epithelium during lactation in vivo though the nature of the influence is not entirely clear and warrants further investigation. Nothing to Disclose: LTS, LPM, DGP

Pub #	P2-359
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Hormone: Physiology, Deficiency (Adult), Delivery Systems, Use in Other Syndromes (1:30 PM-3:30 PM)
Title	The Accuracy of Diagnostic Tests for Growth Hormone Deficiency in Adults: A Systematic Review and Meta-Analysis
Author String	A Hazem, M Elamin, G Malaga, I Bancos, CL Zeballos-Palacios, Y Prevost, ER Velasquez, P Erwin, MH Murad, V Montori Mayo Clinic, Rochester, MN; Universidad Peruana Cayetano Heredia, Lima, Peru
Body	**Context:** The diagnostic accuracy of tests used to diagnose growth hormone (GH) deficiency in adults is unclear. **Objective:** To conduct a diagnostic systematic review and meta-analysis of studies that provided data on diagnostic tests of choice. **Data Sources:** We searched electronic databases (MEDLINE, EMBASE, Cochrane CENTRAL, Web of Sciences and Scopus) through September 2009. Review of reference lists and contact with experts further identified candidate studies. **Study Selection:** Reviewers, working independently and in duplicate, determined study eligibility. **Data Extraction:** Reviewers, working independently and in duplicate, determined the methodological quality of studies and collected descriptive, quality and outcome data. **Data Synthesis:** Twenty-one studies provided diagnostic accuracy data. Studies were all nonrandomized and had fair methodological quality (assessed using the QUADAS checklist) and included 977 patients. Several tests had good diagnostic accuracy (diagnostic odds ratio >50) such as GH-RP6, Acipimox+GHRH test, GHRH+GHRP6, GHRH+GHRP2, hexarelin stimulation test, arginine and ITT (insulin tolerance test).Other tests such serum level of IGF-1 and growth hormone had lower accuracy. The gold standard varied widely between studies. Heterogeneity was significant in most analyses. No studies examined the effect of diagnostic strategies on patient-important outcomes. **Results:** Out of 14 tests of interest covered by included studies, 5 tests proved to be significantly more accurate than the rest. Those tests are; ITT, GHRP6, GHRH+GHRP6, GHRH+GHRP2, Hexarelin stimulation GHRH+Acipimox and arginine stimulation test (AST). This is evidenced by the high calculated diagnostic odds ratio scores achieved by these tests. **Conclusion:** several tests with fairly good diagnostic accuracy are available for the diagnosis of growth hormone deficiency in adults. The supporting evidence however, is indirect and at high risk of bias. Nothing to Disclose: AH, ME, GM, IB, CLZ-P, YP, ERV, PE, MHM, VM

Pub #	P2-360
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Hormone: Physiology, Deficiency (Adult), Delivery Systems, Use in Other Syndromes (1:30 PM-3:30 PM)
Title	Growth Hormone Replacement Therapy in Adults with Growth Hormone Deficiency: A Systematic Review and Meta-Analysis
Author String	A Hazem, M Elamin, G Malaga, CL Zeballos-Palacios, Y Prevost, ER Velasquez, N Abu Elnour, B Irina, J Almandoz, P Erwin, MH Murad, V Montori Mayo Clinic, Rochester, MN; Universidad Peruana Cayetano Heredia, Lima, Peru
Body	**Context:** The benefits and harms of growth hormone (GH) treatment/replacement in adults with presumed deficiency are unclear. **Objective:** To conduct a systematic review and meta-analysis of trials that provided data on outcomes of interest; changes in weight, BMI, BMD, fat content and quality of life. **Data Sources:** We searched MEDLINE, EMBASE, Cochrane CENTRAL, Web of Science and Scopus through September 2009. Review of reference lists and contact with experts further identified candidate studies. **Study Selection:** Reviewers, working independently and in duplicate, determined study eligibility. **Data Extraction:** Reviewers working independently and in duplicate determined the methodological quality of studies and collected descriptive, quality and outcome data. **Data Synthesis:** From each study, we estimated the relative risk (or risk ratio, RR) and 95% confidence interval (CI) for dichotomous outcomes and weighted difference in means (WMD) and 95% CI for continuous outcome. Data were pooled using the random effects model and heterogeneity assessed using the I^2 statistic. The GRADE methodology was used to evaluate the quality of evidence. **Results:** Twenty-six randomized controlled trials reported data sufficient for inclusion in meta-analysis. The trials included over 1400 patients. GH treatment was associated with statistically significant reduction in weight (WMD= -2.39 kg; 95% CI, -2.75, -2.03; I2=0%) and body fat content (WMD= -1.91 kg; 95% CI, -2.92, -0.9; I^2=32%). There was a trend that did not reach statistical significance for reduction in BMI and increase in the occurrence of carpal tunnel syndrome. GH replacement therapy was found to significantly increase the risk of treatment requiring edema (RR: 6.36, 95% CI: 3.8-10.67). There was no statistically significant effect on bone density, recurrence of pre-existing tumors or joint complaints. Quality of life outcomes were infrequently reported and were insufficient for meta-analysis. The quality of evidence was deemed high for the outcomes of weight and body fat and low for the remaining outcomes due to imprecision. **Conclusion:** Growth hormone treatment/replacement in adults with presumed deficiency leads to reduction in weight and body fat content and to increased risk of treatment requiring edema. The evidence regarding other potential adverse effects or benefits is inconclusive and definite answers require studies with longer follow up duration and larger sample size. Nothing to Disclose: AH, ME, GM, CLZ-P, YP, ERV, NAE, BI, JA, PE, MHM, VM

Pub #	P2-361
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Hormone: Physiology, Deficiency (Adult), Delivery Systems, Use in Other Syndromes (1:30 PM-3:30 PM)
Title	Characteristics of Adult Growth Hormone Deficiency (AGHD) Patients Treated with Growth Hormone over 3 Years: Results from the ANSWER Program[reg]
Author String	M Gordon, R Levy, J Goldstein, R Gut, J Germak Allegheny General Hospital, Pittsburgh, PA; Rush Presbyterian-St Luke's Medical Center, Chicago, IL; Novo Nordisk, Princeton, NJ
Body	Adult patients ([ge]18 years) with GHD enrolled in the American Norditropin Studies: Web-Enabled Research (ANSWER) Program[reg] at the discretion of participating physicians as of October 2010 were analyzed for diagnostic categories, growth hormone stimulation tests (GHST) used during diagnosis, baseline demographics, and changes in weight, body mass index (BMI), and serum IGF-1 levels associated with growth hormone treatment (GHT) over 3 years. Cross-sectional analyses were used to examine means across time. A total of 340 patients (135 M, 205 F, mean±SD age 48.8±13.4 years) were included in this analysis, of which 150 (44%) had isolated growth hormone deficiency (IGHD) and 190 (56%) had multiple pituitary hormone deficiency (MPHD). Fewer MPHD patients (47%) underwent GHST compared with IGHD (93%). Among those patients who underwent GHST, the % of patients taking the tests and the mean±SD peak values (ng/mL) are as follows: L-Dopa/arginine (39.5%, 1.4±1.2), glucagon (26.3%, 1.8±1.5), arginine (15.8%, 1.2±1.5), growth hormone releasing hormone (GHRH)+arg (9.2%, 3.9±1.9), insulin tolerance test (ITT) (6.1%, 1.1±1.3), and other tests. The mean duration of GHT was 12.4±10.7 months in IGHD and 17.8±17.4 months in MPHD. The mean GH dose (mg/day) was 0.34±0.21 at baseline, 0.47±0.30 at Year 1, 0.60±0.39 at Year 2, and 0.58±0.23 at Year 3 for IGHD patients. Among MPHD patients, mean GH dose was 0.52±0.43 at baseline, 0.59±0.37 at Year 1, 0.66±0.44 at Year 2, and 0.62±0.40 at Year 3. Baseline BMI (kg/m^2) was consistent with an obese population (33.5±7.7 for IGHD, and 32.6±6.6 for MPHD). Body weight and BMI were stable during the first 2 years of treatment. Longitudinal MPHD patients showed a slight weight loss of 1.2 kg, and a BMI decrease of 1 kg/m^2 after 3 years of GHT. Serum IGF-1 levels (mean±SD; ng/mL) were as follows: baseline (94±41 IGHD; 103±57 MPHD), year 1 (161±81 IGHD; 186±86 MPHD), year 2 (140±79 IGHD; 190±105 MPHD and year 3 (128±49 IGHD; 216±85 MPHD). In conclusion, among AGHD patients enrolled in the ANSWER Program[reg] MPHD was the most common diagnostic category. GHSTs were used more frequently among IGHD patients. L-Dopa/arginine and glucagon were the most commonly used GHSTs and ITT was used less frequently for diagnosis of AGHD in this US based registry. GHT increased serum IGF-1 levels from baseline to year 3 in both IGHD and MPHD patients, and a slight decrease in BMI was observed in MPHD patients after 3 years of therapy. Sources of Research Support: Novo Nordisk. Disclosures: RL: Study Investigator, Novo Nordisk. JG: Employee, Novo Nordisk. RG: Employee, Novo Nordisk. JG: Employee, Novo Nordisk. Nothing to Disclose: MG

Pub #	P2-362
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Hormone: Physiology, Deficiency (Adult), Delivery Systems, Use in Other Syndromes (1:30 PM-3:30 PM)
Title	rhGH Treatment of COGHD in the Age of Transition: Effect on Quality of Life and Endothelium
Author String	S Della Casa, A Capozzi, R Bocale, G Desideri, B Altieri, A Pontecorvi Patologia Speciale e Semeiotica Medica, Rome, Italy
Body	GH has an important influence on growth, bone metabolism, cardiovascular system and quality of life (QoL). GH deficiency (GHD) is associated with a higher incidence of cardiovascular disease because of alterations o cardiac performance and early endothelial dysfunction. GHD patients show impairments in mood and cognitive functioning that rhGH treatment is found to improve. The age of transition is a time to define the persistence of GHD in patients COGHD and to establish the need to continue rhGH. We studied long-term effects of rhGH on psychological well-being and QoL and on endothelium and preclinical atherosclerotic markers. We enrolled 40 patients (mean age: 20.5±2.1 yrs; BMI 21.6±10.9 kg/m2). 23 COGHD patients treated with rhGH were retested and divided into two subgroups according to result of arginine test (GH peak<10 pg/ml): 12 patients confirmed GHD (group1) while 11 patients showed normal GH response (group2). 6 patients of group 1 continued rhGH therapy (1a) while 6 not (1b). Control group consisted of 17 healthy age- mached subjects (group 3). In all subjects we administrated the 36-Items Short Form Health Survey (SF-36) and Psychological General Well-Being Index (PGWBI) for the assessment of QoL and psychological well-being at the beginning of rhGH and after 6 months of treatment in group 1a. ICAM-1, VCAM-1, E-selectin, P-selectin, CD40L, PGF2α, hsCRP were also dosed. There was statistically significant difference in general mental health and mood in SF-36 and PGWBI ($p< 0.05$) between COGHD group and controls. In group 1a SF-36 revealed significant improvements in physical and social functioning, bodily pain From PGWBI we detected amelioration about anxiety, depression, positive well-being and self-control. We found all endothelial biomarkers were more elevated in all COGHD than in the control group ($p< 0.05$). They were less expressed in group on GH than in group without (ns). We matched group on GH and group COGHI with normal GH at retest and we found statistically significant differences between E-selectin, CD40L and hsCRP ($p< 0.05$). Although number of patients was small, results indicate rhGH should prevent premature atherosclerosis in adolescence and should contribute to avoid development of cardiovascular disease in GHD adults. Our findings show patients GHD which continue rhGH treatment reach a significant improvement in both physical and psychological well-being, suggesting GH replacement has benefits also on mood and QoL. Nothing to Disclose: SDC, AC, RB, GD, BA, AP

Pub #	P2-363
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Hormone: Physiology, Deficiency (Adult), Delivery Systems, Use in Other Syndromes (1:30 PM-3:30 PM)
Title	The Effect of Aerobic Exercise on Intramyocellular (IMCL) and Intrahepatocellular Lipids (IHCL) in Hypopituitary Patients with Growth Hormone Deficiency (GHD), Sedentary Control Subjects (CS) and Endurance-Trained Athletes (EA)
Author String	ER Christ, C Stettler, A Egger, S Alleman, P Diem, T Buehler, C Boesch University Hospital of Bern, Inselspital, Bern, Switzerland; University of Bern, Bern, Switzerland; University of Bern, Bern, Switzerland
Body	**Introduction:** Increased levels of IMCL and IHCL are related to impaired insulin action at the skeletal muscle and hepatic level.Conversely, IMCL utilization during exercise is high in EA.The effect of aerobic exercise on IHCL is unknown. **Methods:** Ten GHD patients,10 sedentary CS matched for age, gender, BMI,waist and 10 EA (Vo_{2max}>50 ml/kg body weight) were recruited.VO_{2max} was assessed using a incremental exercise test.Insulin sensitivity was determined with a hyerinsulinaemic euglycaemic clamp.Using MR-imaging total fat mass (FM), visceral (VAT) and subcutaneous fat compartments (SCAT) were determined.IHCL and IMCL were measured before and after a 2-h aerobic exercise at 50-60% of individual VO_{2max} using MR-spectroscopy.Plasma free fatty acid concentrations (FFA) were determined every 30 minutes during exercise and area under the curve (AUC) was calculated. **Results:** Mean±SD VO_{2max} was highest in EA followed by CS and GHD (62.4±7.2 vs 41.5±5.5 vs 35.5±7.3 ml/kg body weight; ANOVA $p<0.001$).The M-value was significantly higher in EA compared to GHD and CS (10.8±2.8 vs 7.4±2.8 vs 7.7±1.4, mg/kg body weight/min; $p<0.007$).FM, SCAT and VAT were similar in CS and GHD.EA had significantly lower total FM, SCAT and VAT compared to GHD and CS.Pre-exercise levels of IMCL were similar in all three groups.IMCL decreased significantly in all three groups following exercise.The highest decrease in IMCL (delta IMCL) was in EA (-26.3±12.6% from baseline) followed by CS (-17.8±12.5%) and GHD (-8.4±16.1%; $p<0.04$).Delta IMCL was negatively correlated with VO_{2max} ($r=-0.38$; $p<0.05$).Pre-exercise levels of IHCL were lower in GHD and EA compared to CS (4.2±5 vs 1.8±0.9 vs 12.4±15, %, $p=0.05$). IHCL significantly increased in all three groups following exercise. The highest increas was observed in CS (+1.3±1.5%) with a similar increase in GHD and EA (+0.6±0.4 and +0.5±0.4, %). Delta IHCL values were positively correlated with FFA availability as assessed by AUC of FFA during exercise ($r=0.35$; $p<0.05$). **Conclusions:** 1) GHD patients do not exhibit features of the classical insulin resistance syndrome (increase in VAT, M-value and IHCL) when compared to sedentary CS matched for age, gender, BMI and waist cirumference. 2) Aerobic physical exercise induces a decrease in IMCL and an increase in IHCL.3) Exercise capacity (VO_{2max}) may be related to IMCL-utilization during exercise whereas FFA availability may be involved in increasing IHCL during exercise. Sources of Research Support: Swiss National Foundation No: 320030-109522, Independant Researach Grant (Pfizer). Nothing to Disclose: ERC, CS, AE, SA, PD, TB, CB

Pub # P2-364

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Hormone: Physiology, Deficiency (Adult), Delivery Systems, Use in Other Syndromes (1:30 PM-3:30 PM)

Title A Longer Interval between Childhood and Adult Growth Hormone (GH) Replacement Is Associated with Lower Bone Mineral Density (BMD) in GH-Deficient (GHD) Adults -- A KIMS (Pfizer International Metabolic Database) Analysis

Author String NA Tritos, AH Hamrahian, D King, SL Greenspan, DM Cook, PJ Jonsson, MP Wajnrajch, M Koltowska-Haggstrom, BMK Biller
Massachusetts General Hospital and Harvard Medical School, Boston, MA; Cleveland Clinic, Cleveland, OH; University of Pittsburgh, Pittsburgh, PA; Oregon Health and Science University, Portland, OR; Pfizer Endocrine Care, Sollentuna, Sweden; Pfizer, Inc, New York City, NY

Body

Adults with childhood-onset (CO) GHD on GH replacement may be at risk for lower BMD as a result of interruptions in GH replacement during the transition period to adult care.
To examine BMD predictors in this population, KIMS was searched using these criteria: CO GHD persisting in adulthood based on stringent diagnostic criteria, baseline BMD data in the posterior-anterior lumbar spine (LS) and/or femoral neck (FN) (used to calculate standardized BMD (sBMD)). Subjects were classified as true na[iuml]ve (starting GH replacement at study entry), semi-na[iuml]ve (off GH replacement for [ge]12 months before study entry) and non-na[iuml]ve (off GH replacement at study entry for <6 months since initiation of pediatric GH therapy).
The search identified 314 subjects, including 74 true na[iuml]ve, 178 semi-na[iuml]ve and 62 non-na[iuml]ve patients (148 women and 166 men). Population characteristics were [median (10^{th} percentile, 90^{th} percentile)]: age at pituitary disease onset: 10.1 years (4.5, 16.2); age at study entry: 27.2 years (19.3, 42.8); number of additional pituitary hormone deficits (% of subjects): isolated GHD (12.4 %), 1 (12.4 %), 2 (9.3 %), 3 (40.1 %), 4 (25.8 %); length on GH replacement at study entry: 4.0 years (0.0, 13.0); interval (gap) between GH replacement in childhood and adulthood: 4.1 years (0.0, 18.1); GH dose: 0.27 mg/day (0.13, 0.70); serum insulin-like growth factor-I standard deviation scores: -3.8 (-6.3, -0.7); LS sBMD (mg/cm^2): 989 (809, 1219); FN sBMD (mg/cm^2): 860 (691, 1129); LS z score: -1.4 (-2.5, 0.7); FN z score: -0.7 (-2.6, 0.9).
There were no differences in sBMD or z scores between true na[iuml]ve, semi-na[iuml]ve and non-na[iuml]ve subjects. On univariate correlation analysis in the semi-na[iuml]ve group, predictors of LS sBMD were: female gender: r = -0.20 (P=0.007) and gap in GH replacement: r = -0.17 (P=0.027). Both predictors remained significant on multivariate analysis. On univariate correlation analysis in the semi-na[iuml]ve group, predictors of FN sBMD were: female gender: r = -0.34 (P<0.001); gap in GH replacement: r = -0.23 (P=0.005); age at study entry: r = -0.20 (P=0.011). Female gender and age at study entry remained significant predictors on multivariate analysis.
In conclusion, a longer interval between childhood and adult GH replacement, female gender and younger age at study entry were associated with lower sBMD. Minimizing the gap in GH replacement during transition to adult care may be considered and might help optimize BMD in this population.

Disclosures: AHH: Speaker, Pfizer, Inc.; Novo Nordisk; Principal Investigator, Pfizer, Inc.; Novo Nordisk; Eli Lilly & Company; Consultant, Novo Nordisk. DK: Employee, Pfizer, Inc. DMC: Advisory Group Member, Pfizer, Inc.; Speaker, Pfizer, Inc.; Principal Investigator, Pfizer, Inc. PJJ: Employee, Pfizer, Inc. MPW: Employee, Pfizer, Inc. MK-H: Employee, Pfizer, Inc. BMKB: Principal Investigator, Eli Lilly & Company; Novo Nordisk; Consultant, Novo Nordisk; Pfizer, Inc. Principal Investigator, Pfizer, Inc. Nothing to Disclose: NAT, SLG

Pub #	P2-365
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Hormone: Physiology, Deficiency (Adult), Delivery Systems, Use in Other Syndromes (1:30 PM-3:30 PM)
Title	The Effect of 24-Month Therapy with Recombinant Growth Hormone on Bone Metabolism in GHD Adults
Author String	J Payer, M Kuzma, Z Homerova, T Koller, P Jackuliak, P Vanuga, Z Killinger University Hospital and Medical Faculty of Comenius University, Bratislava, Slovakia (Slovak Republic); National Institute of Endocrinology and Diabetology, Lubochna, Slovakia (Slovak Republic)
Body	Introduction: Growth hormone (GH) through IGF-I effects on linear bone growth, it influences also peak bone mass (PBM) and bone remodelation. GH induces also chondrocyte proliferation, enchondral bone formation and influences RANK-RANK-L/osteoprotegerin system. More than 2x higher risk of osteoporotic has been proven in women with childhood onset growth hormone deficiency (CO-GHD). Positive influence of therapy with recombinant GH on bone mineral density (BMD) and bone markers was repeatedly shown in GHD patients. The therapy has also influence on risk of osteoporotic fractures. Aim: Monitoring of bone changes in patients with GHD after 24 months recombinant growth hormone treatment. Methods: 40 patients (22 women, 18 men) in age from 19 to 57 (average 35,9). 18 patients from this group were treated hypopituitarism after surgery, 7 patients with congenital deficiency and 15 patients with idiopathic GHD. Other hormonal deficiencies (if present) were adequately treated. We were observing BMD (DXA - Hologic Discovery) of femoral neck and L-spine before the start of the treatment, after 1st year of treatment and after 2nd year of treatment with recombinant growth hormone. In those intervals we have also observed the change in bone resorption marker CTX and bone formation marker osteocalcin. Each patient was adequately treated for at least one pituitary hormone deficiency, average dose were 0.35 mg/day. Levels of IGF-I were in therapeutic range (12.month 142.85 ng/ml, in 24. month 171,86 ng/ml). Results: Significant increase in BMD has been found out during the GH treatment, higher increase in comparison with femoral neck has been recorded in L-spine (10,7% after 2 years). Significant changes have been observed in increasing BMD in men in comparison with women ($p<0.001$). Significant higher increase has been found out in bone markers (at beginning 28,85 ug/l; 12.mth 44,95 ug/l, 24.mth. 49,53 ug/l) and CTX levels (beginning 187,26 ng/l; 12.mth. 222,09 ng/l; 24. mth. 250,87 ng/l) after 2 years GH treatment. Sexual differences have not been shown in bone markers. No clinical fractures have been proven. Conclusion: A significantly higher BMD of femoral neck and L-spine has been proven in every patient (more in men). Higher increase was recorded in L-spine. During the given period, increase in levels of bone markers has been found out. Longterm GH therapy in GHD adults has a positive effect on bone status. Nothing to Disclose: JP, MK, ZH, TK, PJ, PV, ZK

Pub #	P2-366
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Hormone: Physiology, Deficiency (Adult), Delivery Systems, Use in Other Syndromes (1:30 PM-3:30 PM)
Title	Improved Renal Function after Five Years of Growth Hormone (GH) Therapy in GH-Deficient (GHD) Adult Survivors of Childhood Leukemia
Author String	C Follin, T Wiebe, C Moell, EM Erfurth Clinical Sciences, Lund University, Lund, Sweden; Clinical Sciences, Lund University, Lund, Sweden
Body	Introduction: Acute lymphoblastic leukaemia (ALL) is the most common childhood malignancy and accounts for 25% of all childhood (CO) cancer. The survival rate is now 85% which emphasis the importance of long-term treatment complications. GH-deficiency (GHD) is common among these survivors treated with cranial radiotherapy (CRT) and chemotherapy. Renal impairment has been reported in CO cancer survivors and glomerular filtration rate (GFR) is decreased in hypopituitarism. GH therapy to CO GHD patients has been shown to increase GFR and kidney size. Methods: In 44 (21 women) former ALL patients, treated with 24 Gy CRT (18-24 Gy) and chemotherapy and 44 matched controls GFR (mL/min) was investigated. We used Cystatin C (CysC), a small 13 kDa protein, to estimate GFR. The level of CysC in serum is less influenced by body composition than creatinine. Thus, simple GFR-prediction equations based solely upon the CysC level is therefore useful when comparing patients and controls with different proportions of lean mass. The median age was 25 yr (19-31yr) and all patients were either GHD (91%) or insufficient. In a subgroup of 16 GHD ALL patients the effect of 5 years of GH therapy (0.5 mg/day) on GFR was evaluated and compared to 16 matched controls after 5 years. Results: At baseline the ALL patients had significantly lower GFR compared to controls ($P = 0.01$). After 5 years of GH therapy GFR improved compared to baseline among the ALL patients ($P = 0.04$). Two patients had subnormal GFR (< 60 mL/min) at baseline and GFR was normalized in these patients after GH therapy. After 5 years no significant difference in GFR between patients and controls was recorded ($P = 0.2$). When stratified for gender, GFR improved significantly among the men ($P = 0.01$), but not among the women ($P > 0.3$). The female ALL patients reached an IGF-I level of -0.7 SD and in men the level was +0.05 SD. Conclusion: GHD adult survivors of CO ALL have impaired renal function compared to matched controls 20 years after ALL diagnosis. Five years of a low dose of GH therapy improved renal function among the ALL patients, particularly among the men. Whether higher GH doses in women will improve their renal function needs further investigation. Nothing to Disclose: CF, TW, CM, EME

Pub #	P2-367
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Hormone: Physiology, Deficiency (Adult), Delivery Systems, Use in Other Syndromes (1:30 PM-3:30 PM)
Title	Effects of Growth Hormone on Markers of Bone Turnover and Bone Mass in Adults with Prader-Willi Syndrome: A 24-Month Prospective Study
Author String	AP Jorgensen, T Ueland, R Sode-Carlsen, T Schreiner, KF Rabben, S Farholt, C Hoybye, JS Christiansen, J Bollerslev Oslo University Hospital, Oslo, Norway; Oslo University Hospital, Oslo, Norway; Aarhus University Hospita Skeiby, Aarhus, Denmark; Frambu, Center for Rare Diseases, Siggerud, Norway; Karolinska University Hospital, Stockholm, Sweden; Aarhus University Hospital, Aarhus, Denmark
Body	Background: Bone mineral density (BMD) in adult Prader-Willi syndrome (PWS) might be reduced due to high bone turnover. We prospectively investigated effects of 24 months of growth hormone (GH) treatment o BMD and biochemical markers of bone turnover. Design: Forty-two adults (21 women, 22 from Denmark and 20 from Norway), mean (+/-SD) age 28.6 (6.6) y with genetically verified PWS were randomized to GH or placebo treatment for 12 months, followed by open GH treatment for additional 24 months. The Norwegian patients had higher BMI 31.8 (7.2) vs. 24.1 (5.0) kg/cm[sup2], p< 0.001, otherwise the two groups were comparable with respect to gender, age, and sex hormone replacements. The target GH dose was 0.6mg/day respectively 0.8mg/day depending on body weigh below or above 100 kg in the 12months of the controlled study, and doses were adjusted with respect to side effects and insulin-like growth factor I (IGF-I) in the open part of the study. BMD and body composition wer assessed by DEXA at baseline and at 24 months of GH treatment for 37 patients. Serum markers of bone formation (PINP, osteocalcin (Oc)) and resorption (NTx) were measured at baseline and at 12 months of GH treatment. Results: IGF-I increased from mean 114.5 (34.0) to 173.2 (50.6) [mu]g/l, p< 0.001, with a final mean GH dose of 0.6mg (0.25). No difference in final IGF-I levels or GH dose were observed between the genders, BMI classes (<25, 25-30, >30 kg/ cm[sup2]), or the two countries. After 12 months Oc, PINP and NTX were significantly increased. Despite this BMD did not change with GH treatment for 24 months. There was however, an increase in BMD Lumbar Spine in the patients from Denmark as compared to the Norwegian patients; 1.4 (5.5) % vs. -2.7 (2.7) %, p< 0.05. The Danish subjects had a more pronounced increase in Oc 15.5 (12.1) vs. 4.4 (4.9) ng/l, p< 0.01. The observed increase in lean body mass of 2.5 (2.5) kg, p< 0.001, wer also more pronounced in the Danish patients 3.6 (1.6) vs. 1.2 (2.8) kg, p< 0.01. These findings were not due t different BMI in the two groups alone (multiple regression). Conclusions: Despite improvement in markers of bone turnover, BMD did not change with GH treatment for two years in this cohort of PWS adults. The patients from Denmark however, had an anabolic effect of GH treatment not seen in the Norwegians, also after adjustment for baseline BMI. Differences in ward program in the two countries should be evaluated for an explanation. Sources of Research Support: Novo Nordisk Scandinavia AB, Malm[ouml], Sweden and Novo Nordisk, Bagsv[aelig]rd, Denmark; The A.P. M[oslash]ller Foundation for the Advancement of Medical Science; Research Initiative of Aarhus University Hospital; Aarhus University Hospital Skejby Research Foundation; Aase and Ejnar Danielsen Foundation; The Danish Prader-Willi Syndrome Association; The Augustinus Foundation. Nothing to Disclose: APJ, TU, RS-C, TS, KFR, SF, CH, JSC, JB

Pub #	P2-368
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Hormone: Physiology, Deficiency (Adult), Delivery Systems, Use in Other Syndromes (1:30 PM-3:30 PM)
Title	Tamoxifen but Not Raloxifene Reduces GH Secretion and Whole Body Fat Oxidation in Healthy Women
Author String	V Birzniece, A Sata, S Sutanto, KKY Ho Garvan Institute of Medical Research, St Vincent's Hospital, Sydney, Australia
Body	GH plays an important role in the regulation of fat metabolism. It promotes lipid utilization by stimulating fat oxidation. Tamoxifen, a Selective Estrogen Receptor Modulator (SERM), exerts tissue specific estrogen agonist or antagonist effects. It reduces GH secretion and inhibits hepatic IGF-I production (1). Whether other SERMs exert similar effects in therapeutic doses is not known. The aim was to compare the impact of the two most commonly used SERMs, tamoxifen and raloxifene, on the GH-IGF-I axis and on whole body fat oxidation. Ten healthy postmenopausal women were randomised to 2-week sequential treatment with tamoxifen (10 and 20 mg/d) and raloxifene (60 and 120 mg/d) with an intervening washout period of 2 weeks. GH response to arginine stimulation, serum levels of IGF-I, and fasting/postprandial whole body fat oxidation were measured at the end of each treatment. GH response to arginine was significantly reduced by tamoxifen ([Delta] -36% and -88% for 10 and 20 mg, respectively), but not by raloxifene treatment. Mean IGF-I concentrations significantly fell during treatments only with 20 mg tamoxifen and 120 mg raloxifene by ([Delta] -24±5% ($p<0.01$) and -14±6 % ($p<0.05$), respectively). The reduction in IGF-I was significantly greater with tamoxifen compared to raloxifene treatment ($p<0.05$). Neither SERMs significantly affected fasting fat oxidation. Only tamoxifen (20 mg) significantly reduced post-prandial fat oxidation ([Delta] -34.6±10.3%; $p<0.01$). In summary, in the doses used, only tamoxifen reduced GH response to arginine stimulation, whereas both SERMs significantly reduced IGF-I levels with tamoxifen imparting a greater effect. Tamoxifen but not raloxifene significantly reduced post-prandial fat oxidation. We conclude that, at therapeutic doses, tamoxifen is more potent than raloxifene in blunting the GH-IGF-I axis activity and whole body fat oxidation. Long-term treatment with tamoxifen carries a greater risk of inducing detrimental metabolic outcomes than raloxifene. 1) Birzniece et al., J Clin Endocrinol Metab 2010, 95: 3771-6 Sources of Research Support: NHMRC of Australia. We thank Alphapharm for providing tamoxifen. Nothing to Disclose: VB, AS, SS, KKYH

Pub #	P2-369
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Hormone: Physiology, Deficiency (Adult), Delivery Systems, Use in Other Syndromes (1:30 PM-3:30 PM)
Title	Gender Difference in the Suppression of Fat Oxidation by Tamoxifen
Author String	V Birzniece, A Sata, S Sutanto, KKY Ho Garvan Institute of Medical Research, St Vincent's Hospital, Sydney, Australia
Body	GH secretion is stimulated centrally by estradiol derived locally via aromatisation of testosterone in both men and women. In men, the inhibition of LH secretion by testosterone also requires prior aromatization to estradiol. Tamoxifen, a Selective Estrogen Receptor Modulator that blocks central estrogen action, reduces GH secretion in women (1) but not in men, at the same time increasing testosterone levels (2). As GH and testosterone stimulate fat metabolism, we postulated that the effect of tamoxifen in women and men may be different. We determined whether there is a gender difference in the impact of tamoxifen on fat oxidation. Ten healthy postmenopausal women and ten healthy men were randomised to 2-week treatment with tamoxifen (20 mg/d) We measured GH response to arginine stimulation, serum levels of IGF-I, testosterone (men only), and whole body fat oxidation. In women, tamoxifen significantly reduced the GH response to arginine stimulation ([Delta] -88%, $p<0.05$) and mean IGF-I levels ([Delta] -23.5±5.4%, $p<0.01$). Tamoxifen did not significantly change fasting fat oxidation but significantly reduced post-prandial fat oxidation ([Delta] -34.6±10.3%; $p<0.01$). In men, tamoxifen did not significantly change GH response to arginine stimulation but significantly reduced mean IGF-I levels ([Delta] -24.8±6.1%, $p<0.01$). It significantly increased mean testosterone levels ([Delta] 52±14.2%; $p<0.01$). Tamoxifen did not significantly change fasting and post-prandial fat oxidation in men. In summary, tamoxifen attenuated the GH response to stimulation and reduced post-prandial fat oxidation in women but not in men. It increased testosterone levels in men and reduced IGF-I levels to a similar degree in both sexes. We conclude that in therapeutic doses, the suppressive effect of tamoxifen on fat metabolism is gender dependent being greater in women than in men. As testosterone stimulates fat oxidation independently, the compensatory increase in testosterone may counteract the reduction in fat oxidation resulting from suppression of the GH-IGF-I axis activity. 1) Birzniece et al., J Clin Endocrinol Metab 2010, 95: 3771-6 2) Birzniece et al., J Clin Endocrinol Metab 2010, 95: 5443-8 Sources of Research Support: NHMRC of Australia. We greatly thank Alphapharm for providing tamoxifen. Nothing to Disclose: VB, AS, SS, KKYH

Pub #	P2-370
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Hormone: Physiology, Deficiency (Adult), Delivery Systems, Use in Other Syndromes (1:30 PM-3:30 PM)
Title	Body Fat Mass as the Major Negative Associate of Delayed Glucose-Induced GH Release in Healthy Men
Author String	DM Lawson, B Dunn, JD Veldhuis, A Iranmanesh Salem VA Medical Center, Salem, VA; Mayo Clinic, Rochester, MN
Body	Age, adiposity, muscle mass, androgens, and physical activity are reported to variably affect GH release both basally and in response to GH secretagogues. The present study was intended to assess the individual/joint impact of age, body composition, testosterone, and exercise on glucose-induced changes in GH release in 58 healthy men in the age range of 19-78 yrs, BMI of 20-39 Kg/m^2, and percentage body fat of 7 to 39. Each subject was studied on 2 separate occasions after an overnight fast, consisting of either 75 grams of oral dextrose solution or equal volume of water. All sessions started between the hours of 0800-0900 and continued for a total period of 6.5 hrs, with blood collected at 10-min intervals for the GH measurements. Free testosterone was assayed in a single fasting specimen. Body fat mass (FM) and appendicular muscle mass (AMM) were computed by DXA. Fat mass index (FMI) and appendicular muscle mass index (AMMI) were derived respectively by dividing FM and AMM in Kg by height in meter squared. CT scan was used to estimate visceral fat area (VFA). Exercise intensity was quantified by daily duration of work-out (minutes) multiplied by the number of work-out sessions per week. Oral dextrose administration was associated with significant increases in the 6.5 hr sum (83±8.2 v 53±5.8: P<0.0001), and mean (2.1±0.2 v 1.3±0.1: P<0.0001) as well as peak (9.3±0.8 v 6.5±0.6: P=0.0002) GH concentrations ([micro]g/L). Regression analysis did not identify age or muscle mass (AMMI) as a significant correlate of either measures of GH (sum, mean, peak) concentration. On the other hand, VFA was strongly and negatively correlated(R/P) with sum (-0.48/0.0002), mean (-0.47/0.0002), and peak (-0.47/0.0002) GH values. FMI prediction of various GH concentration parameters was identical to VFA. GH profiles were positively associated with exercise intensity (sum: 0.43/0.0006, mean: 0.43/0.0006, peak: 0.36/0.006), and circulating free testosterone (sum: 0.29/0.03, mean: 0.29/0.03, peak: 0.39/0.003). Conversely, VFA had a negative correlation with free testosterone (-0.56/<0.0001), and exercise intensity (-0.36/0.006). In conclusion a reduction in delayed glucose-induced GH release in men is primarily related to increased body fat (being general or visceral), rather than age and/or muscle mass. While a positive role for testosterone and exercise can be speculated, their negative correlation with body fat indices (VFA, FMI) is suggestive of an indirect effect of adiposity. Nothing to Disclose: DML, BD, JDV, AI

Pub #	P2-371
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Hormone: Physiology, Deficiency (Adult), Delivery Systems, Use in Other Syndromes (1:30 PM-3:30 PM)
Title	A Novel Human Growth Hormone XTEN Construct (VRS-317) for Once-a-Month Subcutaneous Administration in a Phase 1a Study of Growth Hormone-Deficient Adults
Author String	JL Cleland, N Geething, B Spink, S Motlagh, Y Yao, J Silverman, MS Kipnes Versartis, Inc, Mountain View, CA; Amunix, Inc, Mountain View, CA; Cetero Research/DGD Clinic, San Antonio, TX
Body	VRS-317 is a novel long acting growth hormone product comprised of a pharmacologically active domain of recombinant human growth hormone (rhGH) and two sequences of natural hydrophilic amino acids fused to the N and C termini (XTEN technology) of rhGH. Preclinical studies indicate that VRS-317 may be dosed at a 5-10 fold lower rhGH mass equivalent or molar dose than daily rhGH to achieve comparable pharmacodynamic (PD: IGF-I and IGFBP-3) and growth responses (weight gain and bone elongation) in monkeys and hypophysectomized rats, respectively. These studies have also demonstrated that a single dose of VRS-317 in a juvenile or adult monkey provides a dose dependent and sustained increase in IGF-I and IGFBP-3 for one month. The half-life of VRS-317 in monkeys is approximately 110 hr. The PD results combined with the pharmacokinetics indicate that a single subcutaneous monthly dose of 0.20 mg/kg VRS-317 (equivalent to 0.037 mg/kg rhGH) in growth hormone deficient (GHD) patients should be sufficient to stimulate an IGF-I response comparable to daily rhGH therapy. GLP toxicology studies in monkeys have been completed for acute (4 week) and long term (13 week) exposure at doses up to 25 mg/kg VRS-317 administered every two weeks. These studies indicated that the proposed human starting dose of 0.05 mg/kg VRS-317 has a minimum of a 161 fold safety factor. No lipoatrophy or anti-VRS-317 antibodies have been detected in the GLP toxicology studies to date. A Phase 1a randomized blinded placebo controlled single ascending dose study of VRS-317 in adult GHD patients currently receiving daily rhGH therapy is ongoing to confirm the safety and tolerability of VRS-317 as well as the effective VRS-317 dose required to sustain an IGF-I level in the normal range for age and sex for 30 days after a single subcutaneous dose administered with a 29G needle. Up to 50 adult GHD patients will be enrolled in the Phase 1a study. Patients are withdrawn from daily rhGH therapy for a minimum of 30 days and must have a pre-determined decrease in their IGF-I standard deviation (SD) score. Patients will be closely monitored with safety assessments including evaluation of glucose and lipid metabolism. In addition, the PK of VRS-317 will be assessed along with IGF-I and IGFBP-3 at selected time points post-dose. If proven to be effective and safe in humans, once-monthly administration of VRS-317 may provide a significant improvement in the treatment of GHD patients. Nothing to Disclose: JLC, NG, BS, SM, YY, JS, MSK

Pub #	P2-372
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Hormone: Physiology, Deficiency (Adult), Delivery Systems, Use in Other Syndromes (1:30 PM-3:30 PM)
Title	Bioavailability of Nasally Administered Human Growth Hormone (CP024) and Induction of IGF-I in Healthy Volunteers
Author String	AL Lewis, FM Jordan, SM Shalet, L Illum Critical Pharmaceuticals, Nottingham, UK; The Christie Hospital, Manchester, UK

Body

Rationale

Patient adherence to growth hormone (GH) replacement therapy is estimated to be as low as 36-49% (1) with 70% of patients unhappy with daily injection(2). Low treatment adherence reduces efficacy(3) and increases healthcare costs. All currently marketed GH products require subcutaneous injection and non-invasive delivery methods should increase adherence and treatment success.

CriticalSorb[trade] is a nasal delivery system that facilitates the absorption of macromolecules across mucosal membranes. Using CriticalSorb[trade] we have developed a nasal spray of GH (product code CP024) able to achieve higher bioavailabilities in preclinical models than any previously reported (4,5), and which more closely mimics endogenous growth hormone secretion in children. These formulations have been shown to be well tolerated in a repeat dose toxicity study. The current study will evaluate the bioavailability and bioactivity of two CP024 formulations administered intranasally to healthy volunteers relative to a subcutaneous injection of Omnitrope[reg].

Trial Design

Single centre, open label, five-way crossover in 8 healthy volunteers. Endogenous GH secretion suppressed by infusion of 40[micro]g/h octreotide.

Inclusion criteria

Healthy males aged 21-55, BMI 18.5-30 kg/m2, IGF-1 levels in the normal range with respect to age (87-358ng/ml)

Objectives

[bull]To determine GH pharmacokinetics after intranasal administration of two prototype CP024 formulations given once or twice a day in comparison with an equivalent subcutaneous dose of Omnitrope[reg]
[bull]To assess the nasal tolerability of two CP024 formulations and CriticalSorb[trade] vehicle
[bull]To determine IGF-1, IGFBP-3 responses for the reference injection of Omnitrope[reg] and prototype CP024 intranasal formulations

Primary Endpoints

The pharmacokinetic (GH) and pharmacodynamic (IGF-1 and IGFBP-3) profiles following intranasal administration of two different CP024 formulations will be compared to a subcutaneous reference control by measuring the following parameters:

[bull]tmax
[bull]Cmax
[bull]AUC to 2 hours (AUC0-2hr) and corresponding relative bioavailability (Frel) for each prototype formulation
[bull]AUC to last measured time point (AUClast) and corresponding Frel for each prototype formulation
[bull]AUC to infinity (AUC[infin]) and corresponding Frel for each prototype formulation
[bull]Terminal half-life (t1/2)

The nasal tolerability of the formulations will be assessed by the evaluation of safety parameters that include adverse events, safety laboratory tests, vital signs and ECGs.

(1) F. Haverkamp et al., Clin Ther 2008; 30(2): 307
(2) Frost and Sullivan. European human growth hormone market (2008).
(3) R.R. Kapoor et al., Arch Dis Child 2008 93: 147
(4) H.R. Costantino et al., Int. J. Pharm. 2007; 337:1
(5) Y.H. Cheng et al., Eur J Pharm Sci. 2005;26(1): 9

Sources of Research Support: The Wellcome Trust; Critical Pharmaceuticals.

Disclosures: ALL: Employee, Critical Pharmaceuticals. FMJ: Employee, Critical Pharmaceuticals. SMS: Ad Hoc Consultant, Critical Pharmaceuticals. LI: CEO, Critical Pharmaceuticals.

Pub #	P2-373
Session Information	POSTER SESSION: CLINICAL/TRANSLATIONAL - Growth Hormone: Physiology, Deficiency (Adult), Delivery Systems, Use in Other Syndromes (1:30 PM-3:30 PM)
Title	Comparison of Device Preference and Usage Errors for a New Growth Hormone Injection Device *vs.* Comparator Devices
Author String	A-M Kappelgaard, K Hartmann, A Pfutzner, GS Fuchs, F Winter, T Rohrer Novo Nordisk A/S, Virum, Denmark; Institute for Pediatric Endocrinology and Diabetology, Frankfurt, Germany; Ikfe GmbH, Mainz, Germany; Novo Nordisk A/S, Soeborg, Denmark; Ikfe CRO GmbH, Mainz, Germany; Saarland University Hospital, Homburg/Saar, Germany
Body	**AIM**: Recombinant growth hormone (GH) is used to treat short stature in children with GH deficiency (GHD) and other conditions. Treatment adherence, which may be poor due to the need for daily injections and treatment length may potentially be improved with easy to use injection devices. We compared patient preference for and usage errors with a new GH injection pen (Norditropin[reg] FlexPro[reg] [Novo Nordisk A/S, Bagsv[aelig]rd, Denmark]) relative to four other pens: easypod[reg] (Serono, Switzerland), Genotropin [reg] pen (Pfizer, New York, USA), Nutropin AQ[reg] NuSpin[trade] pen (Genentech, San Francisco, USA) and Omnitrope[reg] pen (Sandoz, Holzkirchen, Germany). **METHODS**: In two non-interventional, randomized, crossover, comparative studies (INT1 & INT2) children (10-17 years) treated with GH ([ge] 6 months) were randomly assigned to intuitiveness (INT1, INT2) (n=30; n=32) or instruction (n=26; n=32) groups. All subjects performed a usability test involving needle attachment, dose setting and injection into an Eppendorf tube. Intuitiveness groups had brief verbal instruction on device use. Instructed groups, were instructed in full according to the user guide. Patient preference for devices was assessed by a 13-item questionnaire. The number and type of usage errors were recorded. **RESULTS:** FlexPro[reg] was rated as the most preferred device in the majority of items in intuitiveness (INT1/INT2) (9/13; 11/13) and instructed groups (10/13; 11/13) and was the overall most preferred device in both studies (intuitiveness/instructed) (INT1, 15/30; 19/26; INT2, 19/32; 23/32). FlexPro[reg] scored highest for ease of use, easypod[reg] on best delivery feedback, Genotropin[reg] and NuSpin[reg] pens for appearance and quality. Technical errors were generally less with FlexPro[reg] than with comparator devices (Intuitiveness groups: INT1: FlexPro: 1 vs. easypod[reg]: 36; Genotropin[reg] pen: 11: INT2: FlexPro[reg]: 2 vs. Nuspin[reg] pen: 29, Omnitrope[reg] pen: 9. Instruction groups: INT1: FlexPro[reg]: 1, easypod [reg]/Genotropin[reg] pen: 2; INT2: FlexPro[reg]/Omnitrope[reg] pen: 2, Nuspin[reg] pen: 1). **CONCLUSIONS:** Both instructed and uninstructed patients preferred Norditropin[reg] FlexPro[reg] to comparator devices. The number of errors in the intuitiveness group probably reflects the problems/errors patients or carers face when they have not received adequate training or do not understand the training given. Overall, use of FlexPro[reg] was associated with fewer errors than the comparator devices. Disclosures: A-MK: Employee, Novo Nordisk. GSF: Employee, Novo Nordisk. Nothing to Disclose: KH, AP FW, TR

Pub #	P2-374
Session Information	POSTER SESSION: CLINICAL - General Aspects of Pituitary Diseases (1:30 PM-3:30 PM)
Title	Russian Register of Patients with Hypothalamo-Hypophyseal Disorders: Update to December 2010
Author String	N Molitvoslovova, E Przhiyalkovskaya, E Pigarova, L Dzeranova, V Pronin, E Marova, S Arapova, L Rozhinskaya, G Melnichenko Research Center for Endocrinology, Moscow, Russian Federation; Moscow Medical Academy, Moscow, Russian Federation
Body	Electronic databases of patients with chronic diseases are useful for analysis of incidence, prevalence, morbidity and treatment of these diseases. Since 2004 patients with acromegaly had been registered in special electronic database. Since 2006 all patients with pituitary adenomas were included in register. Demographic data, information on symptoms, visual abnormalities, diagnosis, pituitary size, hormone levels, different modalities of treatment and morbidity were collected. On the 6 th of December 2010 there were 4526 patients (pts): 1116 (24,5%) men (m) and 3410 (75,5%) women (w). Nosological structure was: acromegaly- 2584 (57,0%) pts, m/w-724/1860; prolactinomas -1108 (24,5%) pts, m/w-174/934; Cushing disease -259 (5,7%) pts, m/w-38/221; clinical non active pituitary adenomas -302 (6,7%) pts, m/w-98/204; other types of pituitary adenomas -237 (5,2%) pts, m/w-67/170; craniopharyngeomas-36 (0,8%) pts, m/w-15/21. The median age at diagnosis in patients with acromegaly was 45 years, the median period from debut to diagnosis was 5 years. The main symptoms in acromegalics were: head ache -1662 pts (64,3%), change of appearance -1626 pts (64%), arthralgia -1165 pts (45,1%), myopathy -1075 pts (42%), perspiration -975 pts (37,8%), arterial hypertension -609 pts (23,6%), enlargement of extremities -574 pts (22,2%), fatigue -527 pts (20,4%), diabetes mellitus -508 pts (19,7%), visual alterations -418 pts (16,2%), menstrual dysfunction - 318 pts (12,3%), erectile dysfunction -246 pts (9,5%). The size of adenoma was measured in 2119 pts: microadenomas -243 pts, 11-15 mm - 576 pts, 16-25 mm -1072 pts, 26-35 mm - 147 pts, 36-59 mm - 68 pts, >60 mm - 13 pts. The adequate laboratory diagnosis (basal GH, GH/OGTT and IGF-1) was performed in 67%. Among different methods of treatment were: neurosurgical - 756 pts, radiological - 490 pts (gamma-therapy - 383 pts, proton beam - 58 pts, gamma-knife - 7 pts), medical - 1248 pts (dopamine agonists - 710 pts, long-acting somatostatin analogues - 656 pts). 242 (21%) pts needed, but did not receive therapy of long-acting somatostatin analogues. Nothing to Disclose: NM, EP, EP, LD, VP, EM, SA, LR, GM

Pub #	P2-375
Session Information	POSTER SESSION: CLINICAL - General Aspects of Pituitary Diseases (1:30 PM-3:30 PM)
Title	Sellar Lesions: A Clinico-Pathologic Review and Twenty-Year Institutional Experience
Author String	C Pendleton, N Mathioudakis, A Quinones-Hinojosa, R Salvatori, P Caturegli Johns Hopkins University, Baltimore, MD; Johns Hopkins University, Baltimore, MD; Johns Hopkins University, Baltimore, MD

Body

INTRODUCTION: Pituitary adenomas are reported to comprise 90% of sellar lesions(1), yet the differential diagnosis of these lesions is broad. The pathology mix may change among institutions due to population differences and referral patterns. **METHODS**: Of pathology reports from 1987-2010 containing "pituitary" or "hypophysis," we reviewed the diagnoses from cases operated at Johns Hopkins Hospital (JHH) and combined these with referrals from outside institutions (ALL). Patients with repeat surgeries were included once, according to the highest tumor grade. Tumor size was obtained from radiology or clinic documents. For adenomas, diagnoses were based on biochemical evaluation. **RESULTS**: In the ALL group, 1721 sellar lesions included 145 normal (8.4%),1238 adenomas (72), 152 non-adenoma benign (8.8), 50 malignant (2.9), 52 infiltrative (3.0), 14 vascular (0.8), and 70 miscellaneous (4.0). 1129 JHH cases yielded 70 normal (6.2), 906 adenoma (80), 71 non-adenoma benign (6.3), 15 malignant (1.3), 26 infiltrative (2.3), 6 vascular (0.5), and 35 miscellaneous (3.1). Of benign lesions, there were 20 craniopharyngiomas (1.8), 30 Rathke's cysts (1.8), 8 each meningiomas and epidermoid cysts (0.7), 4 pituicytomas (0.3), and 1 oncocytoma (0.9). Of malignant lesions, there were 3 each carcinoma, metastases, and germinomas (0.3); 1 each chondrosarcoma and unspecified malignant tumors (0.09%), and 4 chordomas (0.4). Of infiltrative lesions, there were 12 lymphocytic hypophysitis (1.1), 2 Langerhan's histiocytosis (0.2), 1 each tuberculosis and sarcoidosis (0.09), granulomatous hypophysitis (0.4), 4 chronic inflammation (0.4), and 1 xanthomatous hypophysitis. Of miscellaneous lesions, there were 31 unspecified pathology (2.7), 2 hyperplasias (0.2), and 1 each abscess and Crooke's changes without adenoma (0.09). Of 906 JHH adenomas, 575 were macros (63.5), 83 micros (9.2), 248 unknown tumor size (27.4), and 273 lacked a diagnosis but were presumed non-functioning (30.1). Of confirmed diagnoses, 89 had Cushing's disease (14), 87 prolactinoma (13.7), 81 acromegaly (12.8), 5 TSH-secreting (0.8), 3 gonadotropin-secreting (0.5), 366 non-functioning tumors (57.8), and 2 prolactin and growth-hormone co-secretors (0.3). **CONCLUSIONS**: Our institutional experience demonstrates a lower proportion of adenomas than previously reported; the higher proportion of malignancies likely reflects a referral bias, with rarer sellar lesions encountered more frequently in our academic center.

1.Huang BY, Castillo M. Nonadenomatous tumors of the pituitary and sella turcica. Top Magn Reson Imaging 2005;16(4):289-99.

Nothing to Disclose: CP, NM, AQ-H, RS, PC

Pub #	P2-376
Session Information	POSTER SESSION: CLINICAL - General Aspects of Pituitary Diseases (1:30 PM-3:30 PM)
Title	The Expression of Markers for the Aggressiveness of Corticotroph Adenomas
Author String	JS Lim, KH Park, CR Ku, M-k Lee, SH Kim, EJ Lee Yonsei University College of Medicine, Seoul, Republic of Korea; Yonsei University College of Medicine, Seoul, Republic of Korea; Ilsan Hospital, Ilsan, Republic of Korea; Yonsei University College of Medicine, Seoul, Republic of Korea
Body	**Background** The molecular mechanisms underlying tumor growth in Cushing's disease still remain a challenge for the endocrinologists. In addition, clinical manifestations of these patients may vary depending on the hormonal activity, however, factors involved in the aggressiveness of ACTH-secreting pituitary tumors have not fully been clarified. **Objective** We investigated the association between the expression of cellular markers that are known to be related to tumor progression of pituitary adenomas and hormonal levels in patients with Cushing's disease. **Methods** Tumor tissues from 28 corticotroph adenomas (female 26, male 2, mean age 39.21 ± 10.39 yr) were subject to immunohistochemical study using the following antibodies: pituitary tumor-transforming gene 1 (PTTG1), cyclin D1 (CD1), Epidermal Growth Factor Receptor (EGFR), and Ki-67. Moreover, Ki-67 labeling index (LI) was estimated from mean numbers of cells positive for Ki-67 immunostaining per 1000 tumor cells in the highly positive area. Then, we analyzed the relation between these results and each hormone level, such as 24hr urinary free cortisol, ACTH, and serum cortisol. Statistic analysis was performed using non-parametric test procedures. **Results** PTTG1 was over-expressed in all patients, however, did not reflect the hormonal activity. The level o expression of CD1 was various; positive group was 13 (1+~3+), and negative group was 15. CD1-positive group showed significantly low levels of serum cortisol compared to CD1-negative group (p=0.011). Contrary to expectations, EGFR was all negative. Ki-67 was expressed in 50% of patients, however, Ki-67 LI was not associated with hormonal levels of corticotroph adenomas. **Conclusion** Although PTTG1 seems to play an essential role in development of ACTH-secreting tumors, CD may be better marker to determine clinical aggressiveness of the tumor. The role of cyclin D1 in Cushing's disease is still controversial, therefore, further research on this molecular background will be needed. Nothing to Disclose: JSL, KHP, CRK, M-KL, SHK, EJL

Pub #	P2-377
Session Information	POSTER SESSION: CLINICAL - General Aspects of Pituitary Diseases (1:30 PM-3:30 PM)
Title	Temozolomide in Three Patients with Pituitary Adenoma
Author String	R Cozzi, G Lasio, S Lodrini, M Castiglione, G Felisati, A Salmaggi, A Cardia, A Maccari, R Attanasio Niguarda Hospital, Milano, Italy; Galeazzi Institute, Milano, Italy; Carlo Besta Institute, Milano, Italy; S Paolo Hospital, University of Milan, Milano, Italy; Carlo Besta Institute, Milano, Italy
Body	**Background.** In aggressive pituitary adenomas Temozolomide (TMZ), an alkylating drug to be administered at monthly cycles by oral route, seems to be a new promising tool. Only few data are available. **Case reports.** In 2006 acromegaly was diagnosed in a 56 yo female with sight loss. She had a giant invasive adenoma and GH/IGF-I were 420/607 ng/ml, respectively. Transcranial debulking (TC) did not ameliorate sight damage. Ki67 was 3%. For persisting disease, she started somatostatin analogs (SA) for 2 years, achieving tumor shrinkage and partial GH/IGF-1 control. After switching to pegvisomant, IGF-I normalized, but tumor grew uncontrolled in spite of concomitant administration of SA and dopamine agonists (DA). TMZ was given since November 2009 for 6 cycles: tumor growth remained controlled for a few months but thereafter escape occurred with bilateral amaurosis. In 1995 acromegaly was diagnosed in a 53 yo female with bitemporal hemianopia and huge invasive macroadenoma: GH/IGF-I/PRL were 7/1500/200 ng/ml, respectively. She underwent debulking (a few cells stained for GH and PRL), leaving mild hypersecretion (GH/IGF-I were 0.8/322 ng/ml) and an intracavernous remnant. In 2001 she had hormonal recurrence and tumor growth, underwent TC, followed by conformal radiotherapy. SA treatment achieved partial hormonal control and tumor shrinkage until 2006. After tumor regrowth, pegvisomant was added to SA, obtaining IGF-I normalization, but tumor increased. TMZ was unsuccessfully administered for 2 cycles. In 1996 clinically non functioning pituitary adenoma was diagnosed in a 30 yo female complaining visual disturbances. Between 1997 and 2009 she underwent 7 neurosurgical resections (5 transphenoidal and 2 TC) of a huge macroadenoma by skilled operators, as well as GK radiosurgery (32 Gy in 2000). Histology always showed a pituitary adenoma, occasionally staining for PRL, and Ki67 3-10%. DA had been unsuccessfully administered for long periods. In March 2009 TMZ was started together with SA. Eight cycles were given and well tolerated, achieving progressive shrinkage by 40% of residual tumor. In **conclusion**, TMZ can be given in aggressive pituitary adenoma, and is well tolerated. Anyway, its efficacy to control disease progression has to be individually checked for the variability and unpredictability of its results. Nothing to Disclose: RC, GL, SL, MC, GF, AS, AC, AM, RA

Pub #	P2-378
Session Information	POSTER SESSION: CLINICAL - General Aspects of Pituitary Diseases (1:30 PM-3:30 PM)
Title	Effects of Sunitinib, a Protein Tyrosine Kinase Inhibitor, on a Pituitary Lesion in a Patient with Renal Carcinoma
Author String	L Srikugan, J Powrie Guy's & St Thomas Hospital NHS Trust, London, UK
Body	**BACKGROUND:** Sunitinib, a multi-targeted protein tyrosine kinase inhibitor (PTKI), was approved for use as first-line chemotherapy agent for metastatic renal carcinoma in the UK in March 2009. However, literature regarding the possible effects of PTKI on pituitary tissue is limited. **CASE HISTORY:** A 60 year old gentleman presented with lethargy, cough and hypercalcaemia. Diagnostic CT and MRI revealed right renal tumour with pulmonary, left adrenal and splenic metastases as well as 2cm sella/suprasellar mass with stalk deviation and optic chiasm displacement. He had normal Humphrey Visual Fields, no evidence of diabetes insipidus (DI); morning cortisol 353nmol, ACTH 29ng/l, fT_4 11.3pmol/l (NR=12-22), fT_3 3.6pmol/l (NR=3.1-6.8), total testosterone 2.2nmol/l, LH 1.8IU/l, FSH 2.6IU/l, IGF-1 1.6nmol/l, prolactin 95mlU/l. Pituitary adenoma rather than metastasis was considered because of the MRI appearance, absence of surrounding bone destruction, lack of metastases elsewhere in the brain and absence of DI. Sunitinib 50mg daily was commenced to reduce tumour burden pre-nephrectomy. Nine weeks later CT showed significant volume reduction of renal tumour and metastases (unfortunately pituitary MRI not undertaken at this time). He presented with headaches and diplopia a month later: brain MRI revealed bleeding around the rim of the pituitary adenoma. He was supplemented with hydrocortisone and thyroxine, and Sunitinib was continued. Imaging four months later revealed almost complete resolution of the initial sell mass and further renal tumour regression. **DISCUSSION:** - The parallel responses of the pituitary lesion as well as the primary tumour and other metastases to Sunitinib suggest metastatic pituitary lesion. However initial clinical and radiological impression was of an adenoma; regression of an adenoma in response to Sunitinib remains a possibility. Studies using pituitary adenoma cell cultures have demonstrated reduced [^{3}H]thimidine uptake and DNA synthesis inhibition by PTKI but no in vivo data exists. - Apoplexy has been reported amongst cases of renal carcinoma metastases. However, it is also likely that Sunitinib precipitated pituitary apoplexy either by its known effects on tumour angiogenesis or the precipitation of haemorrhage. This may have, at least partially, been responsible for the tumour regression. Close surveillance of patients with pituitary lesions on PTKI for pituitary haemorrhage is important. Nothing to Disclose: LS, JP

Pub #	P2-379
Session Information	POSTER SESSION: CLINICAL - General Aspects of Pituitary Diseases (1:30 PM-3:30 PM)
Title	Treatment of Delayed Hyponatremia after Pituitary Surgery with Tolvaptan
Author String	RJ Auchus, GE Baldwin, BE Mickey UT Southwestern Medical Center, Dallas, TX; UT Southwestern Medical Center, Dallas, TX
Body	**Background**: Delayed hyponatremia is the most common reason for hospital readmission following transsphenoidal pituitary surgery. The sodium nadir typically occurs one week following discharge, and the incidence is highest in patients with Cushing's disease. Symptoms include malaise, nausea, and confusion; however, loss of conciousness and seizures can occur if unrecognized and untreated. Treatment includes fluid restriction, hypertonic saline, antiemetics, and supportive care. The mechanism of the hyponatremia is unknown, but inappropriate vasopressin excess is a component of the pathogenesis. We hypothesized that tolvapatan, an orally-active vasopressin type 2 receptor antagonist approved for treatment of symptomatic hyponatremia, would be an effective treatment of this condition. **Methods**: Two patients with symptomatic postoperative hyponatremia were readmitted and treated with fluid restriction and/or hypertonic saline in the intensive care unit. After the serum sodium failed to rise above 119 meq/L after 24 hours, each was given a single 15 mg dose of tolvaptan. Urine output, serum and urine sodium and osmolality, and plasma vasopressin were monitored. **Results**: Before tolvaptan treatment, the plasma vasopressin and urine osmolality were inappropriately elevated. The patients responded to tolvaptan with a profound diuresis of 150-500 mL/h over the next 18 h, for a total of 4120 and 3790 mL over 18 hours, respectively. Both patients required infusion of hypotonic intravenous fluids to achieve the goal rate of serum sodium correction. Within 24 hours, both patients returned to normal serum sodium and urine output, expediting discharge. No complications or recurrence of hyponatremia was observed. **Conclusions**: Tolvaptan, given as a single low dose, is a highly effective treatment of delayed hyponatremia following pituitary surgery, suggesting that this condition is fundamentally a syndrome of vasopressin excess. Extreme caution is required during its use, as the induced diuresis is profound and serum sodium rise can be dangerously rapid. We recommend that any patient treated with tolvaptan for this condition be monitored intensely and given simultaneous hypotonic fluid replacement to slow the serum sodium rise. Nothing to Disclose: RJA, GEB, BEM

Pub #	P2-380
Session Information	POSTER SESSION: CLINICAL - General Aspects of Pituitary Diseases (1:30 PM-3:30 PM)
Title	Cognitive Performance and the Hippocampus in Patients with Postoperative Pituitary Radiotherapy: A Detailed Dosimetric Study
Author String	P Brummelman, MGA Sattler, ACM van den Bergh, LC Meiners, RPF Dullaart, G van den Berg, GJ Izaks, J Koerts, O Tucha, BHR Wolffenbuttel, AP van Beek University Medical Center Groningen, University of Groningen, Groningen, Netherlands; University Medical Center Groningen, Groningen, Netherlands; University Medical Center Groningen, University of Groningen, Groningen, Netherlands; University Medical Center Groningen, University of Groningen, Groningen, Netherlands; University Medical Center Groningen, University of Groningen, Groningen, Netherlands; University of Groningen, Groningen, Netherlands
Body	*Context and objective:* We showed previously that postoperative radiotherapy (RT) for nonfunctioning pituitary macroadenoma (NFA) is unlikely to have a major effect on cognition. However, a small effect could not be excluded. Radiation dosimetry offers the possibility to relate radiation exposure of brain areas of interest to cognitive performance, thereby detecting smaller effects potentially induced by RT. The hippocampus has a crucial role in memory and information processing and is known to be sensitive to radiotherapy. Here, we studied the effects of various pituitary RT techniques by relating detailed dosimetry of the hippocampus to cognitive performance. *Design:* Aspects of verbal memory and problem solving were assessed by using standardized neuropsychological test procedures that have been shown to be sensitive to the effects of brain surgery and radiation. We compared dosimetric data of 3 different RT techniques (3-fields, 4-fields and 5-fields technique and a non-irradiated patient group. A reconstruction was made of the different radiation techniques used and the mean left and right hippocampi doses were calculated. *Patients*: 75 patients (61±10 year) underwent transsphenoidal surgery as primary treatment. Irradiated patients (n=30) were divided into 3 groups; 3-fields technique n=10; 4-fields technique n=15; 5-fields technique n=5. All patients received 45 gray (Gy), given in 25 fractions of 1.8 Gy. Cognitive performance data from the different irradiated patients groups and the non-irradiated patient group (n=45) were compared. *Results:* Mean (SD) cumulative dose for the left hippocampus irradiated with the 3-, 4-, and 5-fields techniques were respectively 13.82 (15.11), 18.46 (10.09), and 12.48 (12.41) Gy. Mean cumulative dose for the right hippocampus with the 3-, 4-, and 5-fields techniques were 15.55 (16.61), 22.27 (12.05), and 13.82 (14.48) Gy. No significant differences were found for the different RT techniques and the non-irradiated patients for cognitive tests, particularly memory and information processing which has its anatomical substrate in the hippocampus (Verbal memory, P=0.337). No radiation dose- cognitive response relationship was detected. *Conclusion:* Postopertive pituitary RT has no effect on cognitive performance involving the hippocampus in NFA patients. No dose response relationship could be established, confirming that these RT techniques and fractionated radiation dose regimens are safe with regard to cognition. Nothing to Disclose: PB, MGAS, ACMvdB, LCM, RPFD, GvdB, GJI, JK, OT, BHRW, APvB

Pub #	P2-381
Session Information	POSTER SESSION: CLINICAL - General Aspects of Pituitary Diseases (1:30 PM-3:30 PM)
Title	Measurement of 3x8h Urine Analysis To Follow Cortisol and Melatonin Rhythms
Author String	RW Rivest, D Mercan, R Auckenthaler Unilabs, Coppet, Switzerland
Body	**Background**: To follow the daily rhythm of cortisol (**C**) and melatonin (**M**) can be useful to explain disorders concerning stress, fatigue or sleep[1]. Common protocols are based on repeated blood or saliva samples over 24 hours. However, daily variations do not follow a sinusoidal curve[2]: rhythms of cortisol and melatonin show very irregular profiles with short lasting peaks and troughs. Therefore [ldquo]spot[rdquo] sampling of blood and saliva can be misleading. In this study we evaluated the fluctuation of cortisol and melatonin in urine over a 24 h period. **Material and Methods**: 25 clinicians investigating 200 patients for defined disorders participated in the study. Three consecutive 8 hours urine collections defined as period 1 (morning), 2 (afternoon) and 3 (night) starting at 07:00 hr, stored at 4[deg]C, were obtained and analysed for urinary free **C** (RIA) and **M** (6-sulfatoxy-melatonin, Elisa) within 24 hours after reception in the laboratory or frozen until assayed. Value changes between urines from 8 hour periods were defined as high amplitude ($\pm$ >150%) , median amplitude (> 50% to < 150%) and low amplitude < 50%). Desynchronized fluctuations of cortisol versus melatonin were defined as a shift in one rhythm not observed in the other. Clinical indications were: burn-out, depression following exhaustion, seasonal depression, morning fatigue or unexplained sleeping disorders. In a few cases the effect of treatment with melatonin was evaluated. **Results**: Rhythms of **C** (**M**) were of low amplitude in 30% (28%), medium amplitude in 18% (18%) and of high amplitude in 5% (13%). Highest **C** levels were observed in phase night and morning phases in 35% of cases delayed phase in 40%, no rhythm in 13% and phase advanced in 8% of subjects. For **M**, peaks during the night occurred in 27% of patients, while the peak was extended in the morning phase in 53%, no rhythm in 15% and phase advance in the afternoon in 5% of patients. In 23% of the patients desynchronisation between **C** (**M**) rhythms were observed with **C** peaks displaced in the morning while **M** peaked during the night. In 5 patients the measurement of **C** and **M** fluctuations were repeated 2 times, yielding similar results. The correlation between **C** and **M** fluctuations and clinical data will be presented. **Conclusions**: The analysis of urine collected by 8 hours period is promising to follow the fluctuations and the relationship of **C** and **M** with clinical data. (1) Lieverse R et al., Arch Gen Psychiatry 2011; 68:61 (2) Rivest RW et al., J Clin Endocrinol Metabol 1989; 68:721 Nothing to Disclose: RWR, DM, RA

Pub #	P2-382
Session Information	POSTER SESSION: CLINICAL - General Aspects of Pituitary Diseases (1:30 PM-3:30 PM)
Title	Sleep Disorder Breathing Risk Screening across the Spectrum of Endocrine Disorders in an Adult Population at a Northeast Florida Clinic
Author String	A Vera, PY Casanova-Romero, AB Vera, CA Hernandez, BA Forster, M Garewal Vera Endocrine Associates, Inc, Daytona Beach, FL; Ocean Sleep Disorders Center, Ormond Beach, FL

Body

Background: There have been increased reports of the association of some endocrine and metabolic disorders (EMD) and sleep disorder breathing (SDB). Obstructive sleep apnea (OSA), an SDB, increases the cardiovascular risk in the general population. The aim of this study was to investigate whether there are a higher proportion of individuals with SDB and at [ldquo]high risk[rdquo] for OSA in an adult population referred for endocrine evaluation at a Northeast Florida clinic.

Methods: We performed a questionnaire-based study to determine the proportion of patients with high subjective measures of daytime sleepiness and SDB, using two validated scores - the Epworth Sleepiness Scale (ESS) and the Berlin Questionnaire (BQ). Questionnaires and clinical data were obtained at the first initial visit. We defined those at [ldquo]high risk[rdquo] of OSA as patients scoring more than 11 on the ESS, or falling within the [ldquo]high risk[rdquo] category on the BQ. A Chi-squared test was used to test the proportions for each group.

Results: 1368 patients aged 18-101 and a mean BMI of 28.5 were evaluated. 66.4% were female. 33.7% individuals self-reported excessive daytime sleepiness and 49.6% positive snoring. Panhypopituitarism, but no other EMD, were found in a significant proportion of subjects with excessive or severe daytime sleepiness and a [ldquo]high risk[rdquo] OSA base on ESS, independent of their age, gender and BMI (Chi-square 6.82 df 1 $p<0.01$). In subjects with Panhypopituitarism (Chi-square 5.34 df 1 $p<0.05$), Diabetes Mellitus (DM) with self-reported episodes of Hypoglycemia (Glycemia <60 mg/dL), (Chi-square 13.99 df 1 $p<0.0001$) and Cushing's Syndrome (Chi-square 4.69 df 1 $p<0.05$), there was a significant proportion that fell within the [ldquo]high risk[rdquo] category of SDB on the BQ, independent of their age and gender. The proportion of patients diagnosed with Acromegaly, Hyper- or Hypothyroidism, Metabolic Syndrome, DM without any complications, Hyperparathyroidism, and Male Hypogonadism, falling within the [ldquo]high risk[rdquo] category for SDB on the BQ, were not significantly different from individuals without [ldquo]high risk.[rdquo]

Conclusions: Our data suggests that the use of ESS and BQ to identify individuals at high risk for SDB may not be sensitive enough in EMD, being different in subjects diagnosed with Panhypopituitarism, DM with hypoglycemia and Cushing's Syndrome. It is important to correctly identify SDB during the individual's treatment of an EMD to improve their quality of life and life expectancy.

Nothing to Disclose: AV, PYC-R, ABV, CAH, BAF, MG

Pub #	P2-383
Session Information	POSTER SESSION: CLINICAL - General Aspects of Pituitary Diseases (1:30 PM-3:30 PM)
Title	Prevalence of Metabolic Syndrome among Patients with Pituitary Tumors
Author String	MPK Huayllas, LB Tavares, RW Zilli, PP Mariani, A Cukiert Hospital Brigadeiro, São Paulo, Brazil
Body	**Introduction**: Metabolic syndrome (MS) may be a co-morbidity in patients with pituitary tumors. ATPIII, WHO and IDF introduced different diagnostic schemes for MS. The mechanisms for this association are not fully understood; it would be important to detect it since it is associated to increased cardiovascular risk and increased mortality. Pituitary tumor might present with hormone deficiency or hypersecretion at diagnosis or after treatment. Growth hormone deficiency or excess and glucocorticoid excess are related to a worse metabolic profile. We studied the prevalence of MS in a series of pituitary tumor patients. **Material and Methods**: The prevalence of MS according to different criteria was evaluated retrospectively ir 50 consecutive patients (39 female) harboring pituitary tumors (acromegaly:19; Cushing's disease:14; prolactinoma:8; non-secreting adenoma:7; craniopharyngioma:2) and followed at Brigadeiro Hospital - São Paulo/Brazil from June, 2010 to December, 2010. All patients underwent resective surgery and all had pathological findings confirming the diagnosis. **Results:** The prevalence of the MS's components was: hypertension, 42%; diabetes or glucose intolerance, 30%; dislipidemia, 34%. The median age was 46 years and median BMI was 29 Kg/m2. MS was diagnosed ir 46%, 42% and 22% of the patients according to IDF, ATPIII and WHO definitions, respectively. Patients witl Cushing's disease had the higher prevalence of MS independently of the criteria, followed by those with acromegaly. **Conclusion:** WHO criteria led to the lower rate of diagnosis of MS, and its likely that its usage alone would leave some patients which would actually need to have their cardio-metabolic profile evaluated uninvestigated. MS has high a prevalence in patients with pituitary tumors, independently of the diagnostic criteria used. Nothing to Disclose: MPKH, LBT, RWZ, PPM, AC

Pub #	P2-384
Session Information	POSTER SESSION: CLINICAL - General Aspects of Pituitary Diseases (1:30 PM-3:30 PM)
Title	Coping Strategies in Patients after Treatment for Functioning or Non-Functioning Pituitary Adenomas
Author String	J Tiemensma, AA Kaptein, AM Pereira, JWA Smit, JA Romijn, NR Biermasz Leiden University Medical Center, Leiden, Netherlands; Leiden University Medical Center, Leiden, Netherlands
Body	Context and objective: Coping strategies may affect quality of life (QoL), which is decreased in patients after treatment for Cushing's disease, acromegaly, or non-functioning macroadenomas (NFMA). We aimed to explore coping strategies in these patients, since this has never been done before. Design: This was a cross sectional study. Subjects: We included patients treated for Cushing's disease (n=42), for acromegaly (n=80), and for NFMA (n=61). These patients were compared with three reference populations: an a-select sample from the Dutch population (n=712), patients with chronic pain (n=59), and patients receiving primary care psychology services (n=525). Furthermore, the three patient groups were compared with each other. Coping strategies were assessed by the Utrecht Coping List. The protocol was approved by the Medical Ethics Committee. Results: Compared to the a-select sample, patients with pituitary adenomas reported less active coping ($P<0.0001$), sought less social support ($P<0.0001$), and reported more avoidant coping ($P=0.008$). In contrast, patients treated for pituitary adenomas reported somewhat better coping strategies than patients with chronic pain and those with psychological disease. When patients with different pituitary adenomas were compared, patients treated for Cushing's disease sought more social support than patients treated for NFMA ($P=0.035$). Conclusions: Patients treated for pituitary adenomas display different and less effective coping strategies compared with healthy controls. A targeted intervention might help to stimulate patients to use a more active coping strategy and to seek social support, instead of an avoiding coping strategy. This might, in turn, improv their QoL. Nothing to Disclose: JT, AAK, AMP, JWAS, JAR, NRB

Pub #	P2-385
Session Information	POSTER SESSION: CLINICAL - General Aspects of Pituitary Diseases (1:30 PM-3:30 PM)
Title	Strategies for Screening and Management of Familial Isolated Pituitary Adenomas (FIPA): Our Experience or 17 Families in a Single Center in Brazil
Author String	LA Naves, LA Casulari, MF Azevedo, IB Ventura, AF Daly, A Beckers University of Brasilia, Brasilia, Brazil; University of Liege, Liege, Belgium
Body	**Background**: Pituitary adenomas present clinically as hormonal overproduction or compression symptoms of adjacent structures. Advances have been made in defining culprit genes in familial syndromes, that may be related to mutations of AIP gene, CDKN1B p27(Kip1) gene. We describe here our clinical and molecular experience in 17 families in a single center in Brazil. **Objectives**: To define a strategy to screen clinically and identify FIPA families and to perform molecular test to define candidate genes, in the current practice at a neuroendocrinology unit. **Methods**: This is a case-finding study. The strategy was based in four steps. **Step 1:** We reviewed the medica records of 589 patients who presented various pituitary adenomas phenotypes, treated at University Hospital of Brasilia, from 2006 to 2010. **Step 2:** All patients that had the diagnosis at young age (<20 years), or older patients that had a bad outcome as resistance to medical treatment or rapid tumor growth, were recruited. We questioned for family history of headaches, amenorrhea, infertility, growth disorders, weight gain and educational meetings were scheduled with suspect families. **Step 3:** The patients and relatives signed an informed consent and underwent a clinical evaluation and a selar MRI, if hormonal changes were confirmed. **Step 4:** We screened for mutations on MEN1, AIP and CDKN1p27 genes in all identified FIPA cases. **Results:** We identified 17 families with no previous diagnosis of familial isolated pituitary tumors, in which only the index case was previously treated. Clinical phenotypes and therapeutic characteristics were described Most of patients were male (64%) and 50% of patients had first symptoms as children/adolescents. None menin mutation was found in this cohort, and in 29,41% of patients had mutations in AIP or CDKN1 genes. We identified 4 AIP mutations at positions A195V, FS174, AIPTyr168X, AIPAla299Val in 4 different families, and one aminoacid substitution CDKN1 Ser56Threo in the fifth family. Most tumors were invasive macroadenomas (83.4%).. Somatotropinomas comprised 35,3% of the cohort; *AIP* mutation-related tumors were significantly larger (P=0.0001). **Conclusions**: The spectrum of *FIPA* tumors includes all clinical subtypes. *AIP* mutations confer an aggressive pituitary tumor phenotype with an early age. Evidence suggests that, especially in MEN1 and FIPA, they are more aggressive and affect patients at younger age, justifying the importance of early diagnosis. Sources of Research Support: CNPq, FINATEC, Laboratorio Sabin. Nothing to Disclose: LAN, LAC, MFA, IBV, AFD, AB

Pub #	P2-386
Session Information	POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)
Title	A Novel GH-1 Gene Mutation (GH-P59L) Causes Partial GH Deficiency Type II Combined with Bioinactive GH Syndrome
Author String	V Petkovic, A Eble, AV Pandey, M Betta, P Mella, CE Fluck, F Buzi, PE Mullis University Children's Hospital, Bern, Switzerland; Pediatric General Practitioner, Riva del Garda, Italy; University of Brescia, Brescia, Italy; University of Brescia and Children's Hospital, Brescia, Italy
Body	Despite the differences in the main characteristics between the autosomal dominant form of GH deficiency (IGHD II) and the bioinactive GH syndrome, a common feature of both is their impact on linear growth leading to short stature in all affected patients. The index patient, a boy, was referred for assessment of his short stature (-2.54 SD score) and a delayed bone age of 5.9 yrs at the chronological age of 7.7 yrs. The GHD was confirmed by standard GH provocation tests, which revealed modestly reduced GH and IGF-1 concentrations. Further genetic analysis of *GH-1 gene* identified heterozygosity for GH-P59L mutation. The secretion of the GH-P59L following stimulation with forskolin was investigated and compared to that of the wt-GH after expression of both GH variants in AtT-20 cells. Based on the position of P59L mutation that lies within a patch of residues composing the GH binding site 1 for GHR, we performed the analysis of GH-P59L binding to GHR by *in silico* mutagenesis and molecular dynamics simulations, which suggested possible problems in correct binding of GH-P59L to the GHR. Therefore, the functional characterization of this GH mutant was assessed through studies of GHR binding and activation of Jak2/Stat5 signaling pathway. In line with the clinical data of the patient, which suggest modest GH deficiency, the GH secretion studies revealed a moderate difference in secretion between GH-P59L and wt-GH. Further functional characterizatior of the GH-P59L by studies of GH-receptor binding and activation of Jak2/Stat5 pathway revealed reduced binding affinity of GH-P59L for GHR and decreased bioactivity compared to the wt-GH. The clinical data of the patient combined with the laboratory data support the diagnosis of partial IGHD type II. Since the GH deficiency was not total, additional binding and signaling studies were performed, which revealed that the GH-P59L variant displays some of the common features of bioinactive GH syndrome. Taker together, in this study we report a patient suffering from the combination of two growth disorders caused by a *GH-1 gene* alteration highlighting the necessity of functional analysis of any GH variant, despite the presence of obvious clinical features of IGHD type II. Nothing to Disclose: VP, AE, AVP, MB, PM, CEF, FB, PEM

Pub #	P2-387
Session Information	POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)
Title	Combined Pituitary Hormone Deficiency (CPHD) Due to Gross Deletions in the *POU1F1 (PIT1)* and *PROP1* Genes
Author String	E Bertko, W Oostdijk, A Richter-Unruh, G Mansmann, M Schlicke, H Stobbe, L ten Have, R Pfaffle, J Klammt Hospital for Children and Adolescents, Leipzig, Germany; Leiden University Medical Center, Leiden, Netherlands; Endokrinologikum, Bochum, Germany; University Hospital Duesseldorf, Duesseldorf, Germany; Reinier de Graaf Gasthuis, Delft, Netherlands
Body	Development of the pituitary gland depends on a complex cascade of interacting transcription factors and signaling molecules. Mutations in any of the genes involved in pituitary development may cause persistent functional defects which lead to combined pituitary hormone deficiency (CPHD). Aim of the study was to determine the frequency of copy number variants (CNVs) in genes known to cause CPHD in a cohort of patients with hypopituitarism. We have screened 90 individuals with CPHD employing multiplex ligation-dependent probe amplification (MLPA) to detect CNVs within the transcription factors *PROP1, POU1F1, LHX3, LHX4* and *HESX1*, as well as *GH1* and *GHRHR*. Previously, point mutations were excluded. Breakpoints of deletions were identified by STS mapping, long-range and inverse PCR followed by dideoxy-sequencing. Causative mutations were found in two unrelated consanguineous Turkish families and one family from northern Iraq. The affected boy in one family was born as the 4th child of 7 siblings, of which 3 deceased during infancy. He presented with severe growth retardation (-11.2 SDS at 11 yrs), showed very low basal levels of GH (<0.5 mU/l), TSH (<0.5 mU/l) and PRL (0.1 [mu]g/l) and failed to increase GH and TSH on stimulation. We identified a homozygous deletion of exons 1 and 2 of the *POU1F1* gene. To ascertain adjacent regions affected by the deletion we defined the deleted sequence to ~5 kB including approximately 1.3 kB of the *POU1F1* proximal region. The index patients of two other families presented with deficiency in GH, TSH and FSH/LH. In these patients we discovered a homozygous 15 kB deletion including the complete *PROP1* gene. Breakpoints map within highly homologous AluY sequences possibly indicative for an unequal recombination event. MLPA analysis of 90 CPHD patients revealed the first homozygous *POU1F1* gross deletion. Moreover, for the first time, we were able to assign the boundaries of a novel *PROP1* deletion. No CNVs within the other analyzed genes were found. Thus, gross deletions within CPHD causing genes remain rare. Their clinical manifestation is comparable to the known clinical presentation of carriers of point mutations. Our data prove MLPA to be a valuable tool for the detection of CNVs as cause of pituitary insufficiencies and to provide a molecular basis for genetic counseling. Disclosures: RP: Research Funding, Eli Lilly & Company; Serono; Medical Advisory Board Member, Ipsen. Nothing to Disclose: EB, WO, AR-U, GM, MS, HS, LtH, JK