OR09-1

Neonatal Exendin-4 Normalizes Epigenetic Modifications at the Proximal Promoter of PGC1-α in IUGR Rat Liver.

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Intrauterine growth retardation (IUGR) has been linked to the development of type 2 diabetes in adults. IUGR impairs hepatic mitochondrial function in prediabetic IUGR animals. PGC1-a is a transcriptional coactivator and metabolic regulator that promotes mitochondrial biogenesis and is critical for normal mitochondrial function. Expression is decreased in IUGR liver. Exendin-4 (Ex4), a long-acting agonist of the glucose dependent insulinotropic hormone (GLP-1), improves mitochondria function, normalizes hepatic insulin resistance, and prevents diabetes in the IUGR rat. The objectives were to determine if Ex4 normalizes PGC1- α expression; and whether changes in expression are linked to epigenetic modifications at the PGC1- α promoter. IUGR newborn pups were treated with a 6-day course (day 1-6) of Ex4 and liver was harvested for analyses at 8 weeks of age. Gene expression was measured by quantitative (q)-PCR. Histone modifications were evaluated by chromatin immunoprecipitation assays and q-PCR. PGC1- α gene expression was decreased in adult IUGR animals compared to control by 80% ($p=0.017$) and Ex4 treatment normalized PGC1-expression to levels equivalent to controls ($p=0.36$). Similarly, mitochondrial DNA and protein content were significantly reduced in IUGR liver (62±4.2% of controls, $p=0.012$) and neonatal Ex4 treatment normalized mitochondria content (102±4.6% of controls, $p=0.45$). In IUGR liver, H3 acetylation and H3K4 trimethylation at the proximal promoter of PGC1- α were markedly reduced (16±0.09% and 19±0.05% of controls, respectively ($p<0.05$). p300 is a transcriptional co-activator with histone acetyl transferase activity (HAT) and has been shown to increase acetylation at a number of metabolic genes. In IUGR liver, p300 binding at the proximal promoter of PGC1- α was 12±0.04% of control ($p<0.01$). Neonatal Ex4 treatment of the IUGR pups increased acetylation of H3 by 80% ($p=0.029$), increased trimethylation of H3K4 by 80% ($p=0.045$) and p300 binding by 95% ($p=0.038$) at the PGC1- α promoter. We speculate that Ex4 normalizes hepatic mitochondrial function by increasing HAT activity (via recruitment of p300), which in turn mediates epigenetic modifications at PGC1- α thereby increasing expression of PGC1- α.

Sources of Research Support: NIH Grants DK55704, DK062965 awarded to RAS.

Nothing to Disclose: SEP, YH, RAS

OR09-2

Key Role of AMP-Activated Protein Kinase in Pancreatic Beta Cell Glucose-Sensing and Whole Body Glucose Homeostasis.

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AMP-activated protein kinase (AMPK) is an evolutionarily conserved sensor of cellular energy status and regulator of organismal energy homeostasis. AMPK activates catabolic pathways that generate ATP and switches off ATP-consuming processes through acute phosphorylation of metabolic enzymes and long-term alterations in gene expression. Pancreatic beta cells require metabolism of glucose and ATP production for glucose-stimulated insulin secretion (GSIS). While this link suggests a potential role for AMPK signaling in beta cell function, the precise involvement of AMPK in insulin secretion from beta cells is at present unclear and controversial.
To investigate the role of AMPK catalytic isoforms in glucose homeostasis, we generated *RIPCreα2KO* mice lacking the AMPKα2 catalytic subunit in beta cells and in a population of hypothalamic neurons and *α1KORIPCreα2KO* mice, which were additionally globally deleted for the AMPKα1 catalytic subunit. *RIPCreα2KO* mice displayed normal food intake and body weight. However, they developed age-dependent glucose intolerance and defective *in vivo* GSIS. *α1KORIPCreα2KO* mice displayed a more severe and earlier onset of impaired glucose homeostasis and abnormal *in vivo* GSIS. Beta cell mass was normal in both mouse lines.
In islets isolated from *RIPCreα2KO* and *α1KORIPCreα2KO* mice GSIS was defective. Furthermore, islets displayed increased basal insulin secretion in response to low glucose. Perforated patch electrophysiological studies demonstrated that in contrast to control cells, beta cells from *RIPCreα2KO* and *α1KORIPCreα2KO* mice failed to hyperpolarize when glucose concentration was decreased. Further investigation of the mechanisms underlying this abnormality showed normal expression of *Glut2* and *glucokinase*, normal K_{ATP} channel function and normal mitochondrial density. In contrast, expression levels of *uncoupling protein 2* (UCP2) were significantly reduced. Furthermore, exposure of wild type beta cells to genipin, a UCP2 inhibitor, although not altering beta cell electrical characteristics in the presence of 10 mmol/l glucose, prevented the hyperpolarization normally associated with hypoglycaemic challenge. Together, these results suggest a key role for beta cell AMPK in sensing low glucose levels and suppressing insulin secretion via a UCP2 dependent mechanism. AMPK is also required for GSIS involving mechanisms that remain to be fully elucidated.

Sources of Research Support: MRC.

Nothing to Disclose: KP, CB, HA-Q, MAS, DC, BV, MLJA, DJW

OR09-3

FoxO1 and Notch1 Interact To Promote Hepatic Gluconeogenesis.

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The role of the Notch family of transmembrane receptors in differentiation is well-established, but Notch signaling in developed liver and regulation by metabolic stimuli is unknown. We have previously demonstrated that FoxO1, one of the primary transcriptional regulators of hepatic gluconeogenesis, promotes corepressor clearance from the Notch effector RBP-Jκ, allowing activation of the differentiation program in many cell types. We hypothesized that similar interactions regulate FoxO1-dependent metabolic functions. Of the four Notch receptors, Notch1 and 2 are the predominant isoforms in liver, and both receptors as well as its ligands are metabolically regulated, leading to increased expression of targets of Notch signaling in the fed state. To identify the role of Notch1 signaling in developed liver, we modestly overexpressed its intracellular domain (N1-IC), which signals constitutively in a ligand-independent manner, in primary hepatocytes or in whole liver. In both models, N1-IC transduction increased expression of gluconeogenic genes (G6Pase and PEPCK) resulting in augmented hepatocyte glucose output and elevated serum glucose and insulin levels, indicative of hepatic insulin resistance. Conversely, treatment of either isolated primary hepatocytes or mice with Notch inhibitors improved hepatic insulin sensitivity through inhibition of gluconeogenic gene expression. We created FoxO1-Notch1 double-heterozygous (F/N) mice in order to circumvent the embryonic lethality of homozygous mutation of either FoxO1 or Notch1. F/N mice develop normally and despite unchanged body composition as compared to FoxO1 heterozygote littermate controls, upon challenge with high-fat diet feeding, demonstrate improved glucose tolerance and insulin sensitivity. In hyperinsulinemic-euglycemic clamps, F/N mice show decreased hepatic glucose output and increased muscle glucose uptake as compared to FoxO1 heterozygote mice, implying metabolic synergy between these two pathways. Finally, we created a liver-specific knockout of the Notch effector, RBP-Jκ – these mice are similarly protected from high-fat diet-induced hepatic insulin resistance; further, mice with liver-specific deletions of both FoxO1 and RBP-Jκ, similar to the effects seen in the F/N mice, are more insulin sensitive than liver-specific FoxO1 mice. In summary, we identify Notch signaling as a regulator of systemic insulin sensitivity and a novel molecular target to modify FoxO1 function in diabetic patients.

Sources of Research Support: Endocrine Fellows Foundation Grant to UBP; NIH 1F32DK084583-01 to UBP.

Nothing to Disclose: UBP, VTS, GIS, DA

OR09-4

The Control of Hepatic Glucose Production by Coactivator-p300.

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The cardinal feature of diabetes mellitus (DM) is fasting hyperglycemia due to inappropriate hepatic glucose production (HGP). Glucagon increases hepatic gluconeogenesis via a cAMP-PKA signaling pathway leading to the formation of an activated CREB-p300/CBP-CRTC2 complex and increased transcription of gluconeogenic enzyme genes; insulin disassembles the complex and suppresses hepatic gluconeogenesis. We assessed the relative importance of CREB coactivators in mediating HGP in mice by depleting co-activators individually or in combination using adenoviral shRNAs and found that depletion of either CBP or p300 reduced blood glucose levels during a prolonged fast and knockdown of both CBP and p300 led to a decrease in blood glucose levels during the early stage of fasting. However, depletion of hepatic CRTC2 had no effect on fasting blood glucose levels, demonstrating that both CBP and p300 are important in maintaining blood glucose levels. Inappropriate HGP is a major cause of hyperglycemia in obesity. To test whether the induction of CREB co-activators are responsible for elevated HGP, we placed WT mice on a high fat-diet (HFD). HGP increased after one week of HFD feeding, while insulin resistance occurred later (after 2 weeks of HFD feeding). Strikingly, p300 protein levels were dramatically induced by HFD feeding, as early as 3-days after starting the HFD. The induction of p300 by HFD is at the post-transcriptional level, since p300 mRNA levels were not induced by a HFD. HFD feeding also led to increased p300 binding to the Ppargc-1 promoter. Depletion of p300 by adenoviral shRNA normalized these findings and resulted in a reduction in the mRNA levels of Ppargc-1 and Pck1 genes in mice fed a HFD. Since p300 lacks the insulin and metformin phosphorylation site found in CBP due to a single amino acid difference, we generated p300G422S mutant mice harboring a reconstituted phosphorylation site. These mice displayed hypoglycemia, reduced HGP, and hypersensitivity to insulin and metformin. When placed on HFD, p300G422S mice had equivalently elevated hepatic p300 protein levels as in WT mice fed a HFD, but p300G422S mice exhibited regulated p300 binding to CREs and were more sensitive to insulin and metformin than WT mice. Therefore, as p300 is a critical co-activator for maintaining basal HGP and is an important etiological factor in the HFD-induced hyperglycemia, it may be an important drug target for treatment of patients with Type 2 DM.

Nothing to Disclose: LH, KN, AS, SR, FEW

OR09-5

Metabolically Responsive Hepatic Insulin Gene Therapy Prevents Enteric Neuropathy in STZ-Diabetic CD-1 Mice.

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Diabetic enteropathy (DE) is a syndrome of delayed gastric emptying and altered bowel transit caused by hyperglycemia induced apoptosis of enteric neurons. To determine if (HIGT) hepatic insulin gene therapy (HIGT)can prevent DE we evaluated gastric emptying, absolute and proportional bowel transit, colonic neuronal number and intestinal smooth muscle relaxation in diabetic, HIGT treated (HIGT) non-diabetic (Con), and hyperglycemic diabetic (DM-Con) CD-1 mice (n=10). HIGT diabetic mice received an injection of an AAV vector expressing human proinsulin from a metabolically responsive promoter. Con and DM Con mice received a similar virus expressing eGFP. Mice were sacrificed 8 weeks after virus dosing. Following methylene blue gastric lavage, gastric emptying and intestinal transit were measured via spectrophotometry of gastric lysates and measurement of intestinal dye travel. Counts of inhibitory (nNOS) and stimulatory (acetyl choline) neurons were made histochemically on colon sections. Electrical stimulation induced colonic smooth muscle relaxation was measured using perfusion bath mounted force transducers. Average blood sugar for DM was greater than both Con and HIGT ($p=<0.05$), while HIGT was similar to Con. Gastric emptying was similar in all groups, as was the absolute intestinal transit distance. However, intestinal length was >40% greater in DM than in HIGT and Con ($p< 0.01$). Consequently, proportional intestinal transit was 20% less in DM than either HIGT or Con ($p<0.01$). Neuronal staining showed equal numbers of stimulatory colonic neurons across groups. However, numbers of inhibitory neurons were diminished among DM compared to Con (10.4±3.5 DM vs 21.1±1.9 Con; $p<0.01$). In contrast, HIGT prevented inhibitory neuron loss (23.9±1.6 HIGT vs DM $p<0.01$). As suggested by inhibitory neuron counts, isometrically contracted colonic smooth muscle relaxation was limited in DM (22.0±9%) compared to HIGT (39.0±7%) and Con (38.0±16%) ($p<0.05$), while there was no difference between HIGT and Con ($p>0.05$). Our data indicate that 2 months of STZ-diabetes induces anatomic, histochemical, and functional abnormalities consistent with DE in CD-1 mice. Treatment with HIGT produced near normal blood sugars, and inhibited abnormal bowel prolongation, inhibitory neuron loss, and a decline in colonic smooth muscle relaxation. Taken together these findings indicate that HIGT is able to prevent DE in diabetic mice, and suggests potential efficacy in larger animals.

Nothing to Disclose: SY, MA, SMDd, DJ, DEO, SS, PMT

OR09-6

Hyperinsulinemia Induces Insulin Resistance in Dorsal Root Ganglion Neurons through Decreased Akt Signaling.

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Insulin resistance represents a state of decreased responsiveness of target tissues to normal circulating levels of insulin. It is the major feature of type 2 diabetes, glucose intolerance, obesity, dyslipidemia and hypertension; i.e. metabolic syndrome. Reports suggest that hyperinsulinemia, rather than hyperglycemia, is the major cause of insulin resistance. Insulin resistance studies are mainly focused on peripheral tissues such as muscle and liver. There is, however, little knowledge about insulin resistance in neuronal cells. In the late 1990s, our laboratory introduced the idea that glucose-mediated oxidative stress injures sensory neurons leading to eventual development of diabetic neuropathy. Recently, we started to investigate whether hypersinsulinemia-induced insulin resistance can cause similar injuries to neurons. As a first step we examined insulin signaling using adult rat dorsal root ganglion (DRG) neurons as a model system. Acute insulin treatment (20 nM, 0-2 h) resulted in time- and concentration-dependent activation of the signaling cascade including phosphorylation of the insulin receptor (IR), insulin receptor substrate (IRS), ERK, Akt, p70S6K and GSK-3β. To mimic hyperinsulinemia, cells were pretreated with 20 nM insulin for 24 h, then treated with 20 nM insulin for 15 min. Chronic insulin treatment resulted in increased basal Akt phosphorylation. More importantly acute insulin stimulation after chronic insulin treatment resulted in blunted phosphorylation of Akt, p70S6K and GSK-3 β; the phosphorylation of IR, IRS-1 and ERK were not affected. Interestingly, when the cells were treated with PI3-K pathway inhibitor (LY294002), but not MAPK pathway inhibitor (U0126), chronic insulin treatment did not block acute insulin treatment-induced Akt phosphorylation; in fact Akt phosphorylation was increased by LY294002 pretreatment. In agreement withe in vitro results, we obtain similar results using DRG neurons from BKS-db/db rats, the model for type 2 diabetes. Insulin-induced Akt phosphorylation was lower in DRG neurons from BKS-db/db compared to control BKS-db+ rats. This effect was age-dependent. Our results suggest that hyperinsulinemia may cause insulin resistance by mainly affecting the Akt-mediated pathway. Hyperinsulinemia-induced Akt hyperactivation may be one mechanism for the decreased Akt phosphorylation. Our results may provide new insight into a therapeutic target for diabetic neuropathy.

Sources of Research Support: Grants from the Michigan Diabetes Research and Training Center (NIH 5P60-DK020572), Animal Models of Diabetic Complications Consortium (NIH U01-DK076160), the Taubman Institute, and the Program for Neurology Research and Discovery.

Nothing to Disclose: BK, LM, ELF

OR10-1

CREB Binding Protein (CBP) Phosphorylation Is Necessary for GnRH Regulation of the Pituitary Gonadotroph.

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Normal function of the hypothalamic-pituitary-gonadal axis is dependent on gonadotropin releasing hormone (GnRH)-stimulated synthesis and secretion of luteinizing hormone (LH) from the pituitary. As the transcriptional coactivator CBP is known to interact with Egr-1, the major mediator of GnRH action on the LH-β gene, we sought to determine if CBP was required for normal function of the gonadotroph. In LβT2 gonadotrophs transfected with full-length CBP, LH-β promoter activity was significantly higher in response to GnRH than in cells transfected with empty vector. Conversely, siRNA against CBP renders LβT2 cells nearly unresponsive to GnRH stimulation. These experiments indicate that intracellular CBP is necessary for GnRH-stimulated LH-β expression. To determine a possible mechanism for CBP-mediated induction of LH-β expression, LβT2 cells were treated with GnRH and harvested for mRNA and protein. Quantitative rtPCR demonstrated a rise in LH-β and Egr-1 mRNA at 4 hours but no increase in CBP mRNA. In addition, while Western blotting showed that total CBP protein did not change, there was an increase in serine phosphorylation of CBP site at position 436 after GnRH treatment. In a two-hybrid assay, CBP phosphorylation enhanced recruitment to Egr1. To test the hypothesis that CBP phosphorylation at Ser436 is required for normal gonadotroph function, we studied fertility, estrous cyclicity and hormone levels in a knock-in mouse model that replaces Ser436 with Ala. In breeding studies, the presence of the S436A mutation resulted in fewer litters over 120 days, and fewer mice per litter when compared to WT x WT breeding pairs. Female S436A mice spent a greater amount of time in diestrus and less time in estrus compared to WT mice and had lower surge LH values (17 ng/mL vs. 0.9 ng/mL, $P < 0.05$) in proestrus. Baseline gonadotropin levels were not different between groups. However, in male mice, the rise in LH following subcutaneous administration of GnRH (100ng/kg) was greater in WT than in S436A mice (6.6-fold vs. 1.3 fold, $P < 0.01$). Our studies demonstrate that CBP phosphorylation is required for normal LH-β expression *in vitro*, while *in vivo*, loss of the Ser 436 phosphorylation site renders mice subfertile and unable to respond to stimulation with GnRH. Given the role of CBP as an integrator of cell signaling, our data suggest that CBP plays a key role in coordinating the gonadotropin response, and is central to GnRH regulation of the reproductive axis.

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Nothing to Disclose: RSM, KB, AW, SR, FEW

OR10-2

Gonadotrope-Specific Re-Routing of a FSH Analog into the Regulated Secretory Pathway in Pituitaries of Transgenic Mice.

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Although LH and FSH are synthesized in the same gonadotrope cell, they differ in their mode of secretion from the pituitary. LH is packaged into secretory granules and released in response to GnRH, whereas, FSH is secreted constitutively. Since LH and FSH share a common α subunit the key determinants for hormone-specific intracellular routing must be encoded in the β subunit. Our previous tissue culture studies using the GH3 cell line indicated that the heptapeptide sequence at the C-terminus of the human LHβ is critical for its regulated secretion. Moreover, a novel FSH chimera that contains this peptide - unlike wild-type (WT) FSH - is targeted to the regulated secretory pathway. To test the gonadotrope-specific re-routing of this FSH analog in vivo, and the consequences of FSH secreted via a regulated secretory pathway, we generated multiple lines of transgenic mice that express either a WT or the mutant human *(h) FSHB* transgene specifically in gonadotropes. Subsequently, these *hFSHB* transgenes were introduced onto mouse *Fshb* null background using a genetic rescue approach. This facilitated monitoring the intracellular and secretory behavior of only the human transgene-encoded proteins in the absence of endogenous mouse FSH. RIA and western blot analyses suggested both the WT and mutant transgenes showed a sexual-dimorphic pattern with expression higher in males than females. In addition, the testis size in cases was significantly higher than that in *Fshb* null mice and comparable to that in normal control mice. Immunoprecipitation of proteins in whole pituitaries labeled with [^{35} S] cysteine demonstrated that the intracellular accumulation was greater for the FSH chimera than that seen for WT hFSH. Confocal dual immuno-fluorescence of pituitary sections showed that the mutant - but not WT FSH - co-localized with RAB27, a dense-core granule marker; the WT hFSH primarily co-localized with a golgi marker. The FSH chimera displayed a punctate, non-golgi pattern of localization. Our studies provide the first in vivo evidence for a key gonadotropin-sorting determinant. Current ongoing studies will determine whether the re-routed FSH analog secreted through the regulated pathway rescues *Fshb* null female mice. Thus, our *Fshb* knockout model offers a convenient genetic platform to selectively investigate the physiological consequences of rerouting FSH in vivo.

Sources of Research Support: NIH grants HD056082; AG29531 awarded to TRK and HD061907 awarded to IB.

Nothing to Disclose: TRK, HW, AJ-S, CP, IB

OR10-3

Uncoupling of Kiss and LH Inhibition during Caloric Restriction: Effects of Estradiol and Leptin.

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Negative energy balance leads to a reduction in GnRH and LH release, resulting in anestrus. Decreased leptin levels may drive LH inhibition during negative energy balance and this inhibition appears to require estradiol (E_2) as well. However, E_2 could contribute to LH inhibition indirectly by increasing the severity of negative energy balance either through decreased food intake, increased metabolic drive, or both. The primary goal of this study was to determine whether E_2 is critical for negative energy balance-induced LH inhibition in a caloric restriction (CR) model and whether restoring leptin could relieve LH inhibition. A secondary goal was to determine whether inhibition of the GnRH-stimulating neuropeptide kisspeptin (Kiss) is coupled to LH inhibition during CR. Adult female rats were ovariectomized (OVX) and given silastic implants with low concentrations of E_2 or oil. OVX animals receiving E_2 (OVX+E_2) were CR for 2 weeks to 60% of OVX+E_2 ad libitum food intake, while those receiving no E_2 were CR to achieve weight-loss comparable to the OVX+E_2 CR group. An additional OVX+E_2 CR group was given physiological levels of leptin for the last 48 hours of CR. The ad libitum fed OVX group gained significantly more weight over the 2-week period compared to the OVX+E_2 group despite similar food intake, thus demonstrating an increase in metabolic drive due to E_2 replacement. CR in the OVX+E_2 group resulted in a 19% decrease in body weights compared to ad libitum OVX+E_2 controls. The OVX group was CR to mimic final body weights of the OVX+E_2 CR group and this represented a 32% decrease in body weights compared to ad libitum OVX controls. In the OVX+E_2 group CR resulted in a significant reduction of serum LH levels as well as ARH Kiss mRNA expression, whereas in the OVX group, CR resulted in inhibition of ARH Kiss mRNA expression but no significant decrease in LH. These results indicate that E_2 is critical for CR-induced inhibition of LH, but not Kiss, and that ARH Kiss inhibition is not always coupled to LH inhibition. Leptin levels were significantly reduced in all CR groups and 48-hour leptin replacement increased LH to an intermediate level not significantly different from either the CR or ad libitum group. Leptin replacement had no effect on CR-inhibited ARH Kiss mRNA levels. These results indicate leptin deficiency may contribute to CR-induced LH inhibition through an ARH Kiss-independent mechanism.

Sources of Research Support: NIH Grants HD14643, RR00163, HD18185.

Nothing to Disclose: CAT, MAK, KLG, MSS

OR10-4

Kisspeptin-Cell-Specific Deletion of Estrogen Receptor Alpha (ERα) Results in Advanced Pubertal Onset and Incomplete Pubertal Maturation in Female Mice.

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Cellular and molecular mechanisms that govern activation of the GnRH pulse generator during maturation of the reproductive axis are still poorly understood. The kisspeptin system (Kiss-1/GPR54) has recently surfaced as an important player for both E2 feedback actions within the hypothalamic-pituitary-gonadal (HPG) axis and for regulation of pubertal onset. While E2 through ERα regulates hypothalamic kisspeptin levels and GnRH/LH release, evidence linking activation of ERα in kisspeptin neurons to regulation of GnRH release has yet to be obtained. Here we selectively eliminated ERα in kisspeptin cells (KERKO) in mice and analyzed maturation of the reproductive axis in female animals. Precocious activation of the gonadotropic axis was observed in KERKO mice as evident by advanced vaginal opening (day 15 KERKO vs. day 29.6 WT) accompanied by increased serum luteinizing hormone (LH) levels (1.34 + 0.30 ng/ml KERKO vs. 0.2 ng/ml WT). Higher LH levels in KERKO mice persisted through day 25, while adult levels were not significantly different from WT (0.34 + 0.04 ng/ml KERKO vs. 0.32 + 0.04 ng/ml WT). KERKO mice were also acyclic, showing persistent vaginal cornification at the time of vaginal opening. Corpora lutea were either absent or greatly reduced in number in ovaries of adult KERKO mice. Ovariectomy (ovx) resulted in LH levels that were significantly lower in KERKO mice compared to ovx WT littermates. However, KERKO mice responded to the negative feedback effects of E2, with significantly decreased LH levels in E2 treated animals compared to vehicle-treated controls. We also observed a dramatic decrease in the number of kisspeptin immunoreactive neurons in the AVPV-POA of KERKO females at day 25 and day 35 compared to WT. These observations suggest that activation of ERα in kisspeptin neurons during development has a dual role, serving both as a restraint on pubertal activation of GnRH release, and later being critical for normal cyclic GnRH/LH release in the adult. The ERα-mediated suppression of Kiss-1 expression in arcuate nucleus neurons likely mediates the suppressive effects of E2 on kisspeptin and hence, GnRH release during the prepubertal period. ERα-mediated stimulation of kisspeptin expression in periventricular neurons appears to mediate maturation of kisspeptin neurons that is in turn critical for the establishment and maintenance of adult GnRH secretory patterns.

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Nothing to Disclose: MA, CM, SD, AW, SR, JEL, UB

OR10-5

Neurokinin B (NKB) Expression in the Arcuate Nucleus Is an Early Marker for Pubertal Onset.

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Emerging evidence implicates a neuroendocrine role for neurokinin B (NKB) with kisspeptin (Kiss1) in gonadotropin secretion and initiation of reproductive function. NKB, Kiss1 and the inhibitory neuroendocrine regulator dynorphin (Dyn), are co-expressed within neurons of the arcuate nucleus (ARC), a hypothalamic site important for pubertal onset. Currently, the precise interplay of these neurotransmitters in the initiation of puberty remains to be established. To address the hypothesis that puberty requires the activation of excitatory inputs and the concomitant release of hypothalamic restraint, we measured changes in gene expression of these stimulatory and inhibitory neurotransmitters in the mouse ARC. To bypass the gonadal steroid suppression of Kiss1, NKB and Dyn reported in adult mice, we used hypogonadal *hpg* mice, deficient in GnRH, to prevent the pubertal activation of downstream reproductive hormones. Isolated ARC tissues from *hpg* mice and wild type (WT) littermates (postnatal day (P)10 to P60), were processed for quantitative RT-PCR. Kiss1 expression increased early (P12), prior to the onset of puberty, but only in *hpg* mice. Kiss1 levels in WT remained constant; indicating that sex steroid negative feedback effectively masks the Kiss1 increases. Significant effects on Kiss1 expression were influenced by genotype and age ($P<0.05$, Two-way ANOVA, n=5). In contrast, NKB expression revealed a linear, age-dependent increase in *both* WT and *hpg* mice, beginning by P12 in males and P15 in females, demonstrating a discrete developmental pattern from Kiss1. These results indicated that gonadal status does not affect NKB expression during pubertal onset, and NKB expression was influenced only by age ($P<0.05$, Two-way ANOVA, n=5). These findings further suggest that NKB expression is regulated by a distinct negative feedback mechanism from that regulating Kiss1 expression. Since inhibition of NKB expression by estradiol has been shown in adults, the sensitivity of NKB expression to negative feedback regulation is predicted to develop at ages older than that of Kiss1, a possibility that remains to be tested. Dyn expression in the ARC remained unchanged across development and displayed no differences between WT and *hpg* males or females. **These findings establish early increases in ARC NKB expression as a sensitive marker of pubertal activation and implicate NKB as a key developmental regulator in the initiation of reproductive function.**

Nothing to Disclose: JCG, RSC, UBK

OR10-6

Effects of Central Fatty Acids on Neuroendocrine and Endocrine Control of Reproduction.

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It is well documented that the reproductive axis is closely linked to nutritional status. Obesity, which has become a major public health problem, is involved in various reproductive disorders including anovulation or pregnancy complications. However, the underlying mechanisms at the hypothalamic and pituitary levels are still not clearly understood. There is now growing evidence that fatty acids (FA) can act directly on the central nervous system and contribute as cellular messengers to the control of energy homeostasis. In this study, we thus evaluated the possible incidence of central FA excess on the neuroendocrine and endocrine control of reproduction. Rats were infused for 24h with a fat emulsion (Intralipid®, 20%) through carotid artery towards the brain, a procedure which does not lead to any plasma FA or triglycerides elevation. The expression of various key genes of gonadotrope function was then analyzed by real-time PCR in hypothalamus as well as in pituitary and circulating gonadotropin concentrations determined. We demonstrated that central administration of FA decreased LH and FSH concentrations and down-regulated the expression of several pituitary genes including the GnRH receptor, LHβ as well as the NO synthase type 1 genes. In contrast, the FA receptors, GPR120 and GPR119 that we identified in gonadotrope cells, were up-regulated by FA treatment. In the hypothalamus of FA perfused rats, a 50% decrease of GnRH transcript levels was observed while expression of Kiss1 and GPR54 genes was unaffected. Complementary in vitro experiments were performed on primary rat anterior pituitary cells and on the gonadotrope cell line LβT2 to evaluate direct effects of FA on gonadotrope cell activity. We showed that a short term treatment of either cell type with different long-chain unsaturated FA dose-dependently enhanced basal LH and FSH release with a maximum (~2 fold) at 200 µM FA whereas the same treatment dose-dependently reduced GnRH-induced gonadotropin release. In contrast, the saturated palmitic acid was uneffective on basal or GnRH-induced release. Neither fatty acid treatment affected gonadotrope cells viability as assessed by MTT assay. Altogether, these results demonstrate that hypothalamic GnRH neurons and gonadotrope cells are direct targets of FA which may thus interfere with the regulation of reproductive function.

Nothing to Disclose: GG, VS, CC-G, SM, CD, CM, RC, JC-T

OR11-1

KIT: An Unrecognized Master Regulator of Skeletal Remodeling.

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Osteoclasts (OCs) are multifunctional cells; in addition to resorbing bone, OCs mobilize stem cells for host defense and hematopoiesis. Stem cells are anchored to osteoblasts (OBs) through *Kit* (a receptor tyrosine kinase) on stem cells, and membrane (m) *Kit*-ligand (KL) on OBs. OC-derived proteases cleave mKL to produce soluble (s) KL, thereby resulting in the release of stem cells. Unopposed, this pathway would lead to persistent bone loss. OC precursors and mature OCs also express *Kit*. The present studies suggest that *Kit*-signaling between OCs (and potentially hematopoietic cells expressing *Kit*) and OBs prevents bone loss during stem cell mobilization in part by coupling bone formation to bone resorption. Mice with a loss of function mutation in *Kit* receptor ($Kit^{W/Wv}$) had multiple skeletal abnormalities including self limiting osteopetrosis associated with greatly elevated OC number. Additionally, OB number and bone formation, initially normal, decreased with age as the mice experienced premature age-related bone loss. Compared to wild type, $Kit^{W/Wv}$ mice had increased RANK-ligand expression. However, many of the OCs in $Kit^{W/Wv}$ mice were detached from bone surfaces, suggesting *Kit* is important for OC activity and adhesion to bone surfaces. $Kit^{Sl/Sl\text{-}d}$ mice were studied to determine the respective roles of sKL and mKL in mediating *Kit* signaling. $Kit^{Sl/Sl\text{-}d}$ mice are unable to produce mKL and have a severely osteoporotic phenotype due to uncoupled bone turnover. The inability of $Kit^{Sl/Sl\text{-}d}$ mice to produce mKL increased OC and reduced OB numbers, both *in vivo* and *in vitro*. In contrast to $Kit^{W/Wv}$ mice, the OCs in $Kit^{Sl/Sl\text{-}d}$ mice appeared normal. Administration of sKL worsened the skeletal phenotype of $Kit^{Sl/Sl\text{-}d}$ mice by inducing a further increase in OC number. These findings suggest that mKL suppresses OC and enhances OB differentiation, whereas sKL mediates OC function. This conclusion is further supported by studies in rats demonstrating that continuous infusion of PTH decreases mKL gene expression in bone and increases OC number, a response mimicked in rats by administration of the *Kit* receptor antagonist gleevec. Taken together, these findings suggest that *Kit* plays a previously unsuspected, central role in coupling of bone formation to bone resorption during bone turnover.

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Nothing to Disclose: RTT

OR11-2

IAP Association with SHPS-1 Regulates Osteoclast Fusion.

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The association between extracellular domains of integrin associated protein (IAP) and the transmembrane protein SHPS-1 regulate osteoclast formation however the mechanism is unknown. Bone marrow cells (BMC) were isolated from 12-week old male C57/B6 mice (wild-type) and IAP knock-out mice (IAP-/-). Osteoclast (OC) formation was stimulated by the addition of RANK-L (50ng/ml) and mCSF (25ng/ml). TRAP positive cells with three or more nuclei were counted as OC. Parallel cultures of cells were lysed proteins visualized by immunoblotting. OC formation was reduced by 88 ± 5 % (mean ± SD, n =3) in the IAP -/- BMC compared with wild-type.There was a significant, 80 ± 9 % (mean ± SD, n =3) decrease in bone resorption by the RANKL stimulated IAP -/- BMC compared with wild-type. To determine whether IAP required association with its binding partner SHPS-1, we determined the effect of adding the anti-IAP antibody B6H12 which disrupts the association between IAP and SHPS-1. The addition of anti IAP antibody resulted in a 1.3 fold decrease in OC formation in BMC from wild-type mice. Interestingly when we counted all TRAP positive cells irrespective of whether they had greater than three nuclei the difference between the BMC IAP -/- and +/+ cultures was negated. When we examined markers of osteoclast maturation including the beta 3 integrin and the calcitonin receptor there was no significant difference between the two cultures. This suggested the possibility that IAP and its association with SHPS-1 may play a more significant role in the cellular fusion rather than the differentiation process. IAP association with SHPS-1 is required for SHPS-1 phosphorylation. By examining RANKL stimulated BMC lysates we determined that SHPS-1 phosphorylation was impaired in CD47-/- BMC compared with wild-type BMC. Phosphorylation of the tyrosine residues in the cytoplasmic domain of SHPS-1 is required for the recruitment and activation of the tyrosine phosphatase SHP-1. The recruitment and activation of SHP-1 is required for the dephosphorylation of non-muscle myosin IIA implicated as a regulator of osteoclast fusion. The lack of SHPS-1 phosphorylation and SHP-1 recruitment were associated with an inhibition of non-muscle myosin IIA (MHY-9\\) deposphorylation in IAP -/- BMC. Our data suggests that the association of IAP with SHPS-1 is required for the final step of osteoclast maturation as the differentiated cells fuse to form a mature cell with optimal bone resorptive capacity.

Nothing to Disclose: LAM, CW, CJR, DRC

OR11-3

Primary Hyperparathyroidism (PHPT) in Mice Due to Conditional Deletion of the Extracellular Ca^{2+}-Sensing Receptor (CaSR) Gene in the Parathyroid Glands (PTGs) Produces Gender-Specific Skeletal Effects.

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PTH hyper-secretion results from mutations in several genes, including the CaSR. Animal models that permit the study of PHPT during development have been difficult to achieve. We generated mice with heterozygous knockout (Het-KO) of the CaSR gene in their PTGs and examined the phenotype of these mice over time. The expression of CaSR protein was reduced by ~75% in PTGs of Het-KO mice as determined by immunoblotting. At 2 weeks of age, serum [Ca^{2+}] and PTH levels were elevated in Het-KO (12.7±0.1 mg/dl, 170±27 pg/ml, respectively) vs wild-type (WT) (11.6 ± 0.3 mg/dl, 53±2 pg/ml, respectively) mice. Urine [Ca^{2+}] was increased by ~60% in Het-KO mice, reflecting enhanced Ca^{2+} excretion likely mediated by intact renal CaSRs in the mice. Despite early onset PHPT, skeletal mass in Het-KO mice was comparable to WT through 8 weeks of age by micro-computed tomography (μCT). We, however, observed gender-specific changes in skeletal mass by both μCT and bone histomorphometry at 12 weeks of age. The ratio of trabecular bone volume over tissue volume (Tb.BV/Tb.TV) and trabecular number (Tb.N) were increased by ~40 and 15%, respectively, in male Het-KO vs WT mice. These changes persisted to 6 months of age, supporting an anabolic effect of PHPT on adult male bone remodeling. In contrast, Tb.BV/Tb.TV and Tb.N were decreased by ~30 and 15%, respectively, in 12-week-old female Het-KO vs WT mice, indicating catabolic actions of PHPT on the adult female skeleton. Along with these skeletal changes, serum PTH in female Het mice (8 and 12 weeks old) were 2-3 fold higher than those in male Het-KO mice, suggesting that the degree of PHPT may be a determinant of the gender-differences in the skeletal actions of PTH at these ages. We studied PTH secretion in PTGs isolated from Het-KO and WT mice to determine the Ca^{2+}-setpoints ([Ca^{2+}] required to achieve 50% inhibition of PTH secretion) in PTGs from male and female mice. In both genders, we observed a similar right-shifted Ca^{2+}-setpoint from ~1.2 mM in WT to ~1.5 mM in Het-KO mice (N=10-15 mice/group) but no significant gender-differences in the sensitivity of PTGs to high Ca^{2+}. We conclude that PHPT in this model exerts gender-specific effects on bone potentially due to differences in the steady-state levels of PTH secretion and other factors such as sex hormones acting on bone cells directly.

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Nothing to Disclose: ZC, T-HC, NL, CD, VP, DA, C-LT, DS, WC

OR11-4

Hypophosphatasia (HPP): Enzyme Replacement Therapy (EzRT) for Children Using Bone-Targeted, Tissue Nonspecific Alkaline Phosphatase.

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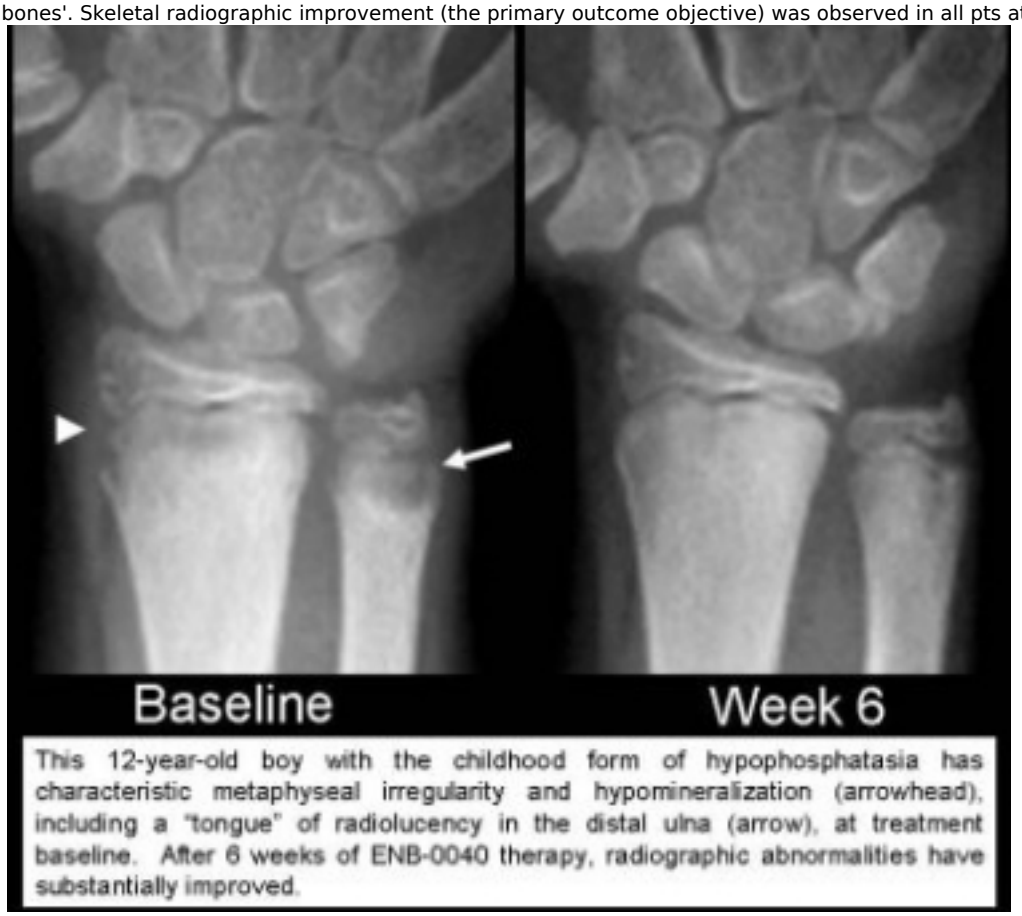
HPP is the inborn-error-of-metabolism characterized by low serum alkaline phosphatase (ALP) activity and caused by deactivating mutation(s) within the gene that encodes the “tissue nonspecific” isoenzyme of ALP (TNSALP). Natural substrates for TNSALP accumulate endogenously and include inorganic pyrophosphate (PPi), an inhibitor of mineralization, and pyridoxal 5′-phosphate (PLP), the principal form of vitamin B_6. Rickets and osteomalacia occur as PPi blocks hydroxyapatite crystal growth within the skeletal matrix. Deranged PLP metabolism reveals TNSALP to be an ectoenzyme. HPP severity spans stillbirth from profound skeletal hypomineralization to osteomalacia presenting late in adult life. There is no established medical treatment. ENB-0040 is a bone-targeted, human recombinant, TNSALP fusion protein that preserved skeletal mineralization and survival in a *tnsalp* knockout mouse model of severe HPP.(1)
Patient trials began in '08. In a 6-mo, open-label study of 6 pts (≤3 yrs) with life-threatening HPP, substantial skeletal remineralization, weaning from respiratory support, and improved motor development occurred with ENB-0040 (IV infusion of 2 mg/kg, followed by 1-3 mg/kg SC 3x/wk).(2)
Here, we report initial observations from a phase II, open-label, North American assessment of ENB-0040 in 13 HPP children, ages 5-12 yr (2 girls, 11 boys) randomized to receive either 2 or 3 mg/kg SC 3x/wk for 6 mo. To date, 8 pts have completed Wk 6, and 3 completed Wk 12. Transient injection site erythema was common, but well tolerated without drug-related SAEs. Wk 6 trough serum ALP activity was 2300-7600 IU/L and PLP corrected in 7 of 8 pts. Increases in circulating PTH from enhanced skeletal mineralization occurred, but without hypocalcemia from 'hungry bones'. Skeletal radiographic improvement (the primary outcome objective) was observed in all pts at Wk 6.



All reported increased strength, endurance, and mobility within a few weeks of EzRT. The 3 pts reassessed at Wk 12 walked 50-110 meters further in a 6-minute walk test (+ 11-29%).
Bone-targeted ENB-0040 is a promising treatment for the childhood form of HPP.

1) Millán JL et al., J Bone Miner Res 2008; 23:777-87
2) In manuscript

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Nothing to Disclose: WHM, KEM, LB

OR11-5

Roles of O-Glycosylation and Furin in Cyclic AMP-Mediated Processing of FGF23.

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Background: FGF23 is a phosphate and vitamin D-regulating hormone that circulates in both active intact (iFGF23) and inactive C-terminal (cFGF23) forms. FGF23 levels are elevated in several hypophosphatemic ricketic disorders including fibrous dysplasia of bone (FD), which is caused by activating mutations in the cAMP-regulating protein, $G_s\alpha$. While total blood FGF23 levels are high in FD, frank hypophosphatemic rickets is uncommon. We postulated this apparent discrepancy may be because of higher levels of cFGF23 due to cAMP-mediated altered processing by O-glycosylation enzyme ppGalNAcT3 and the protein convertase furin, the enzymes believed to be responsible for FGF23 processing.
Methods: iFGF23 and cFGF23 levels were assessed by ELISA and Western blot in FD patients and controls. The effect of cAMP on FGF23 mRNA and protein, and the effect on ppGalNAcT3 and furin enzymatic activity was assessed in primary cultures of bone marrow stromal cells (BMSCs) from FD patients and controls as wells as HEKF cells (a clonal HEK-293 cell-line stably overexpressing human FGF23). HEKF Cells were treated with dibutylryl cAMP or forskolin. These cells were also transiently transfected with wild-type or Gs alpha activating mutations, R201H or R201C. Conditioned media were examined for FGF23 processing. FGF23, ppGalNAcT3 and furin interaction were demonstrated by confocal microscopy and co-immunoprecipitation. Glycosylation patterns were assessed after cAMP treatment. The role of furin in FGF23 processing was demonstrated by examining cells deficient in furin activity after transfection with FGF23.
Results: Patients with FD had elevated levels of cFGF23 compared to disease controls. cAMP treatment or transfection with mutated Gsα resulted in elevated levels of cFGF23, and a concomitant decrease in ppGalNAcT3 and increase in furin enzymatic activity. FGF23, ppGalNAcT3, and furin co-localized in a Golgi-associated pattern as assessed by co-immunoprecipitation and confocal microscopy. cAMP treatment resulted in an altered glycosylation pattern in HEKF cells. Furin deficient cells showed no FGF23 processing.
Conclusions: These data offer several confirmatory and novel lines of evidence demonstrating the roles of ppGalNAcT3 and furin in FGF23 processing, and elucidate the cAMP-mediated mechanism of altered iFGF23 and cFGF23 levels in certain disease states, thus identifying a novel mechanism for the regulation of biological levels of active FGF23.

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Nothing to Disclose: NB, MW, HVP, PA, BC, TB, RIG, MTC

OR11-6

Vitamin D Deficiency in Mice Impairs Colonic Antibacterial Activity and Predisposes to Colitis.

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In recent years it has become clear that vitamin D-insufficiency is a global health issue. Although classically associated with rickets, low vitamin D levels have also been linked to aberrant immune function and associated health problems such as inflammatory bowel disease (IBD). To test the hypothesis that impaired vitamin D status predisposes to IBD, 8 week old C57BL/6 mice were raised from weaning on vitamin D-deficient or -sufficient diets, and then treated with dextran sodium sulphate (DSS) to induce colitis. Vitamin D-deficient mice showed decreased serum levels of precursor 25-hydroxyvitamin D_3 ($25OHD_3$) (2.5 ± 0.1 ng/ml vs 24.4 ± 1.8 ng/ml) and active 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) (28.8 ± 3.1 pg/ml vs 45.6 ± 4.2 pg/ml). Following treatment with DSS vitamin D-deficient mice showed greater weight loss (9% vs 5%), increased clinical evidence of IBD (0.78 ± 0.07 vs 0.56 ± 0.07) and histological evidence of colitis (4.71 ± 0.85 vs 1.57 ± 0.18) compared to vitamin D-sufficient mice. Vitamin D-deficient mice exposed to DSS also exhibited splenomegaly relative to mice on vitamin D-sufficient chow. FACS analyses of lymphocytes from the spleen indicated changes in T- and B-cells that were associated with vitamin D-deficiency even in the absence of DSS-induced colitis. To determine whether vitamin D-deficiency also affected gastrointestinal immune responses prior to inflammatory disease, DNA array analysis was carried out using of colon tissue from n = 4 vitamin D-deficient and –sufficient mice. 27 genes were consistently ($p<0.05$) up- or down-regulated >2-fold in vitamin D-deficient versus sufficient mice, in the absence of DSS-induced colitis. This included angiogenin-4 (Ang4), an antimicrobial protein involved in host containment of enteric bacteria. Immunohistochemistry confirmed that colonic Ang4 protein was significantly decreased in vitamin D-deficient mice even in the absence of colitis. Moreover, PCR analysis of 16S rDNA from the same animals showed elevated levels (50-fold) of bacteria in colonic tissue. These data show for the first time that simple vitamin D deficiency predisposes mice to colitis via dysregulated colonic antimicrobial activity and impaired homeostasis of enteric bacteria. This may be a pivotal mechanism linking vitamin D status with IBD in humans.

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Nothing to Disclose: VL, AVM, NQL, TSL, RFC, YO, SMM, JSA, MH

OR12-1

Overexpression of Cuzd1, a Novel Oncogene That Regulates the Epidermal Growth Factor Signaling Pathway, Leads to Breast Tumorigenesis.

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We have discovered a role for Cuzd1 (CUB and zona pellucida-like domain-containing protein 1) gene in mouse mammary gland development. The Cuzd1 null mice exhibit a severe impairment in ductal side branching and alveolar development during pregnancy and lactation due to a defect in mammary epithelial proliferation. Gene expression profiling of primary mammary epithelium revealed that Cuzd1 controls the production of several growth factors that act via the epidermal growth factor receptor (EGFR). Since the EGFR-driven intracellular signaling contributes significantly to breast tumor growth and progression, we analyzed Cuzd1 expression in human primary breast tumors. We observed significantly elevated levels of Cuzd1 mRNA and protein in ~60% of the tumors compared to normal breast tissues and found that this expression is correlated with EGFR expression.
To further assess the role of Cuzd1 in breast tumorigenesis, we overexpressed this gene in HC11, a non-transformed mouse mammary epithelial cell line. Transduction of lentivirus harboring this gene created HC11-Cuzd1 cells that express high levels of Cuzd1 mRNA and protein. HC11 cells transduced with lentivirus expressing β-galactosidase (lacZ) served as control. When stimulated with serum, the HC11-Cuzd1 cells displayed enhanced rate of proliferation compared to the HC11-lacZ cells. Gene expression profiling of HC11-Cuzd1 cells showed elevated expression of two key growth factors, EGF and betacellulin, confirming a critical functional link between Cuzd1 and the EGFR pathway. Remarkably, the HC11-Cuzd1 cells also exhibited characteristics of transformed cells, such as the ability to form colonies on soft agar, and invasive behavior in Boyden chamber assays. To further assess the oncogenic potential of Cuzd1, the HC11-Cuzd1 or HC11-lacZ cells were xenografted into the mammary glands of adult nude mice. Palpable tumors were observed 2 weeks post injection in 100% of mice that received HC11-Cuzd1 but not in mice injected with HC11-lacZ. The tumors continued to grow and reached sizes between 15-30 mm2 in area by 9 weeks post injection. Further characterization of the nature of these tumors and their metastatic potential is underway.
In summary, our study has uncovered a novel oncogene Cuzd1 that controls mammary epithelial proliferation by regulating the EGFR signaling pathway. Our results strongly suggest that overexpression of Cuzd1 is associated with the genesis and progression of breast cancer.

Nothing to Disclose: LA, QL, AK, ICB, MKB

OR12-2

Progesterone Alters Tumor Cell Composition, Increases Tumor Cell Proliferation and Survival, and Promotes Invasive Behavior in Carcinogen-Induced Mammary Cancer in the Rat.

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Progesterone (P) increases breast cancer risk in postmenopausal women receiving combined estrogen plus progestin hormone replacement therapy, however, the underlying molecular mechanisms are not known. We have investigated the role of P in tumor development and behavior in carcinogen-induced hormone-dependent mammary cancer in the rat, a model for hormone-dependent human breast cancer. Fifty-day-old rats were ovariectomized and treated with exogenous hormones, 17-β-estradiol (E) alone (2.5 mg/silastic implant) or with a combination of E (2.5 mg) plus P (200 mg). To induce mammary cancer, animals were treated with 7,12-dimethylbenz-[α]anthracene (50 mg/kg) intragastrically. We found no substantial differences in the dynamics of tumor development, tumor size, or tumor multiplicity between different hormonal regimens. However, immunohistochemical analysis revealed that tumors grown in presence of E alone had an exclusively luminal phenotype (keratin 18 (K18) and K19 positive), while tumors grown in the presence of E+P exhibited a dramatic expansion of basal/myoepithelial cells, expressing K5 and smooth muscle actin. Comparison of E vs. E+P showed that E+P increased proliferation of both luminal and myoepithelial cell populations, increased PCNA levels and caused redistribution of cell cycle inhibitor p27 from the nucleus to the cytoplasm. Analysis of factors regulating cell survival showed that compared to E alone, E+P treatment decreased mRNA expression of the pro-apototic gene Bad, increased expression of anti-apoptotic Bcl-2 and Bcl-Xl proteins, and increased phosphorylation of Akt, a major pro-survival kinase. Analysis of tumor behavior showed that E+P-treated tumors exhibited loss of epithelial markers K18, K19, and E-cadherin in luminal cells and increased expression of mesenchymal marker Zeb1, features indicative of invasive behavior. Consistent with this, histopathological analysis showed that tumors grown in presence of E+P frequently exhibited invasion into surrounding stroma. These results demonstrate that compared to E alone, E+P may promote development of breast cancer by increasing proliferation rate, increasing tumor cell survival, and also promotes invasive behavior of tumors. The strong stimulatory effect of P on the basal/myoepithelial cell population suggests that P may also contribute to the development of a basal cell–like phenotype of breast cancer.

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Nothing to Disclose: AK, ANK, KNM, SZH

OR12-3

Cell-Specific Targeting of RANKL to the Murine Mammary Gland Induces Branching Morphogenesis and Alveologenesis in the Absence of Progesterone Receptor Expression.

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Receptor of Activated NF-kB Ligand (RANKL) is implicated to comprise one of an increasing number of parallel and perhaps interconnected signaling cascades which execute proliferative and pro-survival programs obligate for progesterone-induced mammary morphogenesis in the early pregnant mouse [(1)]. Notwithstanding the growing support for RANKL as a paracrine effector of mammary progesterone action, the extent to which RANKL can functionally contribute to these morphogenetic events in the absence of other progesterone signaling cues is still unclear. To address this issue, a conditional bigenic system was developed for the doxycycline-inducible expression of RANKL in the mammary epithelium of the progesterone receptor knockout (PRKO) mouse [(2)], an animal model which is devoid of PR expression. With this system, the reverse tetracycline transactivator (rtTA) is specifically expressed under the control of the endogenous PR promoter in the PRKO mouse to induce transgene-derived RANKL expression in the presence of doxycycline. In response to acute doxycycline exposure, mammary RANKL induction in the PRKO was shown to be spatially restricted to the estrogen receptor (ER) positive/PR negative (ER+/PR-) cell type which is a subset of cells that normally express RANKL (and PR) in the mammary epithelium of the wild type (WT) mouse during pregnancy. Remarkably, cell type specific expression of RANKL resulted in spatially ordered mammary ductal side-branching and alveologenesis in the PRKO mouse, morphological changes that normally occur during early to mid-pregnancy. Importantly, these morphological responses contrast with the disorganized branching and extensive hyperplasia that result from indiscriminate targeting of RANKL to the mammary epithelium of the MMTV-RANKL transgenic [(3)]. At the molecular level, RANKL induced mammary epithelial proliferation in the PRKO was shown to be driven in part by the upregulation of cyclin D1 as well as a replication licensing factor both of which have been shown to be induced by progesterone in the mammary epithelial cell [(4, 5)]. Therefore, our findings support the proposal that RANKL signaling represents a critical mediator of the normal mammary epithelial response to progesterone exposure and that tight spatiotemporal control of this effector pathway is required to avoid unwarranted hormone-induced mammary epithelial expansion that could lead to mammary hyperplasia or neoplastic transformation in later life.

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Nothing to Disclose: AM, FJD, JPL

OR12-4

mTOR Inhibition Abrogates Insulin Mediated Mammary Tumor Progression in Type 2 Diabetic Mice.

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Type 2 diabetes is an emerging public health problem and has been linked to an increased breast cancer risk and mortality. Hyperinsulinemia, a major hallmark of type 2 diabetes, has been shown to stimulate mammary tumor growth through both the insulin and the IGF-I receptor. The mammalian target of rapamycin (mTOR) is activated by growth factors such as insulin and IGF-I and is a major regulator of mammary tumor progression. Pharmacological mTOR inhibition with rapamycin or its analogs has been shown to effectively reduce mammary tumor growth in non-diabetic animal models and is a promising new treatment strategy for human breast cancer. However, in individuals with type 2 diabetes, rapamycin treatment may aggravate metabolic disturbances, thereby reducing its potential as a breast cancer treatment. Thus, using an animal model of T2D, we aimed to explore the effect of pharmacological mTOR inhibition on mammary tumor progression in a diabetic milieu.
As a model of type 2 diabetes we employed the female MKR mice. These mice display severe insulin resistance and hyperinsulinemia, which leads to an accelerated mammary tumor progression. To induce mammary tumors, we either crossed MKR mice with MMTV-Polyoma Virus middle T (PyVmT) - transgenic mice or inoculated PyVmT- or Neu/ErbB2-expressing mammary tumor cells orthotopically. Chronic treatment with rapamycin (0.5mg/kg body weight/d) led to a worsening of the diabetic state in MKR mice and to the development of insulin resistance in non-diabetic wild type (WT) mice. Furthermore, the treatment blocked the mTOR pathway in mammary and tumor tissue to the same extent in MKR and WT mice. Most importantly, rapamycin treatment was able to fully abrogate type 2 diabetes-mediated mammary tumor progression in all three cancer models.
Taken together, these data demonstrate that pharmacological blockade of the mTOR pathway is sufficient to abrogate mammary tumor progression in the setting of hyperinsulinemia. Thus, mTOR inhibitors may be an attractive therapeutic modality in breast cancer patients with type 2 diabetes; however, a possible worsening of the diabetic state may limit their clinical application.

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Nothing to Disclose: YCF, RN, AV, DL

OR12-5

Paxillin Regulates DHT- and EGF- Induced MAPK Signaling and Cell Proliferation in Prostate Cancer Cells.

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While transcriptional effects of androgens have been extensively studied, the mechanisms regulating non-genomic actions of androgens are poorly understood. We previously showed (1) in Xenopus oocytes that paxillin, a scaffolding molecule that integrates many intracellular signals, is essential for non-genomic testosterone-induced MAPK signaling and subsequent oocyte maturation. Here we examined non-genomic dihydrotestosterone (DHT)-mediated signaling in prostate cancer (PCa) cells, focusing on whether, as in oocytes, paxillin regulates androgen-induced MAPK signaling and downstream physiologic actions. DHT-induced ERK activation was significantly reduced by pharmacological inhibitors of the androgen receptor (AR), EGF receptor (EGFR), Src, and matrix metalloproteinases (MMPs), indicating that, in addition to modulating nuclear AR actions in LnCAP cells, DHT binds extra-nuclear ARs, resulting in MMP-mediated release of membrane-bound EGFR ligands, which in turn activate the EGFR and subsequent MAPK signaling. siRNA-mediated knockdown of paxillin exerted select effects on intracellular signaling in several PCa cell lines, abrogating DHT- and EGF-induced activation of ERK and MEK, but not the EGFR or Akt. Paxillin ablation also prevented DHT- and EGF-induced proliferation in LnCAP and PC3 cells, respectively, as well as EGF-induced migration and invasion of PC3 cells. All of these processes were rescued by over-expression of constitutively activated MEK or Raf. These data suggest that paxillin functions downstream of the EGFR but upstream of Raf/MEK/ERK. DHT or EGF promoted Src-mediated tyrosine phosphorylation of paxillin (Y31, Y118), which was required for ERK activation and cellular proliferation. ERK in turn phosphorylated paxillin at serines 83,126 and 130, which was also necessary for proliferation. Thus, in PCa cells paxillin is necessary for ERK activation as well as for downstream functions of ERK. Surprisingly, paxillin knockdown, as well as MEK or EGFR inhibition, abrogated DHT-induced prostate specific antigen mRNA expression, indicating that extra-nuclear androgen signaling via EGFR/MAPK crosstalk impacts nuclear androgen signaling. Finally, paxillin expression was higher in human prostate cancer samples compared to adjacent normal prostate. In summary, paxillin is a critical regulator of androgen and/or EGF-induced MAPK signaling and cell growth in PCa, and may be a viable diagnostic or therapeutic target for cancer treatment.

(1) Rasar M et al., J Biol Chem 2006; 281(51):39455-64

Sources of Research Support: NIH Grant DK59913.

Nothing to Disclose: AS, KO, ZW, GVR, DBD, SRH

OR12-6

Studies on the Androgen Receptor Isoform AR-3 Using CTC (Circulating Tumor Cells) and HCA (High Content Analysis).

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The finding that a constitutively active truncated androgen receptors isoform (AR-3) is expressed in cell lines and tumors of men with castration-resistant prostate cancer (CRPC) represents a paradigm shift in the field, and provides a unifying mechanism to explain prostate cancer (PC) transition to castration resistance (CR). In preliminary work we have demonstrated the presence of large quantities of AR-3 in bone marrow (BM) biopsies and circulating tumor cells (CTC) obtained from patients with postchemotherapy stage 4 PC. When compared to prostate cancer cell lines with an androgen-dependent phenotype or to cell lines derived form benign prostatic epithelium, AR-3 was significantly overexpressed in all CTC tested. The elevated level of expression AR-3 correlated with elevated expression of transcripts known to be regulated in an AR-3 dependent way, such as the kinase Akt. In addition, we have demonstrated that a GFP-AR-3 fusion transiently transfected in HeLa or PC-3 cells is constitutively nuclear and transcriptionally active. In line with previous high content analysis (HCA) approaches, to better understand AR function in automated, image-based high throughput assays, we have quantified level, localization and transcriptional activity of GFP-AR-3. As expected, GFP-AR-3 is unaffected by exposure to androgen (DHT) or anti-androgen (bicalutamide); further, initial results with pyrvinium pamoate and harmol hydrochloride also indicate the truncated AR isoform is not antagonized. To better understand the molecular pathways controlling AR-3 function and to identify effectors of its activity, large scale RNAi kinome and nuclear receptor coregulator libraries, and small molecule collections are being screened by HCA.

Sources of Research Support: VA Merit Review; Department of Defense Prostate Cancer Research Program; NIDDK.

Nothing to Disclose: SNM, JYN, HYS, GS, TH, DM, MAM, MM

OR13-1

MicroRNA-155 Is a Novel Inhibitor of Adipogenesis, Mediating the Effects of Inflammatory Cytokines.

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Inflammatory factors attenuate adipogenesis and fat storage and promote lipolysis to increase circulating free fatty acid levels, which can lead to ectopic fat deposition and tissue injury. However, the mechanisms responsible for these adipose tissue effects are still unclear. We used a microRNA (miR) array approach to profile miRs regulated during 3T3-L1 adipogenesis, and found that miR-155 expression was decreased 5-fold in mature adipocytes compared to preadipocytes. We subsequently determined that miR-155 overexpression strongly attenuated 3T3-L1 adipogenesis by inhibiting transcription and translation of C/EBPβ, a key regulator of adipogenesis, and hypothesized that miR-155 could mediate the adipose effects of inflammatory factors. Tumor necrosis factor α (TNFα), interleukin-1β (IL-1β) and interferon γ (IFNγ) had separate and additive effects to increase 3T3-L1 preadipocyte miR-155 expression, while miR-155 knockdown inhibited TNFα-induced attenuation of adipogenesis. Preadipocyte expression of miR-155 was increased in obese vs. lean mice in several mouse models of obesity (chow-fed db/db and Agouti mice and $LDLR^{-/-}$ mice fed high-fat diet), which corresponded with TNFα, IL-1β, and IFNγ expression. In order to examine the relevance of this finding to humans, we obtained visceral (V) and subcutaneous (SQ) adipose tissue from surgical samples of 14 patients (BMI 23-48 kg/m^2) to investigate the expression of miR-155 and inflammatory factors in V and SQ adipocytes and stromal vascular fraction (SVF, which contained preadipocytes) from obese (BMI>30) and non-obese subjects (BMI<30). Obese subjects expressed more monocyte chemoattractant protein 1 (MCP-1) and TNFα, but no miR-155 in V and SQ adipocytes; and more MCP-1, TNFα, IL-1β, and miR-155 in V and SQ SVF, despite revealing no difference in CD68 expression, suggesting that differences in SVF macrophage content did not mediate these differences in inflammation. V SVF miR-155 expression highly correlated with TNFα (Pearson r=0.90, p<0.0001) and IL-1β (r=0.84, p=0.0002). These data suggest miR-155 is a novel effector of cytokine action in fat and may play an important role in inhibiting adipogenesis and fat storage in both human V and SQ adipose.

Nothing to Disclose: TD, CJL, PR, LP, DH, WAH

OR13-2

Global Analysis of Forkhead Proteins Reveals a Critical Role of FOXA3 in Adipogenesis.

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Understanding how fat cells develop and are maintained is becoming increasingly important because of the rising incidence of obesity and its associated disorder, type II diabetes. The process that leads to the conversion of mesenchymal stem cells into terminally differentiated adipocytes consists of sequential steps of transcriptional activation. While it has been clearly demonstrated that the late phases of adipocytes maturation are governed by the transcriptional regulators PPARγ and C/EBPα, the identity of the critical factors controlling early stages of differentiation are still unknown. Previous studies have shown that the master adipogenic transcription factors are under the control of many other transcriptional regulators including members of the Fork-head (Fox) family but no detailed analysis on how this entire class of transcription factors influences adipogenesis exists.
To identify novel transcriptional regulators involved in these early events, we performed a global analysis of the function of Fork-head proteins via siRNA screen and determined the individual contribution of each Fox molecule in adipogenesis. We show here that only loss of function of Foxa3 severely impairs adipogenesis in mesenchymal stem cells in vitro. Gain of function of Foxa3 leads to increased adipogenesis as manifested by increased lipid accumulation and by the induction of the molecular markers PPARγ, aP2, C/EBP and adipokines. Furthermore, in our analysis we revealed that the mechanisms of action of Foxa3 involve the binding of Foxa3 to the proximal region of the PPARγ promoter through a novel, previously unidentified Foxa responsive element and the transcriptional cooperation between Foxa3 and C/EBPβ and C/EBPδ in PPARγ promoter activation.
These data represent the first demonstration of the critical positive role of a Fork-head factor in adipogenesis and establish Foxa3 as a positive regulator of PPARγ and its targets.

Nothing to Disclose: VP, LH, EM

OR13-3

Adipose Specific Ablation of *Hif1b/Arnt* and the Role of Hypoxia in Obesity and Adipose Vascular Function.

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Obesity is a worldwide problem driving much of the epidemic of type 2 diabetes. Vascularization is an important component of expansion of adipose tissue. In obesity, adipocytes distant from vessels become hypoxic, and this may be an important contributor to adipose dysfunction leading to insulin resistance and diabetes. Numerous hypoxia-inducible transcription factors (*Hif1α*, *Hif2α*, *Hif3α*) are expressed in adipose tissue. *Hif1β* (also known as *Arnt*) functions as an obligate heterodimeric partner that is necessary for the action of all Hif proteins. To interrogate the role of hypoxia in adipocyte biology, we created a mouse lacking *Hif1β* in adipose tissue (F-*Hif1β*$^{-/-}$ mice) by breeding *aP2*-Cre transgenic and *Hif1β* floxed mice. After 10 months of chow diet, these mice exhibited at 55% reduction in fat mass, which was due to reduced adipocyte cell size. *Hif1β*$^{-/-}$ mice were also protected from high fat diet-induced obesity with a 48% reduction in fat mass compared to controls. This resulted in protection from obesity-related glucose intolerance ($p<0.001$).

Adipocytes isolated from F-*Hif1β*$^{-/-}$ mice had an 87% decrease in basal and 70% decrease in insulin-stimulated glucose uptake ($p<0.005$). This was secondary to parallel decreases in the expression of glucose transporters *Glut1* and *Glut4*.

Vascular permeability, assessed by Evans Blue extravasation, was decreased by 45% in F-*Hif1β*$^{-/-}$ fat pads, corresponding to a 42% decrease in levels of vascular endothelial growth factor (*Vegf*). Reduction of *Hif1β* in 3T3-L1 cell lines *in vitro* by shRNA-mediated stable knockdown produced similar effects with decreases in basal and insulin-stimulated glucose uptake, decreases *Glut1* and *Glut4* and decreases in *Vegf* expression. sh*Hif1β* knockdown also led to alterations in response to the hypoxia mimic $CoCl_2$. Thus, treatment of control 3T3-L1 adipocytes with $CoCl_2$ led to a 67% reduction in respiratory capacity and this effect was lost in sh*Hif1β* knockdown cells leading to a 2.9-fold increase in respiratory capacity as compared to controls. This was associated with 2-fold increases in cytochrome c1 and cyclooxygenase-4.2 expression. Thus, *Hif1β* plays a pivotal role in adipose tissue function by controlling cell autonomous processes, including glucose uptake, expression of *Vegf*, and regulation of the hypoxic response, and cell non-autonomous processes, such as vascular permeability. Together, these effects control accumulation of adipose mass, and provide a novel target for obesity therapy.

Nothing to Disclose: KYL, SG, JB, XW, FJG, CRK

OR13-4

Genetic Link of Obesity to Adipogenic Collagen Turnover in Mice and Humans.

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OBJECTIVE
In white adipose tissue, adipocytes and adipocyte precursor cells are enmeshed in a dense network of type I collagen fibrils. The fate of this pericellular collagenous web in diet-induced obesity, however, has been unknown. This study aims at identifying the genetic underpinnings of proteolytic collagen turnover linked to obesity progression in mice and humans.
METHODS
The cleavage and degradation of type I collagen at the early stage of high-fat diet feeding was assessed in wild-type or *MMP14 (MT1-MMP)*-haploinsufficient mice using immunofluorescent staining and scanning electron microscopy. The impact of *MMP14*-dependent collagenolysis on adipose tissue function was determined with cDNA microarray. The genetic association of *MMP14* gene polymorphisms (SNPs) with obesity or diabetes traits was examined in a healthy Japanese cohort (n=3,653) using logistic regression analysis and ANCOVA.
RESULTS
In adult mice, type I collagen fibers are cleaved rapidly *in situ* during a high-fat diet challenge (three-fold increase compared to low-fat diet, n=8, $P<0.01$). By contrast, in *MMP14* haploinsufficient mice, animals placed on a high-fat diet are unable to remodel fat pad collagen architecture and display blunted weight gain (reduced by 50%, n=8, $P<0.01$). Moreover, the transcriptional programs of lipogenesis and cholesterol synthesis are disrupted by the lack of collagen turnover (GO analysis: $P<0.01$, respectively). Consistent with a key role played by *MMP14* in regulating high-fat diet-induced metabolic programs, human *MMP14* gene polymorphisms located in proximity of the enzyme's catalytic domain are closely associated with human BMI and fating blood sugar level ($P=0.0017$ and 0.0069, respectively). Risk allele of *MMP14* genotype increases the population's BMI by 0.42 $kg \cdot m^{-2}$ (d=0.13).
CONCLUSIONS
Together, these findings demonstrate that *MMP14* gene, coding for a dominant pericellular collagenase, mediates adipogenic collagen turnover linking ECM remodeling to obesity in mice and humans.

Nothing to Disclose: THC, MI, KS-K, HM, IY, YM, TO, SJW

OR13-5

Treatment with a Soluble ActRIIB Prevents Obese Phenotype and Promotes Favorable Thermogenic Program in White Adipose Tissue in Mice Fed a High-Fat Diet.

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Obesity is a pervasive epidemic in developed countries, where it plays a major role in development of metabolic and cardiovascular diseases. Additionally, compelling research demonstrates that even moderate weight loss can provide significant protection from cardiovascular disease and type II diabetes. Therefore, alterations in body composition to increase lean mass and decrease adiposity may help reduce the risk of obesity-related cardiovascular and metabolic diseases. Growth factors, such as myostatin (GDF-8), that signal via activin receptor type IIB (ActRIIB) act as negative regulators of muscle mass and may also regulate adipose tissue mass. Previous work has shown that inhibition of ActRIIB signaling with a soluble ActRIIB increases muscle mass and decreases adiposity in normal mice. To determine if a soluble form of the ActRIIB can alleviate the adverse effects of diet-induced obesity, high fat diet (HFD)-fed mice were treated for 60 days with either vehicle (VEH) or ACE-435 (2x/wk, 10mg/kg SC), a soluble fusion protein comprised of an optimized form of the extracellular domain of ActRIIB fused to a human Fc. In vivo body composition imaging demonstrated that both VEH and ACE-435 treated mice gained lean as well as fat mass; however, ACE-435 resulted in greater lean tissue gain (10.4±2.0% (VEH) versus 49.8±2.5% (ACE-435)) and lower fat gain (312.1±22.6% (VEH) versus 196.6±26.2% (ACE-435)).Furthermore, ACE-435 prevented HFD-related changes in serum lipid and adipokine levels as well as reduced liver steatosis. ACE-435 also induced a brown fat-like thermogenic gene profile in epididymal white fat, defined by the upregulation of the thermogenic genes UCP-1 and PGC-1alpha by 62-fold and 3.5-fold, respectively ($P<0.05$). Immunohistochemistry for UCP-1 confirmed its localization to cells in the white fat depot. Furthermore, ACE-435 resulted in 2-fold upregulation of Sirt1 and Foxo1 ($P<0.05$) in epididymal white fat of mice fed a high-fat diet. The data suggest that ACE-435, in addition to increasing lean tissue, also increases the thermogenic potential of white fat to offset the effects of HFD-feeding. These findings demonstrate that treatment with ACE-435 can offset many of metabolic and hormonal changes associated with diet-induced obesity and may be beneficial for decreasing risk of metabolic and cardiovascular disorders.

Disclosures: AK: Employee, Acceleron Pharma. RB: Employee, Acceleron Pharma. MC-B: Employee, Acceleron Pharma. MD: Employee, Acceleron Pharma. DS: Employee, Acceleron Pharma. JL: Employee, Acceleron Pharma. RK: Vice President, Acceleron Pharma. KT: Employee, Acceleron Pharma. TB: Employee, Acceleron Pharma. BU: Employee, Acceleron Pharma. TM: Employee, Acceleron Pharma. AG: Employee, Acceleron Pharma. KL: Employee, Acceleron Pharma. KU: Employee, Acceleron Pharma. JU: Employee, Acceleron Pharma. BH: Employee, Acceleron Pharma. JB: Employee, Acceleron Pharma. MS: Employee, Acceleron Pharma. BMS: Advisory Group Member, Acceleron Pharma. JS: Chief Scientific Officer, Acceleron Pharma. JL: Employee, Acceleron Pharma.

OR13-6

Intranasal Delivery of Mouse [D-Leu-4]-OB3, a Synthetic Peptide Amide with Leptin-Like Activity, Improves Energy Balance, Glycemic Control, Insulin Sensitivity, and Bone Formation in Leptin-Resistant C57BLK/6-m *db/db* Mice.

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We have recently shown that intranasal administration of mouse [D-Leu-4]-OB3 reconstituted in Intravail® to male Swiss Webster mice resulted in significantly higher uptake and bioavailability when compared to commonly used injection methods of delivery. In the present study, we examined the effects of intranasal delivery of mouse [D-Leu-4]-OB3 in Intravail® on energy balance, glucose regulation, insulin secretion, and serum levels of osteocalcin, a sensitive and specific marker of bone formation. Genetically obese C57BLK/6-m *db/db* mice were allowed food and water *ad libitum,* and given either Intravail® alone or mouse [D-Leu-4]-OB3 in Intravail® for 14 days. Mouse [D-Leu-4]-OB3 reduced body weight gain, daily food intake, daily water intake, and serum glucose by 11.5%, 2.2%, 4.0%, and 61.9%, respectively. Serum insulin levels in *db/db* mice given mouse [D-Leu-4]-OB3 were approximately 3-fold lower than those in mice receiving Intravail® alone. Mouse [D-Leu-4]-OB3 elevated serum osteocalcin in *db/db* mice by 28.7% over Intravail® treated control mice. The results of our study indicate that intranasal delivery of biologically active mouse [D-Leu-4]-OB3 in Intravail® is feasible, and has significant effects on regulating body weight gain, food and water intake, serum glucose, insulin sensitivity, and bone formation. Moreover, these effects on energy balance and glycemic regulation further suggest that intranasal delivery of mouse [D-Leu-4]-OB3 may have potential application to the treatment of human obesity as well as type 2 diabetes mellitus. In adddition, its effects on bone turnover may also help to prevent and/or reverse at least some of the bone loss which accompanies osteoporosis, anorexia nervosa, cancer, and other wasting diseases not associated with the obesity syndrome.

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Nothing to Disclose: MAW, MCL, DWL, PG

OR14-1

Clinical Risk Factors for Malignancy in Pheochromocytomas and Paragangliomas.

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Background: Sympathetic pheochromocytomas/paragangliomas (PHEO/PGLs) originate in the autonomic nervous system paraganglia localized in the thoracic, abdominal, and pelvic cavities.Ten to 15% of PHEO/PGLs are malignant, most of which present with distant metastases at diagnosis. The 5-year overall survival rates range from 40-72%. The presence of germline *SDHB* mutations is the only clearly recognized risk factor for malignant disease. Information collected from small, retrospective series with limited follow-up has failed to identify histological, molecular, and/or clinical aspects that could help to predict malignancy in these neoplasms.
Methods: We identified and reviewed 371 cases (269 PHEO and 102 PGL) treated at MDACC between the years 1953-2009. The aim of the study was to determine if location and size of the tumors predict metastases. In all cases, diagnosis was confirmed pathologically and tumor location and size were verified by pathologic, surgical, and/or radiographic reports. Frequencies and percentages were reported for categorical variables.Comparisons by disease type were conducted for categorical variables using a chi-square test or Fisher's exact test.
Results: The mean age of diagnosis was 42 years (range 5-83 years) with median follow-up of 8.2 years for both groups (range 0-681 months). Thirty-five percent (131/371) of patients had metastases: 68/269 (25%) PHEOs and 63/102 (60%) PGLs (chi-square $p<0.001$). The odds of metastases was 4.8 times more likely for patients with PGL compared to patients with PHEOs. Fifty-five percent of patients had metachronous metastases and the remaining 45% had synchronous metastases. Further break-downs were as follows: 17/20 (85%) of retroperitoneal PGLs, 9/13 (69%) of mediastinum PGLs, 29/49 (59%) of Zuckerkandl organ derived PGLs and 4/9 (44%) of bladder PGLs had metastases. Median size of the metastatic PHEO/PGLs primary tumors was 8.2 cm (range 1-24 cm) vs. 4.9 cm (range 0.4-21 cm) (Wilcoxon rank sum p-value <0.001) in non-metastatic tumors.
Conclusions: Location not tumor size was more frequently associated with metastases.The incidence of metastases in sympathetic PGLs was greater than that seen in PHEOs. Within this group the risk of metastases was higher for retroperitoneal, Zuckerkandl, mediastinum, and bladder PGLs. Large variability in tumor size existed for both metastatic and non-metastatic tumors, which was demonstrated by the similarity in ranges of tumor sizes for both disease groups.

Nothing to Disclose: MA-R, MH, MAH, NLB, GC, TAR, SW, CJ

OR14-2

Functional Imaging of Head and Neck Paragangliomas: Comparison of ^{18}F-Fluorodihydroxyphenylalanine (^{18}F-FDOPA) Positron Emission Tomography (PET), ^{18}F-Fluorodopamine (^{18}F-FDA) PET/Computed Tomography (CT), ^{18}F-Fluoro-2-Deoxy-D-Glucose (^{18}F-FDG) PET/CT, ^{123}I- Metaiodobenzylguanidine (^{123}I-MIBG) Scintigraphy, and ^{111}In-Pentetreotide Scintigraphy.

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Accurate diagnosis of head and neck paragangliomas is often complicated by biochemical silence and a lack of catecholamine associated symptoms, making accurate anatomical and functional imaging techniques essential to the diagnostic process. The present study compares five different functional imaging techniques [^{18}F-fluorodihydroxyphenylalanine (^{18}F-FDOPA) positron emission tomography (PET), ^{18}F-fluorodopamine (^{18}F-FDA) PET/computed tomography (CT), ^{18}F-fluoro-2-deoxy-D-glucose (^{18}F-FDG) PET/CT, ^{123}I- metaiodobenzylguanidine (^{123}I-MIBG) scintigraphy, and ^{111}In-pentetreotide scintigraphy] in the localization of head and neck paragangliomas. Nine patients (3 with SDHB mutations, 5 with SDHD mutations, and 1 pending), with a total of 18 head and neck paragangliomas, were evaluated with anatomical and functional imaging. The results of the functional imaging scans indicated a sensitivity of 100% (18/18 lesions localized) for ^{18}F-FDOPA PET, 89% (16/18 lesions localized) for ^{18}F-FDG PET/CT, 65% (11/17 lesions localized) for ^{111}In-pentetreotide scintigraphy, 44% (8/18 lesions localized) for ^{18}F-FDA PET/CT, and 28% for ^{123}I-MIBG scintigraphy (5/18 lesions localized). Differences in imaging efficacy related to genetic phenotype, though speculative given the small sample size, included the negativity of ^{111}In-pentetreotide scintigraphy and ^{18}F-FDA PET/CT in all patients with SDHB mutations and the accuracy of ^{18}F-FDG PET/CT in all patients with SDHD mutations, as compared to accuracy of ^{18}F-FDG PET/CT in only 1 patient with SDHB defect. ^{18}F-FDOPA PET proved the most efficacious functional imaging modality in the localization of head and neck paragangliomas with a sensitivity of 100% and may be a potential first line functional imaging agent for the localization of these tumors.

Sources of Research Support: Intramural Research Program of the NICHD/NIH.

Nothing to Disclose: KSK, DKA, MAW, JCR, KTA, PX, HML, CAS, CCC, KP

OR14-3

^{131}I MIBG Therapy in Metastatic Pheochromocytoma.

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BACKGROUND:

^{131}I-Metaiodobenzylguanidine (^{131}I MIBG) can be used to treat malignant pheochromocytoma (PHEO) however the experience with its therapeutic use is limited. We report the institutional experience with ^{131}I MIBG in the treatment of malignant PHEO.

MATERIALS AND METHODS:

We performed a retrospective review of 11 patients with multifocal metastatic PHEO followed at a single institution. The patients were divided in two groups according to the type of treatment: Group 1: 5 patients treated with ^{131}I MIBG and Group 2: 6 patients who received other therapies (surgery [n=4], chemotherapy [n=1] and external radiation [n=1]). Symptomatic, biochemical and tumoral response, as well as survival rate were compared between the two groups (log-ranktest).

RESULTS:

No differences between the two groups were found regarding age, sex, size of tumor and histology. Of the 5 patients treated with ^{131}I MIBG, the median age was 38 years (range 11-64) and 3 were male. The mean single dose of ^{131}I MIBG (±SD) was 231.4 ± 27.3 mCi; and mean cumulative dose (±SD) was 324 ± 112.9 mCi. Partial tumoral response (≥ 50% reduction on CT and ^{131}I MIBG scans) was achieved in 40% of the patients and stable disease (a decrease < 50% or an increase < 25%) in 40%. Complete (normalization of catecholamine levels) and partial (>50% decrease in catecholamine levels) biochemical response were achieved by 60% and 20% of the patients, respectively and 100% of the patients presented symptomatic response. There was a non-significant trend for an increased overall survival among patients treated with ^{131} I MIBG (survival at 100 months 80% *versus* 20%; p=0,097). Progression-free survival almost reached statistical significance (percentage without progression at 100 months 80% *versus* 0%; p=0,052).

CONCLUSION:

Although the differences in the survival rate between the groups did not reach statistical significance, which was probably related to the scarce number of patients followed, this data suggests that symptomatic and biochemical responses can be reached with ^{131}I MIBG treatment for patients with metastatic PHEO and they are probably a predictor of increased survival.

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Nothing to Disclose: APS, MLCC-G, DSF, SPAT, MAAP

OR14-4

Central Obesity and the Metabolic Syndrome Are Associated with Portal and Not Systemic Hypercortisolism Supported by the Raise of Urinary Corticosteroid Metabolites.

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Introduction: The phenotypic similarities between Cushing syndrome, central obesity and the metabolic syndrome (metS), have led to propose that these patients could have splanchnic hypercortisolism by action of the enzyme 11 β-Hydroxysteroid dehydrogenase type 1 (11-βHSD1), which generates cortisol (F) from inactive cortisone (E) in visceral adipose tissue and liver. Cortisol is metabolized by the liver reductases and 3α-HSD to inactive tetrahydrometabolites (THM), which could avoid systemic hypercortisolism despite increased portal cortisol. Objectives: 1. To investigate the urinary excretion of cortisol metabolites and their correlation with anthropometric and biochemical parameters and to explore their association with F and E in plasma and urine. 2. To evaluate the urinary excretion of THM in patients with the presence or absence of metS by ATPIII criteria. Patients and Methods: We recruited 220 subjects [age: 52.5 ± 9.6 years, 76% female, BMI: 29.3 ± 4.4 kg/m2; metS 130/220 patients (59 %)]. We evaluated, in plasma: F, E, glucose and lipid profile; in urine (24 h): free F and E by HPLC-MS/MS and total F and E metabolites: THF, αTHF, THE, Cortol, βCortol, Cortolone, βCortolone by GC/MS. Results: There was a positive correlation between THM with weight ($r=0.44$, $p <0.001$), BMI ($r=0.28$, $p <0.01$), and waist circumference ($r= 0.38$, $p > 0.01$), not observed with F and E in plasma or urine. THM concentration was associated positively with glycemia ($r=0.37$, $p< 0.01$) and triglycerides ($r= 0.18$, $p = 0.06$), and negatively with HDL ($r = -0.29$, $p < 0.01$). Patients with MetS showed higher excretion of THF ($p = 0.01$),αTHF ($p = 0.01$), THE ($p <0.01$), Cortol ($p = 0.02$) and Cortolona ($p <0.001$) than patients without metS, with no differences in F and E in plasma or urine levels between both groups. Conclusions: Our results suggest that obesity and metS are associated with visceral and not systemic Cushing's syndrome. The progressive gain in visceral fat can generate increased production of cortisol by the action of 11β-HSD1, causing splanchnic and portal hypercortisolism that could have a key role in the pathogenesis of associated metabolic disorders. The hepatic metabolism of cortisol explains the similar phenotype of obesity with Cushing's syndrome but with normal plasma and urinary cortisol.

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Nothing to Disclose: RB, CC, CAC, OO, GG, GF, FP, JS, JC, BSM, JMD, LMM, GO, AMK, CF

OR14-5

Utilization of a Proopiomelanocortin ELISA in the Differential Diagnosis of ACTH-Dependent Cushing's Syndrome.

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Accurately distinguishing between pituitary and ectopic causes of ACTH-dependent Cushing's Syndrome (CS) remains a challenge even when the most reliable non-invasive tests are used. Not only are pituitary incidentalomas common but a significant number of the microadenomas that cause Cushing's are not detected by MRI. Therefore clinicians often must rely on inferior petrosal sinus sampling (IPSS) to distinguish between Cushing's disease (CD) and ectopic ACTH syndrome (EAS) even though IPSS is a specialized procedure that is not widely available. Thus identifying additional diagnostic tools that capitalize on tumor specific differences in the processing of the proopiomelanocortin (POMC) precursor from which ACTH derives could prove clinically useful. Abnormal POMC processing and accumulation of POMC precursor might be expected in EAS in contrast to CD with microadenomas. We therefore measured plasma POMC levels in peripheral blood samples from 23 patients with ACTH-dependent CS who presented to our center for IPSS and had either no pituitary lesion or a microadenoma on MRI. POMC precursor was measured using a specific monoclonal antibody based ELISA (1). 15/23 patients had CD; 8/23 had EAS. Mean POMC levels were higher in patients with EAS vs CD (67.4±14.5(SEM) vs 18.6±2.0 fmol/ml; $p<0.001$). All patients with a plasma POMC value greater than 36 fmol/ml had EAS (n=6), indicating that elevated POMC levels had a high positive predictive value for EAS. Mean ACTH levels were also higher in EAS (130±23.4 vs 43.3±6.1 pg/ml, $p<0.001$). However, in 3/8 EAS cases the elevated POMC level suggested an ectopic source despite only mildly elevated ACTH levels. For example, two young men with CS presented with almost identical urine free cortisol and ACTH levels (42 and 55 pg/ml) and negative pituitary MRIs. Their POMC levels were 22 fmol/ml and 132 fmol/ml respectively, and the latter was diagnosed with EAS by IPSS and found to have a bronchial carcinoid. In another case a patient with a clear microadenoma was found to have a POMC level of 107 fmol/ml and was diagnosed with EAS. While IPSS remains the gold-standard test in the differential diagnosis of ACTH-dependent CS, there is still considerable debate about who should undergo IPSS. Measurement of POMC in cases of ACTH-dependent CS with absent or small microadenomas may help clinicians determine which cases should be referred for IPSS and in cases of incidental microadenomas, may prevent unnecessary neurosurgery.

(1) SR Crosby, MF Stewart, JG Ratcliffe, A White. Direct Measurement of the Precursor of Adrenocorticotropin in Human Plasma by Two-Site Immunoradiometric Assay. J Clin Endocrinol Metab 67:1272, 1988.

Nothing to Disclose: GP-W, PUF, TPJ, AGK, JNB, AW, SLW

OR14-6

Medical Treatment of Cushing's Disease with Pasireotide Mono- or Combination Therapy with Cabergoline and Ketoconazole Modulates Somatostatin Receptor Subtype Expression on Corticotroph Tumor Cells.

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Background. The somatostatin receptor (sst) subtype pattern of corticotroph tumor cells is characterized by high sst_5 expression, whereas sst_2 expression is relatively low, presumably due to down-regulating effects of high cortisol levels in Cushing's disease (CD). Next to sst_5, dopamine receptor subtype 2 (D_2) is frequently expressed by corticotroph tumor cells. We performed a prospective study in patients with CD in which the efficacy was examined of pasireotide, a new somatostatin analog with high sst_5 affinity, which was stepwise extended with the D_2 agonist cabergoline and ketoconazole. In a patient subset who underwent pituitary surgery afterwards, corticotroph tumor tissue was examined for sst and D_2 expression patterns.
Methods. 17 CD patients were included and all started with pasireotide 100 mg sc. tid which was increased to 250 mg tid at day 15, according to urinary free cortisol excretion (UFC). At day 28, cabergoline (1.5 mg qod) was added to pasireotide if UFC remained elevated. If UFC had not normalized at day 60, ketoconazole was added (200 mg tid). After day 80, patients could participate in an extension study until pituitary surgery. Sst and D_2 mRNA expression patterns in corticotroph tumor tissue (n=5) were assessed by quantitative RT-PCR analysis using TaqMan Gold nuclease assay.
Results. Biochemical remission was achieved in 29 % of patients with pasireotide monotherapy, in an additional 24 % by addition of cabergoline to pasireotide and in another 35 % with pasireotide, cabergoline and ketoconazole. Corticotroph tumor tissue was obtained from patients with biochemical remission induced by pasireotide (n=1), pasireotide-cabergoline (n=2) and pasireotide-cabergoline-ketoconazole (n=2). Mean time between biochemical remission and operation was 12.8±3.1 weeks. The sst expression pattern was remarkably different from the previously reported sst pattern in corticotroph adenomas, with a relative more prominent sst_2 expression compared to sst_5 expression. Sst_5 and D_2 expression patterns were within the range of previously reported series.
Conclusion. Stepwise medical treatment of CD with pasireotide, cabergoline and ketoconazole resulted in biochemical remission in almost 90 % of patients. Prolonged control of hypercortisolism may modulate the sst subtype pattern with an upregulation of sst_2 expression by corticotroph tumor cells. This, in turn, may have therapeutical implications with respect to application of sst_2 targeting compounds in CD.

Disclosures: AJvdL: Consultant, Ipsen, Novartis Pharmaceuticals, Pfizer Global R&D.
Nothing to Disclose: RAF, CdB, AMP, JAR, RTN-M, ARH, PMZ, DMS-M, WWdH, SWJL, LJH

OR15-1

Comparison of Vascular, Metabolic and Inflammatory Effects of Soybean Oil-Based (Intralipid) and Olive Oil-Based (Clinoleic) Lipid Emulsions in Parenteral Nutrition.

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Despite improving nutritional status, the use of parenteral nutrition (PN) has been associated with increased risk of complications and mortality in hospitalized patients. The underlying causes of these adverse effects are not known but may relate, among other factors, to its lipid component. We previously reported that IV infusion of Intralipid, a soybean oil-based lipid emulsion rich in ω-6 polyunsaturated fatty acids and single lipid emulsion approved for clinical use in the United States, results in the elevation of blood pressure (BP) and endothelial dysfunction in obese subjects. In this study, healthy subjects (N=12, age 41±7 yrs, BMI 32±2 kg/m^2) were studied on 4 separate occasions during 24-hour infusions of conventional PN with Intralipid 20%, PN with olive oil 20%, lipid-free dextrose PN and normal saline. We examined vascular responses [BP and endothelial function by flow-mediated dilation], metabolic responses [glucose, insulin, C-peptide, lipids and free fatty acids (FFAs) and euglycemic hyperinsulinemic clamp], inflammatory (CRP, TNF-α, IL-6) and oxidative stress markers (glutathione), and autonomic activity (heart rate variability). PN-Intralipid increased BP from baseline (peak increment of 14 mm Hg in systolic BP at 12 h, $p<0.05$) that remained higher over the 24 hr period compared to PN-olive oil ($p<0.05$). PN-Intralipid reduced FMD from baseline by 23% at 4 hrs and by 25% at 24 hours, both $p<0.01$; in contrast, PN-olive oil, PN-lipid free and saline infusion did not alter FMD. Compared to saline, Intralipid, olive oil and lipid free PN similarly increased blood glucose by 20-40 mg/dl ($p<0.05$), plasma insulin concentration by 15-30 μU/ml/ml ($p<0.01$), and C-peptide levels by 0.6-0.9 ng/dl ($p<0.01$) at 24 hrs. We observed no significant changes in insulin sensitivity, FFAs, lipid profile, inflammatory markers, or heart rate variability between PN-Intralipid and PN-olive oil infusion.
In summary, PN with Intralipid emulsion results in increased BP and impaired endothelial function compared to olive oil-based and lipid-free PN in healthy subjects. These adverse vascular changes may contribute to worse clinical outcome in subjects receiving Intralipid-based nutrition support. Further randomized controlled trials are needed to determine potential clinical effects of olive oil-based versus soybean oil-based lipid emulsions in patients requiring PN.

Nothing to Disclose: JFS, AG, DS, N-AL, RS, CN, BK, AQ, TZ, PC, GU

OR15-2

Metabolic Syndrome in Kidney Donors and Long-Term Renal Function Outcome.

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Aim: Metabolic syndrome (MetS) may represent an additional burden for the remaining kidney function of living kidney donors. The aim of this study is to evaluate the impact of the presence of MetS in donors before nephrectomy (BN) on renal function at follow-up.
Methods: Data regarding MetS criteria and renal function were obtained in 140 kidney donors BN and at follow-up. For the analysis, donors were divided into 2 groups; those with MetS BN (group 1, n = 28) and those without MetS (group 2, n = 112).
Results: Comparing both groups, a significantly (p = 0.02) higher reduction of glomerular filtration rate (GFR) in the group with MetS [27.5% (19.3 – 33.0) vs. 21.4% (9.6 – 34.1)] was identified. Using general linear model, presence of MetS (beta = 7.7, p = 0.02) was identified as an independent factor related to higher impairment in GFR (F = 36.6, r2 = 0.447; p < 0.001). Additionally, age (beta = 0.42, p < 0.001) and baseline GFR (beta = 0.65, p <0.001) were independent factors associated with a greater decline in the GFR. Individuals with the MetS had a GFR <70 ml/min at a significant (p = 0.001) shorter time (5.6 ± 0.8 years) than those individuals without MetS (12.8 ± 1.0 years).
Conclusions: Kidney donors with MetS have a significantly greater decline in GFR at follow-up. This finding suggests that the presence of MetS before donation can negatively impact donors renal function outcome.

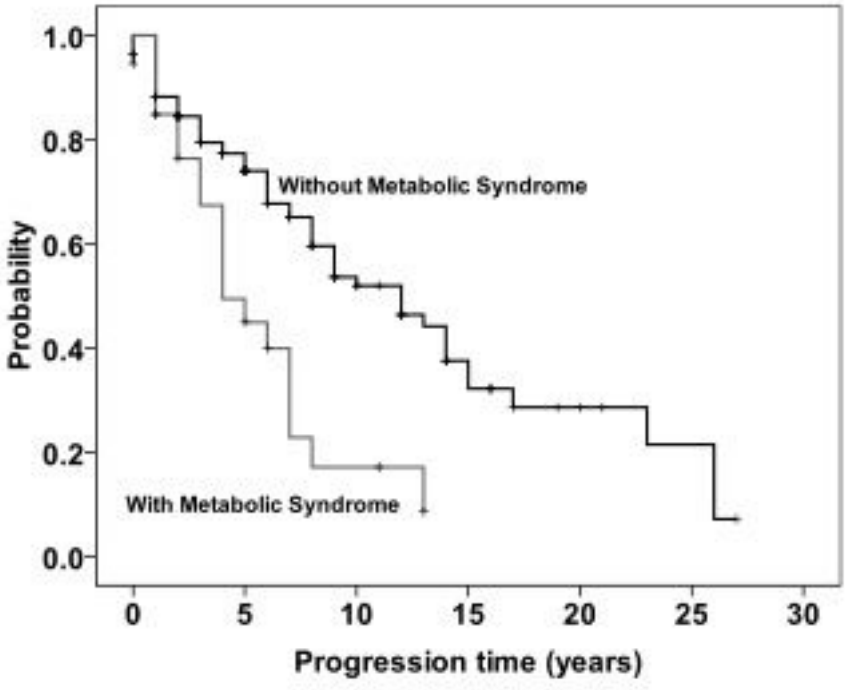

Figure 1. Kaplan-Meier analysis of time to glomerular filtration rate ≤70 ml/min by the metabolic syndrome at time of kidney donation. Log-rank test, p = 0.001

Nothing to Disclose: DC-R, PA-V, JA, MA, JM, LEM, RC-R, BG, MV, FG-N, FJG-P

OR15-3

Pre-Transplant C-Peptide Level Predicts Early Post-Transplant Diabetes Mellitus (PTDM) and Impacts Survival after Allogeneic Stem Cell Transplant (ASCT).

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Background: As long-term survival after ASCT is improving, the importance of metabolic complications is increasing. We conducted a prospective study of 84 patients (pts) undergoing ASCT for hematological malignancies to evaluate incidence and risk factors for PTDM in the first 100 days. **Methods:** ASCT candidates without preexisting DM who met screening criteria were enrolled. PTDM was defined by fasting blood glucose (FBG) ≥ 126 mg/dl or random BG ≥ 200. **Results:** Median age was 46 years (yrs; range 21-66); 44 (52%) were male. Median FBG pre-SCT was 97 mg/dL (range 79-121); median BMI was 27.1 kg/m^2 (range 18.8-49.5). Fifty of 84 (60%) pts developed PTDM at median of 23 days (interquartile range 14.2-33.8.) Acute graft-versus-host disease (aGVHD) was seen in 56 pts (67%). Pre-transplant c-peptide levels were higher in pts with PTDM (median 4.45 vs 2.55 ng/mL, P=0.015). Risk factor analysis showed increased risk of PTDM in pts with c-peptide above median of 3.6 ng/mL (72.5% vs 50% for ≤3.6 ng/mL, P=0.036), myeloablative preparative regimen (66% vs 41%, P=0.024), unrelated ASCT (62% vs 29%, p=0.003), or systemic steroid (SS) dose (>1mg/kg/day) for aGVHD (N=44) (P=0.002). In multivariate logistic regression model, pre-transplant c-peptide level (>3.6 ng/mL; OR=4.3, P=0.012), unrelated donor ASCT (OR=3.0, P=0.052) and myeloablative preparative regimen (OR=3.5, P=0.029) remained independent predictors of PTDM. Univariate survival analysis showed higher pre-transplant c-peptide level (mean survival, ≤3.6-2.9 yrs vs >3.6 ng/mL-1.7 yrs, P=0.012) and PTDM (mean survival, PTDM-2.26 yrs vs no PTDM-2.7 yrs, P=0.021) associated with inferior overall survival (OS). As expected, disease risk impacted OS (P=0.05). AGVHD, regimen intensity, donor type, and SS dose did not impact OS. In multivariate Cox proportional model, high risk disease (HR=3.4, P=0.03) and pre-transplant c-peptide level >3.6 (HR=2.6, P=0.025) were independent predictors of OS. **Conclusions:** Our data show that pre-transplant measurement of c-peptide level independently predicted development of PTDM and OS. This information might allow earlier risk adapted intervention to decrease long term morbidity and mortality associated with PTDM after ASCT. The novel relationship between c-peptide and survival after ASCT needs to be independently validated in larger multicenter studies. Interaction between c-peptide, regulatory T cells, and inflammatory response is a possible link that needs to be explored.

Nothing to Disclose: MLG, AAM, HC, MJ, BE, AK, BNS, MS, SMJ

OR15-4

Hyperglycemia Is Independently Associated with Increased Mortality during Hospitalization in the Non-Intensive Care Setting.

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Hyperglycemia is common in patients hospitalized with and without critical illness. Although treating hyperglycemia has been shown to decrease mortality in randomized trials conducted in ICUs, similar prospective data are lacking in non-ICU settings. Additionally, patients with and without critical illness differ in ways that may influence glucose and possibly outcome, such as glycemic excursions and variability related to meals. Thus, large and well-controlled observation studies may be important in understanding glycemic status and outcomes in hospitalized patients who are not critically ill. We evaluated the relationship between mean glucose (MG) and mortality, independent of illness severity, in a diverse cohort of 1,004,712 admissions to 132 non-intensive care units throughout the US.
A predicted mortality risk (severity index) for each patient was calculated from a validated logistic regression (LR) model that predicts 30-day mortality using admission diagnosis, co-morbidities, laboratory variables, and age. This index was analyzed with MG in a second LR model for all cases and for subsets by medical/surgical units and absence/presence of diabetes. A similar analysis was performed using MG of fasting values.
Hyperglycemia was associated with increased mortality, independent of illness severity, for all patients. Adjusted mortality increased proportionally with MG and was greatest in patients without known diabetes. Analysis with mean fasting glucose produced similar results.
Our findings suggest that hyperglycemia may increase mortality risk in hospitalized patients even without critical illness. While the striking magnitude of mortality risk in patients without diabetes has been reported previously, mostly in the ICU, this finding is particularly important because most hospitalized patients do not have diabetes and are not critically ill. These results support the investigation of hyperglycemia treatment through randomized trials in non-ICU settings.

Adjusted Odds Ratios for Mortality

Cohort	**N**	**Mean Glucose (mg/dl)**			
		111-145	**146-199**	**200-300**	**>300**
MG includes all glucose					
Total	1,004,712	1.43	1.72	1.79	2.21
DM	375,203	0.98*	1.16	1.40	1.77
No DM	629,509	1.60	2.59	3.15	4.47
MG includes fasting glucose					
Total	928,633	1.48	1.84	2.14	3.01
DM	352,749	1.16	1.50	1.97	2.95
No DM	575,884	1.68	2.61	3.47	4.77

ALL statistically significant ($p<0.0001$ AND 95% CI excludes unity) EXCEPT value marked *

Nothing to Disclose: MF, ALC, RWF, PLA, DAD, MLR

OR15-5

Factors Influencing Cardiovascular Disease Progression after Kidney Transplant in Diabetic and Nondiabetic Recipients.

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Background. Diabetes increases risk of cardiovascular events and mortality after kidney transplant (KTX). Carotid intima media thickness (CIMT) has emerged as a noninvasive marker that correlates with vascular risk. **Methods.** In a prospective study, we evaluated potential factors contributing to the progression of CIMT after KTX, according to diabetes status. All recipients had to be ≥6 months after KTX and have an estimated GFR >30 ml/min to be eligible for study. A number of traditional and nontraditional risk factors were collected and evaluated. **Results.** In this cohort (n=308) 24% had type 1 diabetes (DM1), 11% had type 2 (DM2), 15% had post-transplant DM (PTDM), and 50% had no diabetes (nonDM). Mean age was 52.6 ± 0.6 y (± SEM), with 57%M/43%F. Ethnic minorities made up 13% and 87% were non-Hispanic white. Overall, 60% had received a deceased donor and 40%, a living donor graft. Mean time since transplant was 5.92 ± 0.33 y (range 0.5-33.8 y). CIMT was different between DM groups both at baseline (p=0.0011) and at 1 year of follow-up (p=0.0066). The diabetes groups were different in other ways: age was greater in DM2, BMI was greater in DM2 and PTDM, and A1C was greater in all diabetic than nondiabetic groups, while LDL and total cholesterol were lower in all diabetes groups compared to NonDM. Importantly, BMI, smoking status, blood pressure, and hsCRP were not different between the groups (all p<0.05). CIMT increased over the 2-year observation period for the group as a whole (p<0.0001), but there was no difference in change in CIMT between the four DM groups. Among the cohort, we compared those that had an increase in CIMT with those whose CIMT were the same or decreased over the same period. Only highly sensitive C-reactive protein (hsCRP) was shown to be significantly different between these 2 groups (p=0.009), even though many traditional and nontraditional risk factors were evaluated, including diabetes group. **Conclusions.** Many traditional risk factors correlate with and affect CIMT at time of transplant, including diabetes. However, these factors may not significantly affect rate of CIMT progression if or when traditional risk factors are treated to recommended goals, which were similar between diabetic and nondiabetic recipients. Instead, nontraditional risk factors may have a greater impact in vascular risk after KTX.

Sources of Research Support: Grant RO1DK069919 (Clinicaltrials.gov: NCT00374595).

Nothing to Disclose: JMW, TLH, ERL, FY, GCG, RBS, JLL

OR15-6

Increased Plasma Basic Fibroblast Growth Factor Is Associated with Coronary Heart Disease in Adult Type 2 Diabetes.

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Coronary heart disease (CHD) is the leading cause of death in adults in the United States. Dyslipidemia, hypertension, pro-thrombotic and pro-inflammatory factors each contribute to accelerated atherosclerosis in obese type 2 diabetes, yet the precise mechanism(s) accounting for a substantially increased risk for coronary heart disease in adult type 2 diabetes remain unclear. Basic fibroblast growth factor (bFGF) is a potent endothelial and smooth muscle cell mitogen which does not normally circulate. Plasma bFGF increases in microalbuminuric adult type 2 diabetes and in coronary artery disease. In the present study, we tested whether baseline plasma bFGF immunoreactivity (IR) predicts the occurrence of a subset of cardiovascular disease outcomes in adults with advanced type 2 diabetes from the Veterans Affairs Diabetes Trial (VADT) mean: age 59 yrs, diabetes duration 11 yrs, baseline HbA1c 9.5%. Plasma bFGF-IR was determined with a sensitive, specific enzyme-linked immunoassay in 399 patients at the baseline visit. These results were then evaluated as possible predictors of the first post-randomization occurrence of pre-specified cardiovascular or coronary heart disease endpoints. Mean (SD) plasma bFGF was 15.1±25.8 pg/mL in all 399 subjects. There was a borderline significant association ($p = .07$) between plasma bFGF-IR and the main VADT cardiovascular disease outcome. Plasma bFGF-IR was significantly associated with CHD occurrence (myocardial infarction, coronary revascularization, inoperable coronary artery disease, cardiovascular death) ($p = .01$). In univariate regression analysis, age, baseline nephropathy, diabetes duration, high density lipoprotein cholesterol or serum creatinine concentration were each significantly associated ($p < .0001$) with the time to first post-randomization CHD occurrence. Glycemic treatment was not significantly associated with time to CHD occurrence consistent with the main VADT. After adjusting for other risk factors, bFGF (hazard ratio, HR 1.013; 95% CI 1.007-1.019; $p < 0.0001$), prior macrovascular event (HR 3.55; 95% CI 2.154-5.839; $p < 0.0001$), and diabetes duration (HR 1.041; 95% CI 1.012-1.071; $p = 0.0055$) were all significantly associated with time to CHD occurrence. There was no significant interaction between bFGF and glycemic treatment or diabetes duration. These results suggest plasma bFGF is a potentially useful, novel marker of the risk for coronary heart disease in adult men with type 2 diabetes.

Nothing to Disclose: MBZ, RJA, LG, TEM, VADTI

OR16-1

Evidence That Acromegaly Is Associated with Enhanced Renal and Extrarenal Epithelial Sodium Channel (ENaC) Activity in Humans: AcromENaC Study.

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Context: GH exerts a direct stimulatory effect on ENaC-dependent transport both *in vitro* and *in vivo* (1).
Objectives: To investigate the effect of GH excess on renal and extrarenal ENaC activity in acromegalic patients before (P1) and after (P2) treatment by assessing **1)**the natriuretic and kaliuretic responses to amiloride (an ENaC blocker) and to furosemide (a loop diuretic that acutely increases Na delivery to distal tubule) and **2)** intranasal amiloride sensitive potential (ASP) reflecting ENaC activity in nasal epithelium.
Study protocol: 16 acromegalic patients (11M/5F) aged 46 yrs [27; 75] were randomly assigned to receive in a crossover study a single dose of amiloride (20 mg *po*) or furosemide (5mg IV bolus + 2h infusion of 10mg) at 2-7 days interval. Na and K daily intakes were maintained at 150 and 80 mmol/day. Urinary Na/K ratio was measured before and after diuretic administration. ASP was measured before first drug administration. Investigations were repeated before (P1) and after (P2) medical and/or surgical treatment of acromegaly. Results are median [IQR].
Results: Serum IGF-1 concentration normalized in all subjects after acromegaly treatment (798 [387; 1445] *vs* 180 ng/mL [72.0; 265.0], $P<0.0001$). Daily Na and K intakes were similar between P1 and P2. UNa excretion after amiloride or furosemide administration did not significantly differ between P1 and P2 whereas post-amiloride UK excretion was significantly lower ($P<0.001$) and post-furosemide UK excretion tended to be higher ($P=0.07$) on P1 than on P2. The post-amiloride UNa/K ratio was significantly higher on P1 than on P2 (16.8 mmol/mmol [7.0; 36.0] vs 8.1 mmol/mmol [5.0; 20.6], $P<0.001$). In contrast, UNa/K ratio during furosemide administration was significantly lower on P1 than on P2 (6.8 mmol/mmol [4.6; 10.8] *vs* 9.3 mmol/mmol [2.7; 12.9], $P<0.05$). ASP was significantly higher on P1 than on P2 (5.5 mV [3.5; 14.7] *vs* 4.1 mV [8.2; 19.0], $P<0.05$).
Conclusion: Our data suggest enhanced renal and extrarenal ENaC activity in acromegaly as attested before acromegaly treatment by 1) higher antikaliuretic responses to amiloride and higher kaliuretic responses to furosemide, supporting stimulatory effect of GH on ENaC activity in distal renal tubule; 2) higher amiloride sensitive-nasal potential. These observations provide first evidence in humans that ENaC stimulation might contribute to volume expansion and arterial hypertension in acromegalic patients.

(1) Kamenicky P et al, Endocrinology. 2008 Jul;149(7):3294-305

Disclosures: PC: Advisory Group Member, Pfizer, Inc.
Nothing to Disclose: PK, AB, SS, MF, AL, ML, MA

OR16-2
Clinically Silent Somatotroph Adenomas Are Common.

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Background: Somatotroph adenomas are often easy to recognize clinically because in many patients they result in classic features of acromegaly, although in other patients acromegalic features may be subtle. Somatotroph adenomas that are “silent,” ie, do not exhibit either clinical features of acromegaly or elevated IGF-1 but do exhibit immunohistochemical (IHC) evidence of growth hormone (GH) in excised pituitary adenoma tissue, have occasionally been described. “Clinically silent” somatotroph adenomas - exhibiting no clinical features of acromegaly but elevated serum IGF-1 and IHC evidence of GH – have rarely been described. The relative frequency of each of these presentations has not been studied.
Aim: To characterize the spectrum of presentations of somatotroph adenomas by determining the frequency of four presentations: “Classic,” “Subtle,” “Clinically Silent” and “Silent.”
Methods: We identified 100 consecutive patients who had surgically excised and histologically diagnosed pituitary adenomas at one hospital. We reviewed the histology and immunostaining for pituitary hormones of each adenoma and classified each by pituitary cell type. Clinical and biochemical data were then extracted from their records. Somatotroph adenomas were further classified as “Classic” (obvious clinical features of acromegaly and elevated serum IGF-1); “Subtle” (subtle clinical features of acromegaly and elevated IGF-1); “Clinically Silent” (no clinical features of acromegaly but elevated IGF-1); and “Silent” (no clinical features of acromegaly and normal IGF-1).
Results: Of the 100 consecutive pituitary adenomas, the cell type by IHC was gonadotroph/glycoprotein in 29%, somatotroph 24%, null cell 18%, corticotroph 15%, lactotroph 6%, thyrotroph 2% and not classifiable 6%. Of the 24 patients with somatotroph adenomas, Classic accounted for 50%, Subtle 12.5%, Clinically Silent 33.3%, and Silent 4.2% (Table 1).

Table 1. Spectrum of Presentations of Somatotroph Adenomas

Classification	N (%)
Classic	12 (50.0)
Subtle	3 (12.5)
Clinically silent	8 (33.3)
Silent	1 (4.2)
Total	24 (100.0)

Conclusion: Clinically silent somatotroph adenomas are more common than previously appreciated, representing one-third of all somatotroph adenomas in this study. This finding is clinically significant, since an elevated IGF-1 can identify a sellar mass as a somatotroph adenoma even in the absence of clinical features of acromegaly.

Nothing to Disclose: ANW, JB, MSG, KDJ, PJS

OR16-3

Arthropathy in Long-Term Cured Acromegaly; a Comparison with Primary Osteoarthritis.

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Objective: To gain insight in the pathophysiological process of growth hormone (GH) and IGF-I mediated OA, we investigated the OA phenotype in acromegaly by comparing the distribution of osteophytes and joint space narrowing (JSN) in hips and knees between patients with acromegaly and primary OA.
Methods: We utilized 84 patients with controlled acromegaly for a mean of 14.0 years and 189 patients with primary familial OA at multiple sites from the GARP (Genetics, ARtrosis and Progression) study. Hips and knees with a Kellgren-Lawrence (KL) score ≥1 were compared. Study parameters were radiographic severity of knee and hip OA; osteophytes (0-3) and JSN (0-3) were scored in the hips and medial and lateral tibiofemoral joints according to the Osteoarthritis Research Society International (OARSI) atlas. Logistic regression analysis was performed with adjustments for age, sex, BMI and intra-patient effect.
Results: We compared 80 (48%) and 129 (34%) hips with KL≥1 in 42 acromegaly and 91 primary OA patients, and 128 (76%) and 237 (63%) knees with KL≥1 in 72 acromegaly and in 138 primary OA patients.
Acromegalic hips demonstrated JSN in 17% versus 54% primary OA hips (OR(95% CI) 0.3 (0.10 to 0.70), whereas osteophytes were demonstrated in 89% and 60% (OR 4.7 (2.51 to 7.80) respectively.
JSN of the medial knee was less prevalent in acromegaly compared with primary OA (28% *vs.* 37% (OR 0.5 (0.28 to 1.20)). Femoral and tibial osteophytes were more prevalent in acromegaly (OR 1.9 (1.31 to 3.80) and 3.8 (2.42 to 6.32), respectively). JSN of the lateral knee was equally prevalent in acromegaly and OA. Femoral and tibial osteophytes were more prevalent in acromegaly than in OA (OR 4.1 (2.68 to 7.85), and 9.9 (5.75 to 17.85)).
JSN without osteophytes at joint level was statistically less prevalent in acromegaly than in primary OA. On the other hand, osteophytes without JSN was statistically more prevalent in acromegaly than in primary OA.
Acromegaly and OA patients had significantly less self-reported functional disability than primary OA patients ($p<0.001$). Self reported functional disability was associated with JSN rather than with osteophytosis.
Conclusion: Osteoarthritis due to GH oversecretion results in osteophytosis and to a lesser extend in JSN, which can be observed many years after treatment. This observation not only suggests that the GH-IGF-I system may protect against cartilage loss but also is involved in bone formation resulting in osteophytosis.

Nothing to Disclose: NRB, MJEW, JB, AMP, IM, FR, HK, JAR, MK

OR16-4
Treatment Experience in Eleven Patients with Gigantism.

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Gigantism is an extremely rare condition and hence the relevant literature is largely a series of case reports. We present data on patients with gigantism <20 years of age identified from Pfizer's ACROSTUDY registry of patients treated with pegvisomant.
Eleven patients (5 males) were identified (1.2% of all ACROSTUDY patients): IGF-I at diagnosis was 1.76±0.68 xULN, height +5 SDS (1.1-3.8), BMI 26.3±3.4 kg/m^2 and median age 14.5 (4-19) yrs. The 3 youngest (4, 7 and 14 yrs) had pituitary hyperplasia secondary to NF-1 (n=1) or McCune-Albright (n=2). The remaining 8 had macroadenomas and received transsphenoidal surgery (TSS) as first line treatment at 16±2.7 yrs, a mean of 1yr following diagnosis. Two patients had a second TSS, and 1 had two TSS followed by a craniotomy. Four subsequently had radiotherapy (2x 3-field 4500cGy, 2x stereotactic 1800 cGy). Four patients had hyperprolactinemia at diagnosis, 7 were treated post-surgery with cabergoline (median dose=1.75mg/wk (0.25-7)). All patients received octreotide LAR (median duration of 12 months (6-120), dose 30 mg/month (10-40)). IGF-I was elevated in all patients prior to starting pegvisomant. On pegvisomant monotherapy (n=8) or in combination with cabergoline (n=2) or octreotide (n=1) all patients achieved IGF-I within the reference range. A median dose of 20 mg/day (10-30mg) was required and patients have been treated for a median of 4.5 yrs (2.5-6). There have been no abnormalities in LFTs reported. One patient has recent tumour growth demonstrated on MRI after TSS, radiotherapy and six yrs of pegvisomant monotherapy.
Syndromic gigantism is more common in patients with early presentation and in such patients surgery may be avoided. Vigorous and prompt treatment is required to minimise the harm from excessive GH, particularly tall stature. Radiotherapy may be more rapidly effective than in adults (data not shown) although the long-term risks are unknown. Somatostatin analogues and dopamine agonists are effective but despite all conventional treatment a number of patients remain uncontrolled. Experience from 11 patients treated with pegvisomant suggests that it is an effective treatment in this group although as tumours tend to be more aggressive at a younger age close monitoring of tumour size is required.

1 Main K et al., Horm Res 2006; 65:1-5
2 Rix M et al., EJE 2005; 153:195-201

Sources of Research Support: Pfizer ACROSTUDY.

Disclosures: PJT: Lecturer, Advisory Board Member, Research Funding, Pfizer, Inc., Ipsen, Novartis Pharmaceuticals.
Nothing to Disclose: CEH, MK-H, FL

OR16-5

Long-Term Treatment of Acromegaly with Pasireotide (SOM230): Results from a Phase II Extension Study.

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Introduction: Pasireotide (SOM230) is a multi-receptor targeted somatostatin analogue with high affinity for $sst_{1,2,3}$ and sst_5. In a Phase II study of pasireotide in actively acromegalic patients, 27% achieved biochemical control after 1 month of octreotide sc followed by 3 months of pasireotide (month 3). Pituitary tumor volumes decreased by >20% in 39% of patients. Results from the study's extension phase are presented.
Methods: Patients enrolled in the extension phase achieved biochemical control (GH ≤2.5 µg/L and normalized IGF-1) or clinically relevant improvement during the core study. Patients received pasireotide at the dose at which clinical benefit was achieved (200, 400 or 600 µg sc bid), with dose adjustments up to 900 µg sc bid if required. Efficacy and safety were assessed every 3 months. Patients underwent pituitary MRI at core (month -1) and extension baselines and every 6 months.
Results: Thirty patients entered the extension phase; at 9 and 27 months, 26 and 9 patients respectively remained enrolled. At 9 months, 6 patients were biochemically controlled, 1 due to enrollment in the extension phase, 5 others having maintained control from the core study exit. At 27 months, 3 patients were biochemically controlled, all having maintained control from the core study exit. Considering GH control, at 9 months, 12 patients were controlled, 1 due to extension-phase enrollment; at 27 months, 5 patients were controlled, all having maintained control from the core study exit. Considering IGF-1 normalization, at 9 months 13 patients had normalized, 4 due to extension-phase enrollment; at 27 months, 5 patients were normalized, all having maintained control from the core study exit. 16/29 patients (55%) with a core baseline MRI achieved significant tumor volume reduction by the time they exited the extension phase. Mean percent (±SE) tumor volume reduction was 13%±3.9% at extension baseline (n=25), 26%±5.9% by 9 months (n=16), and 38%±11% by 27 months (n=5). All patients experienced an adverse event (AE), with diarrhea and nausea the most common (n=15 each). Two serious AEs with a suspected-drug relationship were worsening of diabetes mellitus and gallbladder polyp. Four patients discontinued the study because of an AE.
Conclusions: Extended pasireotide treatment maintains biochemical control and reduces tumor volume in patients with acromegaly. AEs were mostly mild or moderate.

Disclosures: AJF: Investigator, Novartis Pharmaceuticals, Perceptive Informatics. MR: Employee, Novartis Pharmaceuticals. KW-H: Employee, Novartis Pharmaceuticals. SP: Speaker, Novartis Pharmaceuticals, Ipsen; Medical Advisory Board Member, Novartis Pharmaceuticals, Ipsen.

OR16-6

The Effect of Pegvisomant on Cardiovascular Risk in Patients with Active Acromegaly in Comparison to Matched Data from the General Population.

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Background:
Data on cardiovascular risk in active acromegaly and development during disease control are scanty and lack of clear correlation to epidemiological data. Aim of this study was an evaluation of cardiac risk factors in patients with active acromegaly resistant to other treatment modalities and on treatment with Pegvisomant, a calculation of their Framingham risk score (FRS) in comparison to matched controls of the general population and an evaluation of the effect of IGF-I normalization on cardiac risk.
Patients & Design:
In an University referral center, retrospective, comparative study 133 patients with active acromegaly (65 men, aged 45-74, mean IGF-I 1.74±0.72 fold ULN, disease duration 15.1±7.8 yrs) from the German Pegvisomant Observational study were compared to 665 age- and gender-matched controls from the general population of the Heinz Nixdorf Recall study. Cardiac risk factors were measured at baseline (n=133) and after 12 months of treatment with Pegvisomant (n=62).
Results:
Patients with active acromegaly had increased BMI (30.1 vs 27.9 kg/m^2), increased prevalence of hypertension (67 vs. 41%), increased systolic and diastolic blood pressure (140±22 vs 131±21 and 87±13 vs 81±11 mmHg), increased prevalence of diabetes mellitus (37.6 vs 6.9%) and HbA1c (6.2±0.9 vs 5.5±0.8%) (all $p<0.001$) and decreased HDL (48±17 vs 58±17 mg/dl), LDL (121±46 vs 147±36 mg/dl) and total cholesterol levels (194±63 vs 231±38 mg/dl) (all $p<0.001$). FRS was significantly higher in patients with active acromegaly compared to matched controls (13.6±7.9% vs 10.6±7.7%, $p<0.001$).
After 12 months of treatment with Pegvisomant IGF-I decreased to 0.9±0.6 ULN ($p<0.0001$) and systolic BP ($p<0.05$), HbA1c ($p<0.05$) as well as FRS (13.9 vs 11.3 ($p<0.01$) decreased significantly. In patients with completely normalized IGF-I (n=42) glucose (88±2 vs 109±12, $p<0.05$), systolic (133±2 vs 141±4, $p<0.05$) and diastolic BP (84±2 vs 89±3, $p<0.05$) was lower than in partially controlled patients and FRS at 12 months was non-significantly different compared to controls (11.8±6.6 vs 11.4±8.7%, $p=0.7$).
Summary:
In this large cohort we have found an increased cardiovascular risk in patients with active acromegaly compared to matched controls. Treatment of GH excess with Pegvisomant showed a significant decrease of FRS as compared to non-treated controls, implying a reduced risk for coronary heart disease. This was most significant in those patients who completely normalized their IGF-I levels.

Disclosures: CJS: Advisory Group Member, Lilly USA, LLC, Pfizer, Inc.; Study Investigator, Biopartners; Researcher, Ipsen, Novartis Pharmaceuticals; Consultant, Merck & Co., Novo Nordisk.
Nothing to Disclose: CB, SP, HL, BLH, MB, MD, GKS, UR, NL, SM, KHJ, SM, RE, BS , KM

OR17-1

Higher Endogenous Testosterone Levels Associated with Increased Risk of Coronary Heart Disease in Elderly Men: A Prospective Study.

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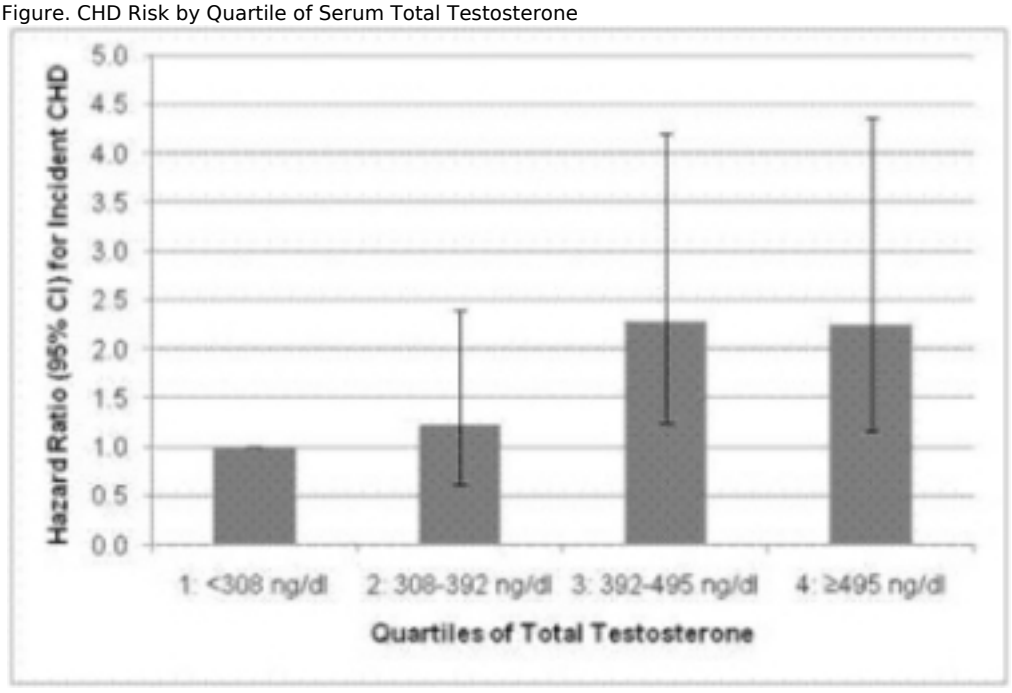
Context: Current data conflict regarding the relationship between serum testosterone (T) levels and cardiovascular disease.
Objective: To examine endogenous sex hormone levels in older men as independent risk factors for coronary heart disease (CHD) events.
Design, Setting & Participants: MrOS is a prospective cohort study of 5995 community-dwelling men age ≥65 years (mean age 72, SD 5.5) recruited in 2000-02 at 6 U.S. centers. At enrollment, fasting serum was collected and stored at -80C for a random sample of 1602 men; 819 of these men participated in an ancillary study from 2003-2005 to identify outcomes of sleep disorders and were contacted every 3 months for CHD events. Participants taking exogenous hormones or missing complete sex hormone measurements were excluded, leaving a sample of 697.
Measurements & Analysis: Baseline sex hormones were measured using liquid chromatography-mass spectrometry. Free and bioavailable testosterone were calculated from mass action equations. Incident CHD events (including acute coronary syndromes, percutaneous or surgical revascularization) were reviewed and adjudicated by a study cardiologist using clinical records. The relationships between quartile of sex hormone and CHD risk were analyzed using Cox models adjusted for age, clinic, body mass index, blood pressure, lipid levels, smoking, hypertension, diabetes and use of lipid-lowering agents.
Results: During an average follow-up of 3.9 years, 100 (14%) suffered an incident CHD event. After adjustment, higher total T was associated with an increased risk of CHD (p-trend=0.003, Figure). Compared to those in the lowest quartile of total T (<308 ng/dl), the risk of CHD was 2.2-fold higher among those in the highest quartile (≥495 ng/dl). Results were similar for bioavailable T (p-trend=0.01) and free T (p-trend=0.01). Higher sex hormone binding globulin was also associated with an increased risk of CHD events, but estradiol and estrone levels were not associated with CHD.
Conclusion: Among elderly men, higher endogenous testosterone levels are associated with an increased risk of coronary heart disease.

Figure. CHD Risk by Quartile of Serum Total Testosterone



Sources of Research Support: NIAMS, NHLBI, NIA.

Nothing to Disclose: KTS, SKE, KEE, GAL, ARH, PDV, MLS, EB-C, KLS, DCB

OR17-2

Association of Endogenous Sex Hormones with Longevity into the 80s and 90s: The Rancho Bernardo Study.

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Background: Interest has grown in identifying modifiable factors associated with exceptional longevity. Endogenous sex hormones have been linked with a number of diseases of aging and may play a role in maintaining vitality and promoting longevity.
Objective: To determine the sex-specific association of serum testosterone (T) and estradiol (E2) levels with long term survival and exceptional longevity among older community-dwelling adults.
Methods: 835 men and 724 non-estrogen using women (median age 75 yrs) who had hormone measurements in 1984-87 were followed for mortality for 25 yrs. Participants must have been old enough (56 yrs) at baseline to have the potential of achieving age 80 during follow-up. Accelerated failure time models were used to determine the association of hormones with survival time; linear regressions to determine associations with lifespan for those who lived to age 80+; logistic regressions to evaluate survival into the 80s versus the 90s.
Results: During follow-up, 1146 deaths occurred (74% mortality). Average age at death was 86.8 yrs. Quintile analyses suggested low threshold effects for both sexes, thus hormones were modeled as the lowest quintile (<20%) versus all higher. Among men, in models adjusting for age, BMI, waist, physical activity, alcohol use and smoking, low T and low E2 were associated with 15% and 20% decreases, respectively, in survival time and 1.1 and 1.3 yr decreases in lifespan among those who lived to 80+ (all $p<.01$). Men with low T or low E2 who survived to age 80 were 50% less likely to live into their 90s than those with higher hormone levels($p<.05$). Among women, low T and low E2 each predicted ~10% shorter survival time ($p<.05$), but were not significantly related to years of life expectancy among those with long life or with living to age 90+. For the 10% of men and women with low levels of both hormones, survival time was decreased 21% and 16%, respectively ($p<.01$). In men, risk estimates for low T, but not low E2, were reduced by additional adjustment for markers of health status, the metabolic syndrome or prevalent CVD.
Conclusions: Sex hormone insufficiency is associated with reduced long term survival in elders of both sexes, and with shorter lifespan among men who live into the 9th and 10th decades of life. Estrogen insufficiency is more important in terms of longevity for older men than older women, and is not explained by poor overall health or pre-existing cardiometabolic disease.

Sources of Research Support: Sandra A. Daugherty Foundation; NIA AG007181; NIA AG028507; NIDDK DK31801.

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

OR17-3

Low Free Testosterone Level Predicts Frailty in Older Men. The Health in Men Study.

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Background and objective
Frailty is generally defined as a decline in multiple organ systems leading to loss of function, diminished capacity to cope with stressors, and increased risk of death and disability. As men grow older, the prevalence of frailty increases while testosterone levels decrease. Testosterone deficiency results in loss of muscle and bone mass hence may predispose to development of frailty. We aimed to clarify the relationship between endogenous testosterone levels and frailty in older men.
Design, setting and participants
We conducted a prospective cohort study. Between 2001-04, frailty was assessed in 3,616 community-dwelling men aged 70-88 years resident in metropolitan Perth, Western Australia. In 2008-09, frailty was re-assessed in 1,586 of these men then aged 76-93 years.
Main outcome measures
Frailty was assessed with the FRAIL scale, comprising 5 domains: fatigue; difficulty climbing a single flight of stairs; difficulty walking more than 100 metres; presence of more than 5 illnesses; or weight loss of more than 5%. Testosterone, sex hormone binding globulin (SHBG) and luteinizing hormone (LH) were assayed in early morning sera collected at baseline. Free testosterone was calculated using mass action equations.
Results
At baseline 15.2% of men (n=548) were frail, increasing to 23.0% (n=364) at follow-up. At baseline, each 1 standard deviation decrease in total or free testosterone levels was associated with increased odds of frailty (odds ratio [OR]=1.23; 95% confidence interval [CI] 1.11, 1.38 and OR=1.29; 95% CI 1.15, 1.44 for total and free testosterone respectively). Lower LH levels were associated with reduced odds of frailty (OR=0.88; 95% CI 0.81, 0.95). Adjustments were made for age, body mass index, smoking status, diabetes, social support and other covariates. In longitudinal analysis, only lower free testosterone levels at baseline predicted frailty at follow-up (OR=1.22; 95% CI 1.05, 1.42).
Conclusions
Low free testosterone was independently associated with frailty at baseline and follow-up. Other studies have reported cross-sectional associations between low testosterone and frailty, but this is the first to demonstrate that low testosterone predicts frailty in a longitudinal analysis. Interventional studies are needed to establish whether testosterone therapy might prevent the development of frailty in ageing men.

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Nothing to Disclose: ZH, LF, OPA, GJH, KM, SAPC, BBY

OR17-4

Dutasteride Reduces Prostate Size and Prostate Specific Antigen in Hypogonadal Men with Benign Prostatic Hyperplasia Undergoing Testosterone Replacement Therapy.

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Introduction and Objectives: Benign prostatic hyperplasia (BPH) and hypogonadism are common disorders in aging men. There is concern that androgen replacement may increase prostate size, resulting in increased incidence and severity of BPH. We hypothesized that the addition of the dual (type 1 and 2) 5 α-reductase inhibitor dutasteride (D) to testosterone (T) replacement therapy in older hypogonadal men with symptomatic BPH would reduce androgenic stimulation of the prostate compared to T alone by inhibiting conversion of T to dihydrotestosterone (DHT) within the prostate.
Methods: We conducted a randomized, double-blind, placebo-controlled trial in 45 men ages 51-82 with symptomatic BPH (International Prostate Symptom Scores [IPSS] 8 to 20), prostate volume > 30mL, PSA 1.5 to 10 ng/mL, and total T < 2.8 ng/mL. All subjects received transdermal 1% T gel 7.5g daily (adjusted to T level 5-10 ng/mL) and were randomized to receive either placebo (T-only) or 0.5mg oral D daily (T + D) for 6 months. Prostate volume by MRI, serum PSA and hormones, and IPSS were obtained at baseline and after 6 months of treatment.
Results:

	T-only (± SD)		T+D (± SD)	
	Baseline	Month 6	Baseline	Month 6
Total T (ng/mL)	2.1 ± 1.1	6.1 ± 3.7∗	2.1 ± 1.0	6.1 ± 4.8∗
Total DHT (ng/mL)	0.21 ± 0.62	0.56 ± 0.50∗	0.10 ± 0.07	0.03 ± 0.03∗§
Prostate Volume (mL)	52.3 ± 35.4	55.3 ± 36.1∗	44.7 ± 19.8	38.6 ± 18.4∗§
PSA (ng/mL)	3.2 ± 3.2	3.5 ± 3.1	2.0 ± 1.3	1.3 ± 1.1∗§
IPSS	13.6 ± 2.8	11.1 ± 5.2∗	13.3 ± 3.1	10.2 ± 6.6∗

∗ P<0.05 versus baseline, § P<0.05 versus T-only

Both groups achieved serum T levels in the mid-normal range with treatment. Serum DHT increased in the T-only group but decreased substantially in men receiving T+D. Prostate volume increased slightly in the T-only group compared to baseline. In contrast, prostate size decreased in men treated with T+D and was significantly smaller at Month 6 compared to the T-only group. Similarly, serum PSA decreased in the T + D group and was lower than in men treated with T-only. Both groups experienced significant improvements in IPSS scores.
Conclusions: In older hypogonadal men with symptomatic BPH, co-administration of T+D reduces PSA and prostate volume compared to T alone. The inclusion of a 5 α-reductase inhibitor with T appears to spare the prostate from androgenic stimulation during androgen replacement therapy in older hypogonadal men with BPH.

Disclosures: LAH: Clinical Researcher, GlaxoSmithKline, Solvay Pharmaceuticals, Inc. STP: Clinical Researcher, GlaxoSmithKline, Solvay Pharmaceuticals, Inc. MD: Clinical Researcher, GlaxoSmithKline, Solvay Pharmaceuticals, Inc. BTM: Clinical Researcher, GlaxoSmithKline, Solvay Pharmaceuticals, Inc. AMM: Clinical Researcher, GlaxoSmithKline, Solvay Pharmaceuticals, Inc.

OR17-5

Y-Chromosome Microdeletions Patients Display an Increased Incidence of X-Chromosome Duplications and Deletions.

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The Y chromosome encodes pseudoautosomal regions (PAR) which pair with the X-chromosome during meiosis. The PAR genes, identical on the X and Y chromosomes, are inherited in a Mendelian manner. PAR1, on the telomere of the short arm, harbors 24 genes that escape X-inactivation. PAR1 deletion is associated with male infertility. PAR2, on the telomere of the long arm, encodes 5 genes that exhibit a lower frequency of pairing and recombination than PAR1. *SHOX* is the only known disease locus in the PAR region. *SHOX* deletion is linked with short stature. *SHOX* duplication is rare with normal to high height. In the non-recombining region, genes in Yq11 are required for fertility and these regions are classified as azoospermic factor (AZF) a, b, c. We hypothesized that men with Y-deletions have chromosomal rearrangements in other areas, particularly in the PAR regions. We performed comparative genomic hybridization using 385K and 720K microarrays (aCGH) from Nimblegen, as well as a 135K custom array encompassing only the X and Y chromosomes to study 10 non-obstructive azoospermic (NOA) men with a normal Y chromosome, 27 NOA men with Y-deletions and 4 fertile men. Analysis was performed with Nexus Copy Number. QPCR copy number variant Taqman-assays validated each aCGH gain or loss. aCGH confirmed each Y-deletion defined by multiplex PCR. Abnormalities in PAR1 and/or PAR2 were detected in 33% of Y-deletion men, but not in the other 2 groups. To confirm the array findings, 4 genes in PAR1 (*PLCXD1, GTPBP6, PPP2R3B, SHOX*) and 1 in PAR2 (*SPRY3*) were analyzed. In PAR1, the gene closest to the telomere, *PLCXD1*, displayed the most gains (1) and losses (5). Four men exhibited losses in PAR2 and 3 men had anomalies in both regions. 4/5 AZFb men had rearrangements in PAR versus 2/11 AZFc men (p=.04). Two men had *SHOX* duplications with stature ranging from 1-10th centile. Many Y-deletion men can achieve a pregnancy using assisted reproductive technologies. Sons will inherit the Y-deletion leading to infertility, but daughters were believed to be normal. Our work demonstrates that Y-deletions are more complex than expected, and defects in both the X- and Y-chromosomes may be transmitted to the offspring. Although function of most PAR genes is unknown, PAR rearrangements could lead to phenotypic abnormalities in offspring. Y-deletion men may have a more complex microdeletion/microduplication condition and should seek genetic counseling before planning to attempt a pregnancy.

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Nothing to Disclose: CJJ, JWW, AS, LIL, DJL

OR17-6

Low Testosterone Level Is Associated with Significant Increase in All Cause and Cardiovascular Mortality in Men with Type 2 Diabetes.

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Background: In men with type2 diabetes there is high prevalence of low levels of testosterone (TT). There is accumulating evidence that low TT levels are associated with greater morbidity and mortality. There is no published data regarding mortality among men with type2 diabetes in relation to TT levels.
Aim: We report preliminary findings from our 4-7 year follow up study to examine the effect of baseline TT on mortality in men with type 2 diabetes.
Methods: A total of 591 patients with type2 diabetes who were screened for hypogonadism between 2002 and 2004 were identified from the database. The mean follow up was 5.8years (SD1.2). Cause of death was obtained from hospital records, the local and national registry. After excluding the deaths occurred in first 6 months (n=4) patients with TT levels 8mmol/l or above (Normal TT group) were compared with that below 8mmol/l (Low TT group). Results were analysed using Cox regression model and Kaplan Meir plots were compared.
Results: Of the 587 patients 21% (n=127) had Low TT. Mean age at the screening 59.4 ($\pm$11). There were 72 deaths (cardiovascular 34, Cancer 17, Respiratory 16, others 8). Death rate was 21% in low TT group (27out of127)and 10% (45 out of 460) in the normal TT group. Mean survival in the normal TT group was 90 (SE0.75; 88-91) months as compared with 79 (SE 2; 95% CI 75-83) months in the low TT group p=<0.0001. Hazard ratio was 2 (95%CI 1.2-3.2 p=0.003) for low TT after adjusting for age.
Cardiovascular mortality analysis showed decrease in mean survival in the low TT group (93vs 85 months (p=0.01) in normal and low TT group respectively). Age adjusted hazard ratio was 2 (95%CI 1.04- 4.1 p= 0.038).
Respiratory mortality (94 vs 88 Months p=0.02) and Cancer mortality (94 vs 88 months p=0.035) showed decrease in mean survival in low TT group but both failed to reach significance when adjusted for age (p=0.69 and 0.61 respectively).
Conclusion: This is the first study to show significant increase in all cause and cardiovascular mortality in men with type2 diabetes and Hypogonadism. There is also a trend towards increase in cancer and respiratory disease mortality but the numbers were small to reach significance. This study together with the results from other mortality studies confirm more wider role of TT in men with metabolic syndrome and diabetes and the deleterious effect of low testosterone on longevity. Further studies are needed to look at effects of testosterone replacement on the mortality.

Nothing to Disclose: VM, HM, THJ

OR-LB1

Prevention and Reversal of Diabetic Neuropathy by Treatment with Ghrelin.

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Ghrelin, an acylated peptide produced in the stomach, increases food intake and growth hormone (GH) secretion, suppresses inflammation and oxidative stress, and promotes cell survival and proliferation. Polyneuropathy is the most common complication of diabetes mellitus, but none of agents approved for clinical use. Ghrelin's diverse functions raise the possibility of its clinical application; clinical trials with ghrelin for anorexia nervosa, diabetic gastroparesis, and cachexia have commenced. We investigated the pharmacological potential of ghrelin in the treatment of diabetic polyneuropathy in rodents and humans. Experimental diabetic polyneuropathy was induced by streptozotocin injection in C57BL/6N mice, ghrelin receptor knockout mice (GHS-$R^{-/-}$), and GH deficient rats. Ghrelin or desacyl-ghrelin, which lacks the acyl modification, was administered daily for 4 weeks immediately after disease onset or 4 weeks after streptozotocin injection. Ghrelin administration did not alter food intake, body weight gain, or blood glucose levels, in C57BL/6N mice when compared with mice administered saline or desacyl-ghrelin. Ghrelin administration prevented and alleviated motor and sensory polyneuropathy in C57BL/6N mice in both preventive and therapeutic studies. Ghrelin reduced the plasma concentrations of 8-iso-prostaglandin 2α and ameliorated reductions in fiber number and fiber density of sciatic nerves. In all experiments, desacyl-ghrelin failed to show any effect. Ghrelin prevented the reduction of nerve conduction velocities in GH deficient rats, but not in GHS-$R^{-/-}$ mice. We have completed preliminary clinical application of ghrelin to type 2 diabetic patients without insulin therapy. Ghrelin at 1 μg/kg BW was administered iv after breakfast for 2 weeks. Ghrelin did not alter glucose metabolism or body weight. Ghrelin increased plasma GH level 60 ng/ml in healthy subjects at 15 min after iv injection, while ghrelin increased it to 16 ng/ml in diabetics. Ghrelin improved motor conduction velocity of the tibial nerve, sensory conduction velocity of the sural nerve, and polyneuropathy-related symptoms in all the patients. Thus, ghrelin's effects represent a novel therapeutic paradigm for the treatment of this otherwise intractable disorder. Ghrelin also prevented and alleviated muscle atrophy in a mouse model of disused muscle atrophy. Ghrelin is highly likely to be used as a novel peptide drug for diabetes mellitus and cachexia.

Nothing to Disclose: MN

OR-LB2

Genetic Deletion of Neuronal Peroxisome Proliferator-Activated Receptor Delta Alters Body Composition.

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Peroxisome Proliferator activator receptors (PPARs) are lipid activated nuclear transcription factors that regulate expression of lipid oxidative genes. PPAR delta (PPARd) is the least characterized PPAR isoform and the most widely expressed PPAR in the central nervous system (CNS) where it is enriched in the hypothalamus, a brain region involved in energy homeostasis regulation. Hypothalamic lipid accumulation and CNS insulin and leptin resistance are implicated in the pathogenesis of diet-induced obesity which is marked by fat mass accumulation, resulting in altered body composition and higher adiposity (% fat). Due to the ability of PPARd to increase lipid oxidative genes we hypothesized that PPARd plays a protective role in central insulin and leptin sensitivity. Consistent with the role of PPARd as an insulin sensitizer, neuronal deletion of PPARd (KO) using Nestin Cre-mediated recombination of a floxed PPARd allele results in mice that have altered body composition with elevated fat mass and decreased lean mass on a low fat diet (LFD). Despite lower food intake, consistent with a smaller body size, KO mice had higher feed efficiency (weight gain/kcal) consistent with slower metabolic rates. Total energy expenditure is reduced but normalized energy expenditure (to body weight or lean mass) was not different. Locomotor activity in the dark period was significantly lower in KO mice vs. control (CNT). The altered body composition on LFD is not accompanied by HPA axis dysfunction; diurnal and stress induced corticosterone levels are similar to CNT although KO mice exhibit an exaggerated diurnal rhythm. To assess peripheral anabolic and catabolic rates, acute changes in body weight and body composition were measured in response to fasting and refeeding. No differences in fasting induced reductions of body weight, fat or lean mass (% pre fast) were observed, suggesting rates of lypolysis and muscle catabolism are similar. Following a 24 hour *ad libitum* refeeding period, CNT mice recovered their lost body weight (within 1%), while KO mice weighed 9% less than before the fast ($P<0.05$). Fat mass recovery was not different although CNT mice tended to have increased fat post refeeding. CNT mice recovered their starting lean mass, while KO mice had 10% less lean mass than before the fast ($P<0.05$), indicating compromised anabolic capacity. These data indicate that neuronal PPARd deletion is marked by altered body composition and defective peripheral metabolism.

Sources of Research Support: NRSA/NIDDK Predoctoral Fellowship DK083222; NIH grant 5RO1DK069927-05.

Nothing to Disclose: HEK, RLP, GNL, LJM, KDN

OR-LB3

A Novel Human Prostate Cancer Model Using Adult Prostate Stem/Progenitor Cells.

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Strong evidence exists for the presence of both normal and cancer stem cells in human prostate. Malignant tumors may originate from transformation of resident normal tissue stem cells that both self-renew and differentiate into abnormal progeny that continuously seed tumor growth. The progression of prostate cancer to androgen independence and the disease relapse are increasingly being attributed to prostate cancer stem cells, which remain untouched by conventional therapies. Consequently, we have developed a method to isolate and characterize human prostate stem cells that can regenerate human prostate tissue *in vivo*. Prostate stem cells were isolated and enriched from primary human prostate epithelial cells (PrEC) using a 3-D culture system. Approximately 0.2% of the PrECs, which were characterized as a basal epithelial cell phenotype, can **self-renew** and form prostaspheres. **Regenerative capacity** of these sphere cells was demonstrated by origination of human prostate gland tissue *in vivo* following tissue recombination and renal grafting. **Differentiation** of prostasphere cells in grafts was confirmed using prostate basal and luminal epithelial cell markers. The human origin and functionality of the regenerative grafts were confirmed by expression of human anti-nuclear antigen and secretion of PSA, respectively. Furthermore, we were able to induce prostate cancer in these tissues by exposing to testosterone and 17-β-estradiol during its development. We have generated a true model of human prostate cancer initiation and progression from normal prostate stem cells to malignant. Detailing conversion and progression of normal prostate stem cells to cancer stem cells including epigenetic mechanisms can prove to be a more targeted means of developing future therapeutics that could be more prophylactic than curative.

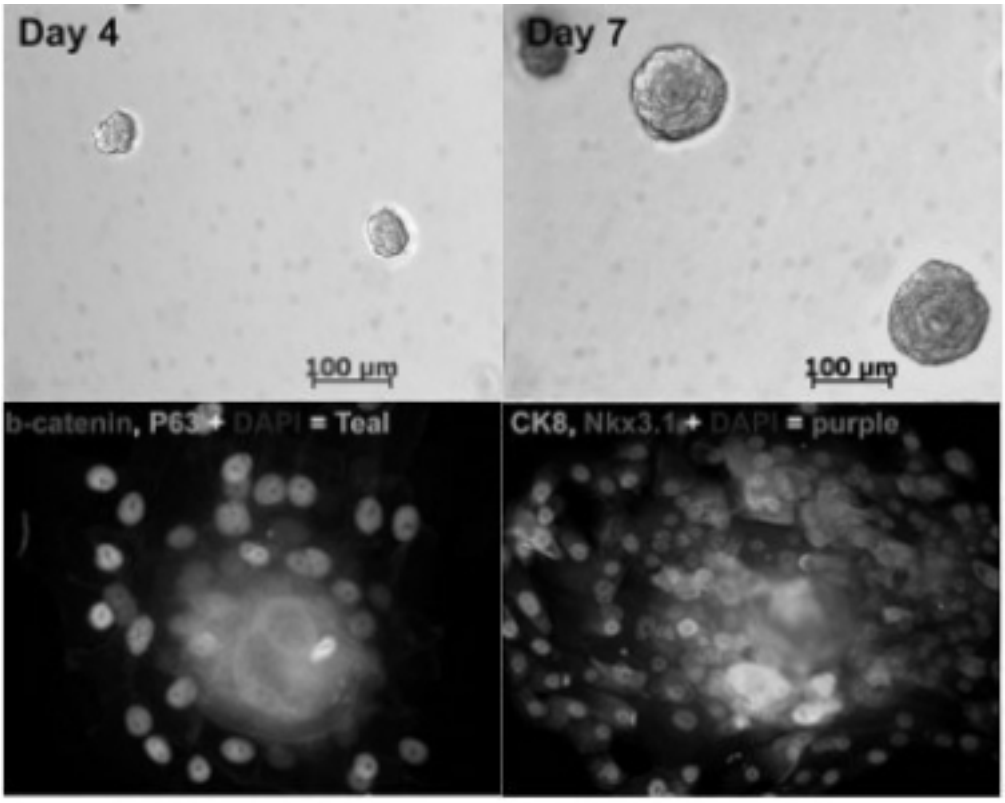

Figure 1 (Left). Upper panel:Day 4-10 prostaspheres. Lower panel:IF staining of β-catenin, p63, CK8, and Nkx3.1. Cells localized to the outer layers of prostaspheres stained positive for p63 but negative for CK8, representing basal type progenitor cells

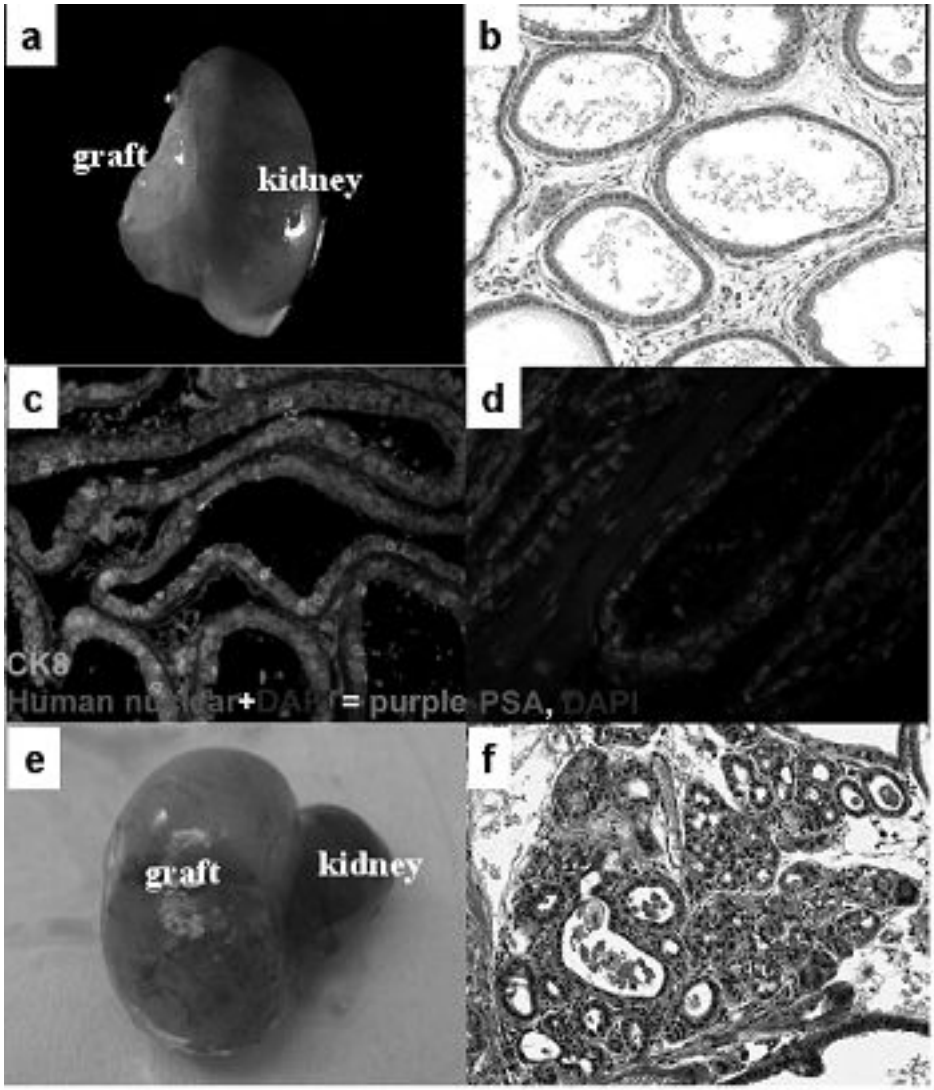

Figure 2 (Right). Human prostate adult stem cells form normal prostate gland structure. Positive staining of human nuclear marker and PSA indicated the human origin and the functional differentiation of the graft (a,b,c,d). Hormone-induced prostate cancer in regenerated grafts (e,f).

Nothing to Disclose: WYH, GBS, ICM, DPH, GSP

OR-LB4

Mineralocorticoid Receptor Signalling in Cardiomyocytes Is Essential for DOC-Induced Cardiac Pathology.

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Mineralocorticoid receptor (MR) signalling in the cardiovascular system has well-described pathological consequences, although the precise mechanisms responsible remain largely unknown. Cardiomyocytes express MR but not the MR-specificity conferring enzyme, 11βHSD2, thus under normal circumstances MR in cardiomyocytes are occupied by endogenous glucocorticoids. The present study explored the role of cardiomyocyte MR in an *in vivo* model of mineralocorticoid/salt-induced cardiac pathology. Cardiomyocyte MR-null mice ($MR^{flox/flox}/MLC2v^{Cre/-}$) were generated using the Cre-*loxP* system of gene deletion and their response to acute (8 days) and chronic (8 weeks) administration of deoxycorticosterone (DOC)/salt was compared with those of control mice ($MR^{flox/flox}$). The acute study explored the role of cardiomyocyte MR-signalling in the early vascular inflammatory events in the DOC/salt model, whereas the chronic study investigated cardiomyocyte MR-signalling in MR-mediated tissue remodelling and hypertension. In the acute model, loss of MR-signalling in cardiomyocytes did not alter DOC/salt-induced monocyte/macrophage recruitment, whereas it prevented early, DOC/salt-induced collagen deposition. Microarray analysis on whole heart homogenate revealed novel gene expression profiles in the different treatment groups at 8 days. In particular, the gene expression profile of the untreated cardiomyocyte MR-null mice highlighted a role for cardiomyocyte MR-signalling in regulating basal gene expression. Immunohistochemical analysis of known DOC/salt-induced inflammatory mediators identified no significant changes in response to deletion of MR from cardiomyocytes at 8 days. In contrast, in the chronic model, loss of cardiomyocyte MR-signalling prevented DOC/salt-induced monocyte/macrophage recruitment, collagen deposition and elevated systolic blood pressure. Although further studies are required to characterise the expression profile of pro-inflammatory mediators at 8 days, the data thus far suggests a direct role for cardiomyocyte MR-signalling in basal gene expression and early, DOC/salt-induced tissue remodelling. The findings of the chronic study suggest that cardiomyocyte MR-signalling may be important during a secondary inflammatory phase in the model, by maintaining monocyte/macrophage recruitment to the myocardium. These data also demonstrate a distinct role for cardiomyocyte MR-signalling in DOC/salt-induced tissue remodelling and blood pressure regulation.

Nothing to Disclose: AJR, JM, MJY

OR-LB5

A Selective Negative Allosteric Modulator of FSHR Suppresses FSH-Induced cAMP and Progesterone Production While Increasing Estradiol Synthesis and FSH Binding.

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Advances in combinatorial chemistry and innovative screening technologies are facilitating the discovery of novel allosteric modulators of cellular G-protein coupled receptors (GPCR). In the present study a unique negative allosteric modulator (NAM, ADX61623) of follicle stimulating hormone receptor (FSHR) is described. Detailed in vitro pharmacological studies of ADX61623 demonstrated (i) no effect when applied alone on different cellular systems, (ii) partial block of human follicle stimulating hormone (hFSH) EC80-induced Ca2+ signal or cAMP accumulation in HEK cells expressing recombinant rat FSHR (rFSHR) or human FSHR (hFSHR), and (iii) results from Schild-plot experiments and reversibility assay at the target on cellular assays were compatible with negative allosteric modulator properties. Further examination showed ADX61623 increased cell-surface binding of 125I-hFSH in a dose-dependent manner revealing potentially unappreciated cooperativity barriers to full ligand binding. Significantly, internalization of the hFSHR-hFSH complex proceeded in the presence of the FSHR NAM clarifying that agonist induced activation is not required for endocytosis of this GPCR. Increased FSH binding in the presence of this NAM was also observed, to a lesser degree in primary cultures of rat granulosa cells, and to HEK293 hFSHR membrane preparations. Thus conformational changes induced in the receptor by this allosteric modulator occurred with physiological levels of receptor and independently of any contribution from the intracellular space respectively. ADX61623 was found to completely suppress hFSH-induced cAMP and progesterone production in primary cultures of rat granulosa cells. Yet, surprisingly, ADX61623 was ineffective in suppressing hFSH-induced estradiol production by rat granulosa cells at a dose that suppressed cAMP and progesterone (10 uM). This unexpected and novel finding illustrates a potential for selective pathway modulation by NAMs and shows that FSH action is parsed out to an additional pathway influenced less by activated protein kinase A. These data illustrate that ADX61623 is a useful tool for selectively impairing one aspect of the FSH signaling without disrupting the whole, which may lead to a more complete understanding of the role FSH plays in estradiol production in granulosa cells. Such data will inform the development of contraceptives, as well as attempts to ameliorate bone loss and estrogen dependent disease.

Sources of Research Support: Addex Pharmaceuticals; NIH HD18407.

Disclosures: BC: Employee, Addex Pharmaceuticals. BB: Employee, Addex Pharmaceuticals. SMP: Employee, Addex. VM: Employee, Addex Pharmaceuticals.
Nothing to Disclose: JAD, RMT, JW, BW

OR-LB6

The miR-200/429 Family Serves as a Potent Regulator of Myometrial Contractility during Pregnancy and Labor.

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Throughout gestation, the uterus is maintained in a quiescent state via the action of progesterone (P4)/progesterone receptor (PR). Near term, increased expression of myometrial genes encoding contractile and gap junction proteins is associated with an enhanced inflammatory response and a decline in PR function. The mechanisms whereby P4/PR and inflammation regulate contractile genes in the pregnant uterus remain unclear. In this study, we investigated the role of miRNAs in the modulation of uterine contractility during pregnancy and labor. Using miRNA and gene expression microarray analyses of uterine tissues of pregnant mice at 15.5 dpc vs. term, we identified a conserved family of miRNAs, miR-200/429, that is highly induced at term, as well as 50 downregulated genes that are predicted targets of this family. Two downregulated miR-200/429 targets encode the zinc finger E-box binding homeobox proteins, ZEB1 & ZEB2, which act as transcriptional repressors and are induced by P4/PR. Illustrating conserved mechanisms from mouse to human, miR-200/429 was upregulated and ZEB1 downregulated in laboring human myometrium, as compared to myometrium of non-laboring women at term. Our findings that overexpression of miR-200/429 in cultured human myometrial cells suppressed expression of ZEB1 & 2 established that ZEBs serve as targets of the miR-200/429 family in myometrium. In two mouse models of premature parturition, intraamniotic LPS injection and maternal RU486 (PR antagonist) treatment, pronounced upregulation of miR-200/429 and downregulation of ZEB1 & 2 were observed in myometrium. Moreover, overexpression of ZEB1 & 2 in cultured human myometrial cells markedly suppressed expression of the contractile genes, oxytocin receptor and connexin-43. To directly evaluate effects of ZEB1 & 2 on myometrial contractility, we conducted collagen gel contraction assays, in which human myometrial cells transduced with recombinant adenoviruses overexpressing ß-galactosidase, ZEB1 or ZEB2 were treated ± oxytocin. Importantly, overexpression of ZEB1 or 2 significantly inhibited oxytocin-mediated contraction of the cultured myocytes. Together, these findings implicate the miR-200/429 family and their targets ZEB1 & 2 as novel regulators of uterine quiescence and contractility during pregnancy and labor and shed new light on their dysregulation in preterm birth.

Sources of Research Support: NIH P01-HD011149; Prematurity Initiative Grant 21-FY07-601 from the March of Dimes Birth Defects Foundation.

Nothing to Disclose: NER, CCC, RDG, CRM

P2-1

The Androgen Receptor Complexes with RNA Splicing Factors – Implications in Prostate Cancer Progression.

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The androgen receptor (AR) is important in the development and progression of prostate cancer (CaP). Clinically, CaP progression is associated with the patient's response to hormone therapy and is often associated with loss of androgen dependence. The mechanism of androgen-independence development is poorly understood, however, the transformation to metastatic CaP has been linked to several somatic mutations and interacting proteins which in turn increase the activity of the receptor. To address the mechanism of AR-mediated transactivation and AI, we have developed a methodology to isolate and characterize AR complexes. It is clear through our initial MS and proteomics analysis that the AR is involved in number of important molecular functions, one of which is RNA splicing.
Steroid receptors have been shown to display non-distinct RNA splicing properties. We have identified AR-interactive proteins with specific transcript splicing selection properties (Sam68, DDX5, and KHSRP). This strongly suggests that the AR may mediate distinct RNA spicing events. We have been able to confirm by reverse co-IPs that these splicing factors interact with AR in an RNA-dependent manner. Furthermore, tissue microarray analysis of Sam68, DDX5 and KHSRP all show increase expression profiles correlating with disease progression.
Using generic *in vitro* mini-gene splicing constructs, we have shown that AR can influence exon selection. Our data also suggest a much more robust effect of the AR on RNA splicing, with the option of having several known RNA splicing factors at its disposal.
We have initially addressed the effect of RNA splicing in CaP by overexpressing a functional GFP-tagged-Sam68 construct in LNCaP cells. LNCaP cells transfected with GFP-Sam68 showed a reduced expression of AR versus mock transfections. Interestingly, this drop in AR expression corresponded with an increase in PSA expression. This reduction in AR protein levels upon overexpression of GFP-Sam68 could be a result of a regulatory function of Sam68 on post-transcriptional AR gene expression, via an alternative splicing event leading to a more active receptor. This is an extremely important observation as there is so little known about regulation of AR gene expression at either the transcriptional or translational levels. The significance of these results are that the AR interacts with transcript specific RNA splicing factors, suggesting that the **AR may play an autoregulatory role in its own expression.**

Nothing to Disclose: MP, RL, LK, MT

P2-2

A 12-Week Pharmacokinetic and Pharmacodynamic Study of Two Selective Androgen Receptor Modulators (SARMs) in Postmenopausal Subjects.

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BACKGROUND/AIMS: SARMs target the androgen receptor in different tissues to elicit a desired androgen effect (increased lean body mass [LBM] and muscle strength) without causing mechanism-based adverse events. This study was designed to identify the SARM-like profiles of MK-3984 and MK-2866 (Ostarine) in healthy postmenopausal (PMP) women by evaluating specific pharmacodynamic endpoints.
METHODS: This was a randomized, double-blind, placebo-controlled, parallel group study in 88 healthy, PMP women (mean age 57 yr, range 46-72) randomized to 4 treatments in a 1:1:1:1 ratio (see Table for procedures).

Procedure	Day
DEXA (LBM)	1 and 84
MRI-thigh muscle volume	1, 28 and 84 (~1/2 of subjects)
One repetition maximum bilateral leg press	1 and 84
5 mm skin punch biopsy	1†, 14‡,84†
TVU	1 and 84
†For sebaceous gland volume and qPCR	
‡For qPCR only	

RESULTS: After 12 weeks of treatment, both MK-3984 and MK-2866 significantly increased LBM from baseline. A mean difference of LBM from baseline versus placebo was observed as 1.54 kg (p-value < 0.001) for both 3 mg of MK-2866 and 50 mg of MK-3984, and 1.74 kg (p-value < 0.001) for 125 mg of MK-3984. All three doses of the SARMs led to an increase in thigh muscle volume compared to placebo at 4 weeks, persisting through 12 weeks of treatment. In addition, there was a dose dependent increase for MK-3984 in leg muscle strength after 12 weeks compared to placebo. MK-3984 and MK-2866 did not significantly increase total sebaceous gland volume compared to placebo. No difference in endometrial thickness as assessed by transvaginal ultrasound (TVU) or in vaginal bleeding post-medroxyprogesterone acetate challenge between either dose of MK-3984 or MK-2866 and placebo was observed. Elevations in liver enzymes of > 3X upper limit of normal at both the 50 and 125 mg qd dose of MK-3984 resulted in the discontinuation of 7 subjects, but did not occur for the 3 mg dose of MK-2866.
Conclusion: Both MK-3984 and MK-2866 are generally safe and well tolerated in PMP women, with the exception of the aforementioned liver enzyme elevations observed with MK-3984. Pharmacodynamic results from this study indicate that both MK-3984 and MK-2866 exhibits SARM-like properties in their ability to increase LBM, thigh muscle volume and leg strength, while having little to no effect on markers of skin androgenization (sebaceous gland volume, sebum excretion rate, assay of androgenization hair follicle gene expression) or endometrial proliferation.

Disclosures: EEM: Employee, Merck & Co. REW: Employee, Merck & Co. YD: Employee, Merck & Co. YX: Employee, Merck & Co. JK: Employee, Merck & Co. YW: Employee, Merck & Co. PHW: Employee, Merck & Co. FL: Employee, Merck & Co. JAC: Employee, Merck & Co. JAW: Employee, Merck & Co. SAS: Employee, Merck & Co.

P2-3

Accelerated Atherosclerosis Associated with Hypertriglyceridemia, Insulin Resistance and Obesity in Female Mice Lacking the Androgen Receptor.

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Objectives: In women, pathologically elevated androgen levels are frequently associated with an adverse metabolic risk profile. However, it remains unknown whether physiological androgen receptor activation influences the metabolic risk profile and atherosclerosis in females. Previous experimental studies are few and the results are inconsistent. In the present study, we investigated the development of atherosclerosis in female mice lacking the androgen receptor.
Methods and Results: Female mice with ubiquitous knockout of the androgen receptor (ARKO) on ApoE-/- background were fed a high fat diet from 8 weeks of age. At 16 weeks of age, atherosclerosis was assessed *en face* and expressed as the percentage of the aorta covered by lesions. Atherosclerosis was increased by 40% ($p<0.05$) in ARKO compared with wild-type (WT) females. This was associated with a marked elevation of serum triglycerides (+45% versus WT; $p<0.05$), while serum total cholesterol levels were unchanged. An insulin tolerance test showed that the ARKO females were insulin resistant (reduction of blood glucose to 48% of initial values in ARKO versus 61% in WT; $p<0.05$), and dual X-ray absorptiometry performed at the end of the study showed increased fat mass in the ARKO females (+64% versus WT; $p<0.001$).
Conclusions: Female ARKO mice display accelerated development of atherosclerosis with hypertriglyceridemia, insulin resistance and obesity as potential underlying factors. These results demonstrate important metabolic effects of androgens in female mice and that the androgen receptor system protects against atherosclerosis in females.

Nothing to Disclose: JB, AW, CA, KDG, GV, AH, AK, CO, AT

P2-4

Anabolic Steroids Activate Calcineurin-NFAT Signaling in Denervated Muscle, and by This Mechanism Reduce Denervation Atrophy.

CP Cardozo MD[1,2], J Pan[1], Y Wu MD[1], WA Bauman MD[1,2] and W Qin MD, PhD[1,2].

[1]James J Peters VA Med Ctr Bronx, NY and [2]Mount Sinai Sch of Med New York, NY.

Nandrolone, an anabolic steroid, slows muscle atrophy after nerve transection, when begun 28 days after denervation and administered together with replacement doses of testosterone. Microarray analysis has demonstrated that one gene downregulated by nandrolone during this period is the calcineurin inhibitor RCAN2, associated with increased calcineurin phosphatase activity in muscle lysates. Calcineurin is a calcium-calmodulin-dependent phosphatase that activates the transcription factor NFAT resulting in nuclear translocation and transcriptional activity of the latter. In L6 myoblasts stably expressing androgen receptor (L6.AR cells), nandrolone reduced RCAN2 and increased calcineurin phosphatase activity. Here we have tested whether these nandrolone-induced changes in calcineurin activity alter nuclear localization and transcriptional activity of a major calcineurin target, NFAT, in denervated gastrocnemius muscle. In this model, the sciatic nerve is transected, and, beginning on day 29 after transfection, animals are administered either nandrolone or vehicle for an additional 28 days. Denervation dramatically reduced nuclear levels of NFATc4 assessed by subcellular fractionation and Western blotting, an effect that was partially reversed by nandrolone. Nandrolone significantly increased mRNA levels for the NFAT-dependent mRNA MCIP1.4 in denervated gastrocnemius muscle. To test whether activation of calcineurin signaling by nandrolone contributed to protection against muscle atrophy, the ability of cyclosporine A, a calcineurin inhibitor, to block protection against denervation atrophy by nandrolone was examined. Cyclosporine A completely blocked the previously noted muscle-preserving effect of nandrolone after denervation. When administered alone, cyclosporine A did not change denervated muscle size. These findings indicate that nandrolone activates calcineurin NFAT signaling in denervated skeletal muscle and suggest that this action of nandrolone contributes to protection against continued denervation atrophy.

Sources of Research Support: The Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development, Rehabilitation Research and Development Service grants B4162C, B3347K and I01 RX000186.

Nothing to Disclose: CPC, JP, YW, WAB, WQ

P2-5

Androgen Receptor Mutants Selected by Prostate Cancer Therapy Impose Differential Gene Regulation.

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Androgen binding to the androgen receptor (AR) is required for prostate cancer initiation and progression, thus androgen ablation is the common treatment for disease. Unfortunately, tumors become resistant to therapy and levels of AR remain high. AR gain-of-function mutations are one mechanism of resistance and arise in response to treatment. We have identified several therapy-selected AR mutants that use diverse mechanisms to promote tumor growth. AR-E255K affects an E3-ubiquitin ligase interaction domain and shows increased receptor stability and nuclear localization in the absence of ligand. Remarkably, an analogous mouse mutation (AR-E235G) is sufficient for tumorigenesis as a prostate-targeted transgene. Another mutant, AR-R753Q, causes androgen insensitivity when germ-line. However, when expressed in PC3 cells AR-R753Q is robust on canonical, but not selective, androgen response elements. This suggests that AR-R753Q activates a subset of AR targets that, while insufficient for male development, provide gain-of-function in tumorigenesis. Contrasting the differential promoter and cell-specific activities of these AR mutants may identify common gene pathways altered in prostate cancer progression and response to therapy.
Our goal is to link differential promoter activation by AR to distinct biological outcomes and gene regulation in prostate cancer. We first generated malignant PC3 and nonmalignant RWPE cells stably harboring wild type (wt) or mutant ARs to compare their hormone-induced growth. Wt AR modestly stimulated PC3 cell growth but suppressed that of RWPEs. AR-E255K was more growth promoting than wt AR. AR-R753Q had the most profound effects, producing the greatest stimulation of PC3 cells and the least inhibition of RWPEs. Lentiviral vectors with mutant ARs have been generated to better probe these aberrant effects. The mutant ARs are being stably expressed in benign (RWPE) vs. malignant (VCaP) cells, with simultaneous knockdown of endogenous AR in VCaP cells. Growth and invasive characteristics of these cell lines will be compared to those with wt AR. PCR arrays provide initial views of differential gene regulation by the mutant and wt ARs, prior to more extensive expression profiling. A better understanding of the phenotypic and gene profiles elicited by these mutant ARs may highlight key nodes in pathways of disease progression rather than differentiation, ultimately allowing selective targeting of AR functions in prostate cancer.

Nothing to Disclose: EWL, JEH, EMS, DMR

P2-6

Small Molecule Inhibitors of Androgen Receptor Identified Using High-Throughput Screening.

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Androgen receptor (AR) bound to androgenic hormones plays a critical role in the growth and distribution of primary and recurrent prostate cancers. Androgen ablation by blocking androgen production, surgical castration, and/or the administration of AR antagonists that compete with androgens for binding to AR, are widely used in prostate cancer therapy. However, the development of resistance to current antagonists underscores the need to develop new types of small molecule inhibitors of AR action. We used protein-based and cell-based high-throughput screens to identify new AR inhibitors. Using purified AR and the fluorescence anisotropy microplate assay we identified a small molecule that inhibits binding of AR to purified androgen response element (ARE) DNA with little or no ability to inhibit binding of estrogen receptor (ER) or progesterone receptor (PR) to their respective DNA response elements. This compound shows moderate potency as an inhibitor of a prostate specific antigen (PSA)-luciferase reporter in intact cells, does not inhibit other steroid receptors and is non-toxic. Using a cell-based assay, we screened ~180,000 small molecules for inhibitors of AR and are currently evaluating the most promising compounds to emerge from this screen. New small molecule inhibitors that act outside the ligand-binding pocket of AR will prove to be powerful new research tools for dissecting AR action.

Sources of Research Support: DOD PCRP.

Nothing to Disclose: MTC, CM, SKN, EMW, DJS

P2-7

Hormone-Refractoriness of Prostate Cancer Cells Is Supported by the Androgen Receptor without Direct Binding to DNA Response Elements.

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Aberrant androgen receptor (AR) signaling pathways play a central role in prostate cancer biology. In the classical view of AR signaling AR binds to its cognate response elements in the target genes to activate transcription. In the present study the role of androgen response elements (ARE) in hormone independent transcriptional signaling was investigated using an androgen independent variant of the LNCaP cell line (LP50 cells). In these cells, proliferation in the absence of hormone was profoundly dependent on endogenous AR and the receptor was not up-regulated. The cells were 'truly' hormone-refractory in that they were not hyper-sensitized to androgen. In these cells AR was unable to bind to AREs in the absence of androgen, although the cells had not lost their typical androgen-responsive gene expression profile which involved androgen-dependent binding to AREs. On the other hand, in the same cells, in the absence of hormone AR activated a different complement of genes that primarily supported cell division. Candidate AR tethering proteins were identified in LP50 cells. Taken together the data imply that AR tethering mechanisms play a critical role in hormone independent transcriptional signaling in prostate cancer.

Sources of Research Support: NIH R01 grant CA 140690 and the Harold & Helen McMaster Endowment to M.R.

Nothing to Disclose: MG, JZ, MdS, HC, AS, VR, RT, MR

P2-8

Truncated Androgen Receptor Gene Expression Is Regulated by Androgen.

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Background: The androgen receptor (AR) is a member of the nuclear receptor super-family, functions as a ligand-inducible transcription factor, and is involved in prostate cancer (PC) growth. If prostate cancer recurs after resection, androgen ablation therapy is the primary mode of treatment. Androgen ablation therapy eventually fails, however, and the resulting castration resistant prostate cancer (CRPC) is the second leading cause of cancer death in men. Truncated AR isoforms (T-ARs), which result from aberrant splicing of AR mRNA, may explain the persistence of AR signaling in CRPC despite androgen ablation. We investigated the mRNA expression of one T-AR isoform, AR3, in prostate cancer cell lines in the presence and absence of androgen.
Hypotheses: We hypothesize that androgen suppresses gene expression of AR3 in prostate cancer cell lines.
Methods: Quantitative PCR for wild type AR, AR3, and GAPDH was carried out in prostate cancer cell lines 22RV1, C4-2, and LNCap. PC3 and HeLa cells were used as negative controls.
Results: The prostate cancer cell line 22RV1 showed the greatest expression of AR3, with levels approximately 40-fold greater than LAPC4. 22RV1 showed a decrease in AR3 mRNA of approximately 30% in the presence of 2 nM dihydrotestosterone (DHT). LAPC4 showed no supression of AR3 expression in the presence of DHT. Cell line C4-2 showed minimal amounts of AR3 expression, with a variable level of supression in the presence of DHT.
Conclusions: We show that in the prostate cancer cell line 22RV1, AR3 mRNA expression is suppressed in the presence of physiologic concentrations of dihydrotestosterone.

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Nothing to Disclose: SNM, HS, MM

P2-9

The Thyromimetic GC-1 Causes Dramatic Fat Loss While Sparing Lean Tissue by Activating Brown Fat.

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Endogenous thyroid hormones, T3 and T4, are key metabolic regulators. However their use as anti-obesity agents is limited due to lean tissue loss and cardiac effects. We tested the thyromimetic GC-1 for its ability to cause weight loss in LDLR-/- and ob/ob obese mice. GC-1 caused dramatic weight loss with little loss of lean tissue and no effect on heart rate. Mice treated with GC-1 had elevated body temperature, elevated metabolic rate, and decreased respiratory exchange rate, indicating a shift from carbohydrate to fat oxidation. Gene expression profiling of tissues from treated mice suggested brown adipose tissue (BAT) to be the primary target of GC-1's metabolic actions. UCP1 levels increased 5-fold and a large increase in the metabolic activity of BAT was observed via PET imaging of GC-1 treated mice. Thus GC-1 appears to elicit an adaptive thermogenic response, and we suggest brown fat to be the primary tissue responsible for the anti-obesity effects of GC-1 and potentially other thyroid hormones.

Nothing to Disclose: KJP, JZL, LJM, AAG, ZZS, WAH, PW, JDB

P2-10

The Effect of Thyroid Hormone Signaling on Testicular Steroidogenesis.

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A role for thyroid hormones has long been implicated in mammalian testicular function. Thyroid hormones affect the steroidogenesis in Leydig cells at the transcriptional level of steroidogenic enzymes, although its molecular mechanism has not been well understood. To investigate the molecular action of thyroid hormones in the steroidogenesis, we executed several experiments using a mouse Leydig cell line. Thyroid hormone receptors are expressed in Leydig cells which produces steroid hormones in the testis. T_3 signaling in Leydig cells differently affect the expression of steroidogenic enzymes; it increased the expression of P450c17, while it decreased the expression of StAR and 3β-HSD. Transient transfection analyses with promoter-reporters also showed that TRα/T_3 singaling affects the promoter activity of steroidogenic enzyme genes in a similar way. To understand this differential effect of TRα/T_3 signaling, we explored its direct and/or indirect actions on the steroidogenic enzyme promoteres by analyzing TRE in the promoters and the protein-protein interaction with transcription factors that regulate the expression of steroidogenic enzyme genes. Sequence and functional analyses revealed that the P450c17 promoter contained the thyroid response element (TRE), while others did not, which would mediate a direct action of TRα/T_3. As one of indirect actions of TRα/T_3 signaling, we investigated its interaction with the orphan nuclear receptor Nur77, which is one of major transcription factors that regulate the expression of steroidogenic enzyme genes in Leydig cells. The results revealed that TRα interacted with Nur77 and modulated its transactivation on steroidogenic enzyme gene promoters. All together, Thyroid hormone/thyroid hormone receptor affects the expression of steroidogenic enzyme genes via direct and indirect actions, resulting in the modulation of the steroidogenesis in testicular Leydig cells. This suggests the important role of thyroid hormone signaling in testicular steroidogenesis.

Nothing to Disclose: YK, KL

P2-11

Isoform Specific Gene Regulation by Thyroid Hormone Receptors.

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Despite high homology between TR isoforms, they evoke differential responses in animal models. While TRα mediates harmful effects on heart rate, TRβ appears responsible for beneficial effects on cholesterol and bodyweight. To investigate the reason for these differential responses we created cell lines capable of induced expression of TRα or TRβ. In contrast to results obtained from whole tissues, gene expression profiling of cell lines artificially expressing either TRα or TRβ revealed >95% of genes to be regulated similarly by both TR isoforms. Additionally, both isoforms responded comparably to TR agonists such as T3. We conclude that isoform selective gene regulation by TRs is rare and suggest that the beneficial effects of isoform selective TR modulation may arise from tissue specific distribution of the TR isoforms.

Nothing to Disclose: JZL, JDB, PW

P2-12

Modeling, Synthesis and Biological Evaluation of Potential Retinoid X Receptor (RXR) Selective Agonists for the Treatment of Cutaneous T-Cell Lymphoma.

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Retinoids, a class of compounds derived from vitamin A, can be used clinically to treat a variety of skin disorders and certain cancers. An isoform of vitamin A, 9-cis retinoic acid (9-cis RA), binds to the nuclear retinoid X receptor (RXR) and induces RXR homodimer formation and RXR-mediated transcription. Bexarotene (Bex) is a synthetic ligand (rexinoid) modeled after 9-cis RA that is indicated for treatment of cutaneous T-cell lymphoma (CTCL). Bex can stimulate RXR homodimer formation and modulate the activity of 9-cis RA target genes. However, Bex can also dysregulate other RXR-requiring pathways since other nuclear receptors (e.g., retinoic acid and vitamin D receptors) form heterodimers with RXR. Therefore, we sought to model (via docking studies) and synthesize novel analogs of Bex, including nitro- and fluoro-substituted compounds, that bind RXR and mediate regulation of anti-tumor genes without disrupting other RXR pathways. Employing both a mammalian two-hybrid system, and an RXRE-mediated transcriptional assay, we tested 19 analogs of Bex and discovered three compounds that best induce homodimerization and RXR-mediated transcriptional activity (20-120% of Bex). These three analogs also stimulate significant apoptosis and cytotoxicity in CTCL cells, and have similar (analog 16 and 18) or better (analog 20) Ki and EC50 values when compared to the Bex parent compound. These observations are consistent with modeling studies that predicted analogs 16 and 18 would possess the best binding affinities. We also evaluated Bex and the three analogs for their residual retinoic acid receptor (RAR) agonist activity employing expression of human RAR and a retinoic acid responsive element (RARE)-luciferase reporter system and found that these three analogs are selective RXR agonists (especially analog 18). Taken together, these results suggest that modification of Bex with a halogen atom on the aromatic ring that bears the carboxylic acid may reduce the activation of RAR (analog 18), or increase its ability to activate RXR (analog 20). Based on these novel findings, we have synthesized ten new halogenated compounds and preliminary results reveal that RXR agonist activity is attenuated with decreasing electronegativity but increasing size of the halogen functional group, further refining our hypothesis. In conclusion, our experimental approach suggests that rational drug design can be employed to develop rexinoids with improved biological properties.

Nothing to Disclose: JKF, CEW, AvdV, SLB, PAM, PWJ

P2-13

Sentrin/SUMO Specific Proteases as Novel Tissue-Selective Co-Activator Proteins That Modulate Signalling by Vitamin D Receptor and Retinoid X Receptor Alpha.

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Employing a reporter assay-based screen, we investigated the role of reversible sumoylation within the activities of a selected panel of nuclear receptors. We report that co-expression of sentrin/SUMO-specific protease 1 (SENP1), that can function to remove small ubiquitin-like modifier (SUMO) from target proteins, can strikingly potentiate ligand-mediated transactivation by the vitamin D receptor (VDR) and retinoid X receptor alpha (RXRα) with comparatively little impact upon the responses of other closely related nuclear receptors. In extending our analysis to include additional members of the SENP family, we find that their modulation of VDR activity is cell line-dependent, with SENP1 and SENP6 eliciting strongest effects in Caco-2 colon cancer and HEK-293 embryonic kidney cells, respectively, while in MCF-7 breast cancer cells the vitamin D response is unaffected by any tested SENP. In addition, when investigating the endogenous activities of such vitamin D target genes as CYP24 in Caco-2 cells, we observe that their calcitriol-induced expression is remarkably enhanced as a result of SENP1 overexpression. We also report that in its unliganded state, RXRα is a SUMOylated protein and that this modification is reversed upon binding of its 9-*cis* retinoic acid ligand, thus supporting the concept that deSUMOylation is a pivotal component within the transactivation of this receptor. In contrast with a previous report, we note that among the isoforms we evaluated, activation of RXRα by its 9-*cis* retinoic acid or bexarotene ligands is heightened most effectively through SENP1, a feature we consistently observe in the context of all cell lines used in this study. In support of a potential function as novel co-activators of VDR and RXRα, we find that SENP1, 2 and 6 are capable of forming complexes with each receptor. In combination, our results reveal that SENPs may represent a novel class of VDR and RXR-interacting proteins that likely serve to modulate apparent repressive effects of sumoylation upon the vitamin D and rexinoid signaling pathways and which could have important implications to the functionality of both RXR-containing homo and heterodimeric complexes. Finally, as several tumours display altered SENP expression and modified protein SUMOylation patterns, we hypothesize that enhanced VDR and/or RXRα SUMOylation status in select human cancers may limit the antiproliferative actions of vitamin D during tumorigenesis.

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Nothing to Disclose: WPL, DD, JF, TW, PJ, PT

P2-14

Stimulation of Sirt1-Regulated FoxO Protein Function by the Hormone-Bound Vitamin D Receptor.

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Hormonal vitamin D, 1,25-dihydroxyvitamin D (1,25D), signals through the nuclear vitamin D receptor (VDR). 1,25D regulates cell proliferation and differentiation, and has been identified as a cancer chemopreventive agent. FoxO proteins are transcription factors that control cell proliferation and survival. They function as tumor suppressors and are associated with longevity in several organisms. Accumulating data has revealed that 1,25D and FoxO proteins similarly regulate a set of common target genes. We have found that the ligand-bound VDR regulates post-translational modification and function of FoxO proteins. 1,25D treatment enhances binding of FoxO3a and FoxO4 within 4h to promoters of FoxO target genes, and blocks mitogen-induced FoxO protein nuclear export. The VDR coimmunoprecipitates with FoxO proteins and their regulators, the Sirtuin 1 (Sirt1) class III histone deacetylase (HDAC) and protein phosphatase 1. In addition, phosphatase activity and trichostatin A-resistant HDAC activity coimmunoprecipitate with the VDR. 1,25D treatment rapidly (within 4h) induces FoxO deacetlyation and dephosphorylation, consistent with activation. In contrast, ablation of Sirt1 expression markedly enhances FoxO3a phosphorylation, consistent with the coupling of FoxO acetylation and phosphorylation. 1,25D regulation of common VDR/FoxO target genes is attenuated by blockade of phosphatase activity, or by siRNA-medicated knockdown of Sirt 1 or FoxO protein expression. Finally, 1,25D-dependent cell cycle arrest is attenuated or blocked in FoxO4 or FoxO3a-deficient cells, respectively, indicating that FoxO proteins are key downstream mediators of the antiproliferative actions of 1,25D. These studies link 1,25D signaling directly to Sirt1 and FoxO function and provide a molecular basis for its cancer chemopreventive actions.

Nothing to Disclose: BSA, LET-M, VD, JHW

P2-15

Vitamin D Regulation of the Epidermal Differentiation Complex To Maintain Healthy Skin and Curb Inflammatory Epidermal Diseases.

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In this study we investigate the ability of the 1,25-dihydroxyvitamin D (1,25D)-liganded vitamin D receptor (VDR) to directly control the transcription of genes within the Epidermal Differentiation Complex (EDC). This approximately 1.7 Mb cluster on human chromosome 1q21 encodes at least 65 genes, largely conserved among mammalian species, that are essential for proper cutaneous differentiation and barrier function. Dysregulation of genes within the EDC locus is associated with inflammatory skin diseases such as psoriasis and atopic dermatitis. We have generated microarray and preliminary real-time PCR data from cultured human epithelial tissues that demonstrate VDR modulation of 16 EDC genes. Two additional genes are reported in the literature to be VDR-dependent (1). Taken together, these results indicate that nearly 28% of the cluster is transcriptionally influenced by liganded VDR. We have confirmed 1,25D regulation of multiple genes, including S100A2, S100A8 and S100A9, by real time PCR, with effects ranging from a 1.4-fold upregulation after 18 hrs (S100A2) to a 1.9-fold downregulation after only 3hrs of 1,25D treatment (S100A8). A bioinformatics approach was used to identify candidate vitamin D responsive elements (VDREs) within the EDC locus. Direct binding of VDR to candidate VDREs was then demonstrated via gel mobility shift assays. VDREs shown to bind VDR were subsequently cloned into luciferase reporter constructs for verification of their ability to confer 1,25D regulation onto a heterologous reporter gene. These results assume added significance in view of the well-documented use of 1,25D analogs in the clinical treatment of psoriasis and other skin conditions. Given the incomplete nature of the data so far, we propose that there are likely many additional vitamin D target genes at this locus. Indeed, we hypothesize that further regions of the EDC may be regulated via 1,25D and VDR, establishing a novel mechanism whereby a nuclear hormone receptor may exert control over entire clusters of related genes to orchestrate coordinated bioresponses that, in the case of the EDC, maintain epidermal barrier integrity and healthy skin.

(1) Hawker et al., J Invest Dermatol 2006: 127:874

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Nothing to Disclose: EWM, JSP, GKW, CAH, J-CH, DH, PWJ, MRH

P2-16

Tumor Necrosis Factor Alpha Inhibits Intestinal Steroidogenesis Via c-Jun and NFκB That Suppress LRH-1-Mediated Cyp11a1 Expression.

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Glucocorticoids are steroids that suppress inflammatory immune response. Recent findings show that LRH-1 (NR5A2) is an important transcription factor for the maintenance of mucosal homeostasis and protection against inflammatory bowel disease (IBD) by controlling proliferation and local glucocorticoid synthesis of intestinal crypt cells. However, the mechanism for the control of LRH-1 activity and intestinal glucocorticoid synthesis has not been clearly defined. Here we show that intestinal glucocorticoid synthesis is inhibited by a pro-inflammatory cytokine TNF-α, which plays an important role in the pathogenesis of IBD. TNF-α suppresses intestinal glucocorticoid synthesis by inhibiting LRH-1-mediated expression of the Cyp11a1 and Cyp11b1 genes, which encode enzymes in the glucocorticoid synthesis pathway. JNK and NFκB pathways are involved in this repression, as inhibitors of JNK and NFκB blocked the effect of TNF-α. The downstream mediator of the JNK pathway, c-Jun, as well as NFκB proteins repressed LRH-1 transcriptional activity and promoter activities of Cyp11a1 and Cyp11b1. The inhibition by c-Jun was eliminated when the LRH-1-binding site of the Cyp11a1 promoter was mutated. In addition, LRH-1 physically interacted with c-Jun or NFκB proteins, as shown by coimmunoprecipitation analysis. These results indicate that c-Jun and NFκB may act as co-repressor of LRH-1. Mice with a mutation in the LRH-1-binding site of its Cyp11a1 promoter secreted less intestinal glucocorticoids and suffered from more severe IBD. Thus TNF-α contributes to gut mucosal inflammation by down-regulating intestinal glucocorticoid synthesis through the JNK and NFκB signaling pathways. These findings provide a molecular mechanism underlying intestinal chronic inflammation by TNF-α, and define the regulators of LRH-1 transcriptional activity in the intestine.

Nothing to Disclose: SCH, BCC

P2-17

Regulation of the Inhibin α Subunit Gene by the NR4A Orphan Nuclear Receptors in Ovarian Granulosa Cells.

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Nuclear receptors play important roles in regulating gene expression in response to hormonal ligands. Some nuclear receptors exhibit ligand-independent binding and regulation of gene expression. One such family is the NR5A nuclear receptors steroidogenic factor 1 (SF-1) and liver receptor homolog 1 (LRH-1), which play key roles in regulating many ovarian genes and are important for female fertility. One target of the NR5A proteins is the inhibin α subunit gene, which is dynamically regulated by follicle-stimulating hormone (FSH) and luteinizing hormone (LH) during the rodent estrous cycle. We previously found that the NR4A orphan nuclear receptor nerve growth factor inducible B (NGFI-B) can act to repress inhibin α expression. In this study, we sought to more broadly investigate roles for the NR4A orphan nuclear receptors, including nuclear receptor related 1 (NURR1) and neuron-derived orphan receptor 1 (NOR-1), in inhibin α regulation.
We demonstrate that all three NR4A mRNAs are rapidly and robustly induced in the rat ovary in response to hCG treatment *in vivo* and this up-regulation coincides with the down-regulation of the inhibin α mRNA. The NR4A mRNAs are also rapidly induced by forskolin in a mouse granulosa cell line, GRMO2. Transient transfections in GRMO2 cells show that the NR4A factors are capable of repressing inhibin α promoter activity. We mapped the promoter region required for NR4A repression to the -166 bp proximal promoter, which contains binding sites for the NR5A receptors and CREB flanked by upstream (5') and downstream (3') GATA factor binding sites. Gel shift assays and chromatin immunoprecipitation failed to reveal binding of the NR4A proteins to this region. Amino acid mutations were made that have been shown to structurally disrupt DNA-binding. All of the mutants were expressed comparably to wild-type proteins, but were unable to bind a consensus NR4A binding site or transactivate a reporter construct containing three consensus binding sites. The mutant proteins still repressed the inhibin α promoter, confirming that DNA-binding is not required for NR4A repression. Mutation of the 5' GATA site inhibited repression by the NR4A proteins. Ongoing experiments are investigating NR4A interactions with GATA proteins to understand the mechanism of repression. The expression and functions of the NR4A factors in human granulosa-lutein cells are also being examined, where inhibin A remains elevated following the mid-cycle LH surge.

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Nothing to Disclose: KMM, WBP, ADB, KEM

P2-18

Estrogen Inhibits Cardiac Fibrosis through ERbeta Rapid Signaling.

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Progression from cardiac hypertrophy to cardiac failure is the most common cause of death in humans. Estrogen (E2 as 17-beta-estradiol) prevents important aspects of the development of cardiac hypertrophy in-vivo and in-vitro. One aspect is preventing fibrosis that accelerates cardiac muscle thinning, dilation, and failure. To understand the molecular details, we carried out studies in freshly isolated, neonatal rat cardiac fibroblasts. Angiotensin II (Ang II) and endothelin-1 (ET-1) are the most important hypertrophic agents in humans and we found that 10^{-7}M AngII or ET-1 induces fibroblast to myofibroblast transition, identified by morphology changes and alpha-smooth muscle actin expression. This was accompanied by enhanced production of vimentin and fibronectin proteins, all significantly inhibited by 1-10nM E2 or the ERbeta specific agonist, DPN, reversed by ICI182780 (ER antagonist). ERbeta was found to be expressed in the fibroblasts and myofibroblasts. AngII and ET-1 stimulated production of the fibrosis-inducing factor, TGFbeta1, a cytokine that we showed caused phosphorylation of the transcription factor SMAD3 at serines 423/425, resulting in translocation of SMAD to the nucleus of the myofibroblast. As a result, AngII, ET-1 and exogenous TGFbeta1 caused strong production of Type I and Type III collagen synthesis in myofibroblasts, the major collagens underlying fibrosis in heart disease. All these events were substantially and equivalently prevented by E2 or DPN. E2 or DPN stimulated rapid (5 minute) cAMP production in fibroblasts, blocking collagen synthesis in part due to inhibiting AngII, ET-1, or TGFbeta- induced ERK and JNK signaling that produces fibrosis. In WT ovariectomized female mice infused with AngII for 2 weeks, E2 replacement prevented many of these events for cardiac hypertrophy and fibrosis. However, E2 was unable to do this in ERbeta KO mice. In summary, fibrosis is a key element of progression to cardiac failure and is almost completely inhibited by rapid signaling from E2 engaging ERbeta in cardiac fibroblasts. Our results suggest that ERbeta agonists might prevent certain forms of heart disease and would not cause breast or uterine proliferation that is mediated through ERalpha. These events may also underlie the known ability of E2 to prevent fibrosis resulting from inflammatory liver and perhaps lung diseases.

Sources of Research Support: NIH Grant CA100366; VA Merit Review Grant awarded to EL.

Nothing to Disclose: AP, MR, DL, EL

P2-19

Progesterone and Progestins Influence PI3K/AKT/GSK3b Signaling and Cell Proliferation of Myometrial and Leiomyoma Cells in Culture.

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Leiomyoma causes significant morbidity in 25% of women in their 30's. Progesterone (P), PI3K, and AKT are thought to be important for tumor growth although detailed signaling leading to proliferation are unknown. We hypothesize that leiomyoma and myometrial cells will exhibit distinct patterns of PI3K/AKT activation and cell proliferation in response to P. We first examined immortalized leiomyoma (DD-HLM) and myometrial (myo-hTERT) cell proliferation in the presence of R5020 (R5) and LY294002 (LY). Treatment with 100nM R5 for 5 days promoted cell proliferation of DD-HLM cells while myo-hTERT cells experienced a decrease in cell proliferation. Interestingly, myo-hTERT cells grown in the presence of LY alone and with R5 showed a significant decrease in cell proliferation compared to untreated controls, while LY had no effect on DD-HLM cell proliferation.
We next examined activation of AKT and GSK3b following treatment with P and LY. AKT phosphorylation (p-AKT) and GSK3b phosphorylation (p-GSK3b) increased in a time dependent manner in myo-hTERT and DD-HLM cells following treatment with 100nM P. To verify that AKT, and GSK3b phosphorylation in response to P was due to activation of PI3K, we treated cells with LY, washed away the inhibitor, and added fresh media with or without P. P enhanced p-AKT and p-GSK3b levels after LY was removed compared to vehicle treated cells. Overexpression of wild type p85a, the regulatory subunit of PI3K, enhanced p-AKT and p-GSK3b in response to P and at the basal level, while dominant negative p85a reduced p-AKT and p-GSK3b. In conclusion, progestins and P can influence immortalized leiomyoma and myometrial cells' proliferation and PI3K signaling. Differences between tumor and normal cells were seen at the level of cell proliferation while PI3K and AKT signaling in response to P were similar. A possible reason for the similarities is that P and PI3K may regulate different effectors to ultimately influence cell proliferation over time while rapid signaling remains similar between the cell types. These results also indicate that P can activate PI3K and that AKT and GSK3b are activated downstream. The distinction between myo-hTERT and DD-HLM cell proliferation in response to R5 and LY warrants further study of leiomyoma and myometrial cells' PI3K biology and P regulated signaling.

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Nothing to Disclose: ECS, JJK

P2-20

Characterization of a New Natural Compound Evodiamine as an Estrogen Receptor Down Regulator in Cancer Cells.

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Evodia rutaecarpa is a popular multi-purpose herb traditionally used in China for the treatment of headaches, abdominal pain, postpartum hemorrhage, dysentery and amenorrhea. Evodiamine, a biologically active alkaloid isolated *Evodia rutaecarpa*, exhibits antioxidant, anti-inflammatory, and anticancer activities. Recently, evodiamine-containing herbal products are employed as a nutritional weight-loss supplement by reducing the uptake of fat and increasing the natural rate of burning fat. The current study investigates the anti-estrogenic properties of evodiamine, including its ability to bind to estrogen receptors, its impact on breast and uterine endometrium cancer cell growth, and its inhibitory effects on the estrogen target genes, compared with other antiestrogenic agents such as tamoxifen and fulvestrant. Our studies showed that evodiamine suppressed the 17β-estradiol (E_2)-induced human breast and endometrial cancer cell growth. Evodiamine also inhibited the E_2-induced ERE-luciferase reporter gene activity and the expression of estrogen target genes such as c-myc and cyclin D1 in MCF-7 cells. We also found that evodiamine bound to and induced nuclear matrix immobilization of endogenous estrogen receptor alpha (ERα) in MCF-7 cells, resulting in a decrease in the levels of ERα expression. Interestingly, the down regulation did not occur with ectopic ERα transfected into Hela cells. We conclude from the data that evodiamine is a estrogen receptor down regulator that inhibits estrogen signaling in human cancer cells through the reduction of E_2-ER binding and the decreased expression of the ERα.

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Nothing to Disclose: AKWT, JT, MH, SVN, XZ, WB

P2-21

Estrogens Increase Cyclic AMP Response Element Binding Protein (CREB) Phosphorylation and Cyclin D1 Expression in Rat Sertoli Cells.

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Introduction: 17β-estradiol (E2) activates a translocation of estrogen receptors (ESRs) to the plasma membrane mediated by Src (Src family of tyrosine kinases), which results in the activation of EGFR (epidermal growth factor receptor) and Erk1/2. Activation of ESRs by E2 is also involved in proliferation of immature Sertoli cells (1) and may suppress differentiation (2), and activation of ESRs by 5α-androstane-3β, 17β-diol modulates gene transcription in mouse Sertoli cells line (3). E2-stimulated growth is mainly through the induction of G_1 to S-phase transition that is associated with upregulation of c-myc, which controls Cyclin D1 expression (reviewed in 4). In the present study, we report the effect of E2 on cyclic AMP response element binding protein (CREB) activity and Cyclin D1 expression in rat Sertoli cells. **Methods and Results:** Primary cell culture of Sertoli cells was obtained from 15-day old Wistar rats (1). Western Blot assays were carried out to determine CREB activity and Cyclin D1 expression. E2 (10^{-10} M, 35°C) induced a rapid and transient increase in the phosphorylation state of CREB. Peak CREB phosphorylation occurred at 10 min of E2 treatment (4-fold increase), with CREB activity returning to basal levels after 30 min. The activation of CREB phosphorylation induced by E2 was blocked by pre-treatment with EGF receptor kinase inhibitor AG 1478 (50 µM, 15 min) and MEK1/2 inhibitor U0126 (20 µM, 30 min), indicating that EGFR and Erk1/2 are upstream components regulating this effect. E2 treatment for 24 hours (10^{-10} M, 35°C) also increased Cyclin D1 expression 78% above basal level, suggesting that the mitogenic effect of E2 triggers upregulation of cyclin D1 in the Sertoli cells. ICI 182,780 also blocked the effect of E2 on Cyclin expression, indicating that ESRs are upstream components regulating this effect. **Conclusion:** These results indicate that in Sertoli cells E2-ESR may regulate gene expression involved with cell proliferation. ESR may mediate E2 actions important for Sertoli cell function and maintenance of homeostasis.

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Sources of Research Support: FAPESP, CNPq.

Nothing to Disclose: CR, TFGL, MFML, CSP

P2-22

Elucidating a Functional Role for Site-Specific Phosphorylation of the Mouse Progesterone Receptor Serine 191.

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Progesterone receptor (PR), a hormone activated transcription factor, is expressed as two isoforms, PR-B (933 a.a.) and PR-A, which lacks the first 164 a.a. of PR-B. The primary PR target tissues are uterus, mammary gland, and ovary, although actions in other tissues including brain and thymus are also well established. PR is required for a variety of reproductive functions and mouse models lacking PR exhibit a variety of abnormalities in the mammary gland, uterus, and ovary and are infertile. Our long term goal is to understand how site-specific protein phosphorylation regulates the activity of PR. Phosphorylation is an integral regulator of protein/protein interactions. Understanding PR function requires the elucidation of how these complex interactions are regulated. Ten phosphorylation sites have been identified previously in human PR, all but one found in Ser-Pro motifs that are targets of proline directed kinases. This study sought to elucidate the site-specific role of one of these sites, Ser190 which is phosphorylated in both PR-A and PR-B and is increased in response to hormone. Comparative genomics reveal that this Ser-Pro site is conserved in the mouse and immunoblotting mouse uterus lysate with a phospho-specific antibody to the corresponding human site verified this site *in vivo*. To test the function of PR Ser190 phosphorylation *in vivo*, we engineered a PR-S191A mutation in the mouse PR that corresponds to the human PR Ser190. $PR^{S191A/S191A}$ mutant female mice showed a typical uterine decidual response and mammary ductal elongation, branching and lobuloalveolar development. However, our results reveal a potential functional role for PR Ser191 phosphorylation as $PR^{S191A/S191A}$ mutant mice have decreased fertility and slightly extended estrous cycles. Specifically, mutant females exhibit a 32% increase in the time between litters over a period of 6 months although there is no delay in the arrival of the first litter nor is there a shortened reproductive lifespan. Analysis of the estrous cycle over a period of 60 days reveals a 6% increase in the length of cycles which may partially explain the fertility defect. Future studies will attempt to determine which PR target genes may play a role in this defect and whether this apparent decrease in fertility is due to defective implantation or aberrant reproductive behaviors or both.

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Nothing to Disclose: RDW, JDD, J-SK, JWJ, AM, HLF, FJD, JPL, DPE, NLW

P2-23
Effects of Androgens on Vascular Cell Physiology.

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Atherosclerosis forms the pathological basis of most (over 90%) clinical cardiovascular disease. The condition affects both males and females but males are more affected, suggesting a likely role for both male and female hormones. Although considerable data are available regarding the effects of estrogens on the physiology of vascular cells much less is known about the effects of androgens. This study sought to examine the impact of testosterone (T), dihydrotestosterone (DHT) and dehydroepiandrostenedione (DHEA) on vascular endothelial cells (EC) and vascular smooth muscle cells (VSMC) cultured in vitro. EC and VSMC were incubated with T, DHT or DHEA at physiological (5nM) and supraphysiological (50nM) concentrations. Cell growth and death were assessed by cell number counting, DNA synthesis by [3H]-thymidine incorporation assay, collagen synthesis by [3H]-proline incorporation assay, and gene protein expression by Western blotting analysis. It was shown that: (1) DHEA, but not T or DHT, protected EC from superoxide (H2O2) injury (p=0.03 cf. control) and the effect was not blocked by the androgen receptor (AR) antagonist flutamide (100nM), suggesting an AR-independent mechanism; (2) T stimulated DNA synthesis and cell growth in EC (p=0.008 & 0.03 respectively) via AR-independent mechanism(s), while DHT inhibited DNA synthesis and cell growth (p=0.05 & 0.03) in an AR-dependent manner; (3) T activated mitogen-activated protein kinase ERK1/2 activity via AR-independent mechanism(s); (4) T stimulated VSMC proliferation (p= 0.005), but DHT and DHEA did not, the effect being independent of AR; and (5) T and DHT inhibited collagen synthesis in VSMC (p= 0.001 & 0.008) in an AR-dependent manner. We conclude that androgens produce multiple effects on vascular cells via either AR-dependent or AR-independent mechanisms. Some effects, such as protection of EC injury by DHEA, enhancement of EC growth by T and inhibition of collagen synthesis by T and DHT, are potentially beneficial in that they may inhibit aspects of the atherosclerotic process. In contrast, other effects, such as reduction of EC growth by DHT and stimulation of VSMC proliferation by T, are potentially harmful in that they may promote it. Further study is needed to elucidate the significance of these in vitro effects of androgens with respect both to physiological and clinical outcomes.

Nothing to Disclose: SL, LN, LN, PAK

P2-24

Gonadal Steroids and Experimental Myocardial Infarction.

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Ischemic heart disease remains a major cause of morbidity and mortality despite therapeutic percutaneous coronary intervention strategies. Gender differences have been noted for the myocardial response to ischemic injury with men more likely to have an ischemic coronary event than women and at an earlier age. There is considerable controversy over the cardioprotective effects of estrogen administration. Slight reductions in androgen levels have also been reported after menopause and there is even less information about the direct cardiac effects of physiological levels of androgens. **Aim:** To determine the role of gonadal steroids in modulating cardiac damage during myocardial ischemia/reperfusion (I/R.). **Methods:** Mature age-matched male and female Sprague Dawley (SD) rats were used intact or surgically gonadectomised (Gx); half the gondectomised rats received subcutaneous replacement testosterone (T) or estradiol (E) implants for 21 days. Animals were killed under anesthesia and hearts isolated and subjected to ischemia (30min) followed by reperfusion (2.5hr) in a Langendorf apparatus. At the completion of reperfusion, hearts were stained with monastral blue and triphenyl-tetrazolium to highlight the area-at-risk (AAR) and infarct area (IA) respectively. Apoptosis in the myocardium was assessed by both in-situ nick end-labelling (TUNEL) and 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) on tissue sections. **Results:** Hearts from intact male rats showed significantly larger infarct size (44 ± 1%, N=6) than those from female rats (39 ± 1%, N=5, $p<0.05$). Preliminary results show that gonadectomy aggravated infarct size (44 ± 1%, N=5) in females, but in males lessened the extent of damage (39 ± 3%, N=6), abolishing the gender-difference in response seen in intact rats. Serum levels confirmed surgical Gx: T levels were reduced <0.7 nmol/L (n=8) and estradiol levels in female rats fell to <37 pmol/L (n=6) following gonadectomy. In yet to be completed analysis, testosterone replacement restored infarct size to levels not different from control (42 ± 4%, N=6 versus 44 ± 1%, N=6). Measurements of apoptosis are in progress. **Conclusion:** Our results show that I/R damage is aggravated by physiological levels of testosterone whereas estradiol limits the extent of damage.

Nothing to Disclose: ASM, TYLL, MM, AA, JWF

P2-25

The Effect of ERa and ERb Specific Agonists on Cell Proliferation and Energy Metabolism in Human Vascular Smooth Muscle Cells.

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In cultured human vascular smooth muscle cells (VSMC) estradiol-17b (E2) induced a biphasic effect on DNA synthesis, i.e., stimulation at low and inhibition at high concentrations, whereas the specific activity of the brain isozyme of creatine kinase (CK) was dose- dependently stimulated. We now investigate the effects of ERa and ERb specific agonists compared to E2 on different parameters in vascular cells. VSMC were treated with 0.3 or 30nM E2, 42 or 420nM 2,3-bis (4-hydroxyphenyl)-propionitrile (DPN ;ERab specific agonist) and 39 or 390nM 4,4',4''-[4-Propyl-(1H)-pyrazol-1,3,5-triyl]tris-phenol (PPT;ERa specific agonist) and the effects on DNA synthesis, CK, the expression of mRNA for ERs, 12 lipooxygenase (12LO), 15 lipooxygenase (15LO), 1a vitamin D hydroxylase and ROS production were analysed. Treatment with PPT at both concentrations increased DNA synthesis, while DPN at both doses inhibited DNA synthesis, and the effect of E2 on cell proliferation was biphasic. PPT and DPN similar to E2 stimulated dose-dependently CK. Raloxifene (Ral), a specific ERa antagonist, inhibited the stimulation of DNA synthesis by either PPT or by low dose of E2, but did not affect the decreased cell proliferation by either DPN or by high dose of E2. LO inhibitor baicalein inhibited E2 and DPN effects but not those of PPT. Real-time PCR revealed that PPT had no effect on ERa but DPN stimulated it. Both PPT and DPN inhibited ERb, while E2 did not affect any ER. E2 stimulated the expression of both 12 and 15LO, whereas PPT inhibited 12LO with no effect on 15LO and DPN inhibited 12LO and stimulated 15LO. E2 increased mRNA for 1a vitamin D hydroxylase whereas PPT had no effect and DPN inhibited its expression. ROS production was induced by all hormones as well as by 12 and 15HETE and was inhibited by DPI which also abolished E2 and DPN induced inhibition of proliferation. In conclusion, we provide herein evidence for the separation of mediation via ERa and ERb pathways in the different effects of E2 on VSMC. The exact mechanism has still to be analysed in future experiments.

Nothing to Disclose: DS, OS, EK, MG-C, NS

P2-26

Gluconeogenesis Is Necessary for cAMP-Mediated Steroidogenesis in Testicular Leydig Cells.

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PEPCK (Phosphenolpyruvate carboxykinase) is well-known for its function as a gluconeogenic enzyme in the liver and kidney. Cyclic AMP(cAMP) is known to induce steroidogenic enzyme gene expression and produce testosterone in Leydig cells. It has been reported that glucose uptake is essential for testosterone production in rat testis and mouse tumor Leydig cell line. However, gluconeogenesis in testicular Leydig cells has not yet been investigated. Here, we have identified that gluconeogenesis is essential for cAMP-mediated steroidogenesis in testicular Leydig cells. Our immunohistochemical staining and other expression analysis have shown that PEPCK gene is expressed in mouse testicular Leydig cells and it is increased after puberty. Transient transfection assays and expression analyses showed that cAMP increased PEPCK gene expression and glucose output in mouse testicular Leydig cells via the activation of CREB (cAMP response element binding). Moreover, cAMP-mediated induction of PEPCK gene expression was blocked by adenovirus-mediated overexpression of DAX-1(dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1), a negative regulator of nuclear receptors. PEPCK inhibitors, 3-MPA (3-Mercaptopropionic acid) and OAD (Oxalic acid dihydrate), blocked cAMP-mediated PEPCK gene expression and glucose output. Interestingly, PEPCK inhibitors decreased cAMP-mediated promoter activities and gene expression of steroidogenic enzyme such as StAR, P450scc and 3β-HSD. Moreover, cAMP decreased AMPK (AMP-activated protein kinase) activation by increasing the cellular ATP level. Treatment of AMPK activator or the expression of CA-AMPK (constitutively active AMPK) inhibited steroidogenic enzyme promoter activities and gene expression via the inhibition of liver receptor homolog-1 (LRH-1). Finally, hCG-induced prepubertal mouse testis showed significant increase in gluconeogenic enzyme gene expression. Overall, this study suggests that gluconeogenesis is necessary for steroidogenesis in Leydig cells.

Nothing to Disclose: SWA, H-SC

P2-27

1,25-Dihydroxyvitamin D_3 Is a Novel Transcriptional Regulator of the Anti-Aging Gene Klotho in Human and Mouse Kidney Cells.

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Klotho (kl), a gene expressed in the kidney, directs the synthesis of a protein (Kl) that serves both as a suppressor of age-related phenotypes and as a coreceptor [with the fibroblast growth factor receptor (FGFR)] for FGF23 to regulate calcium and phosphate excretion/reabsorption. The action of FGF23, as mediated via Kl/FGFR, is to prevent hyperphosphatemia that could arise after the action of 1,25-dihydroxyvitamin D (1,25D), which stimulates both calcium and phosphate absorption in the intestine and reabsorption at the kidney. Hyperphosphatemia is prevented by the action of 1,25D, acting through the vitamin D receptor (VDR), to activate the production of FGF-23, which then downregulates the phosphate transporters NaPi-IIa and NaPi-IIc to decrease kidney phosphate reabsorption. FGF23 also attenuates the synthesis of 1,25D to close the endocrine loop. We hypothesized that, in addition to regulating FGF23, 1,25D/VDR also directly regulates kl to optimize calcium/phosphate homeostasis and reduce aging-related illness. Quantitative real time PCR data in transfected human proximal tubule (HK-2) and in mouse inner medullary collecting duct (IMCD3) cells revealed a modest (1.5-1.7-fold) 1,25D- and VDR-dependent upregulation of kl mRNA. Bioinformatics analysis revealed candidates for vitamin D responsive elements (VDREs) upstream of and within the first intron of the kl gene. Oligonucleotides corresponding to these VDREs were functionally tested in gel mobility shift assays for VDR binding capability. Several VDREs that exhibited potent binding to VDR were then cloned as dual copies into a pLuc-MCS luciferase reporter vector and tested for their ability to mediate transcriptional activation in response to 1,25D, employing transfected HK-2 cells. Our results suggest that adequate 1,25D may not only regulate bone mineral homeostasis but may also ameliorate certain aging related phenotypes via direct upregulation of kl.

Nothing to Disclose: REF, CLL, GKW, CAH, PWJ, MRH

P2-28

New Vitamin D3 Derivatives: 20-Hydroxy- and 20, 23-Dihydroxyvitamin D3 Inhibit Growth and Induce Apoptosis in Vertical Growth Phase and Metastatic Human Melanoma Lines.

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The biologically active form of vitamin D3, 1α,25-dihydroxyvitamin D3 (1,25(OH)2D3) has antiproliferative effect in malignant melanoma cell lines. Also, some authors proposed that the vitamin D3/ VDR (vitamin D receptor) axis can affect behavior of malignant melanomas. However, the hypercalcemia/hypercalciuria effects of classical vitamin D metabolites limit their therapeutic applications. Previously we have documented that 20(OH)D3 and 20,23(OH)2D3 inhibit proliferation and stimulate differentiation of epidermal keratinocytes. In this study the actions of low calcemic analogues of vitamin D3: 20(OH)D3 and 20,23(OH)2D3, have been examined in two cell lines: WM 164 metastatic and WM 1341 vertical growth phase (VGP) amelanotic melanomas. Both cell lines express similar amounts of NFκB-p65 protein, but constitutive activation of nuclear factor- kappa B (NF-κB), a hallmark of melanoma, differ, being more activated in WM164 cells. The amount of vitamin D receptor protein is higher in WM164 than in WM 1341. 20(OH)D3 and 20,23(OH)2D3 inhibited cell proliferation in both cell lines with WM164 showing higher response to the treatment. Also 20(OH)D3 appeared to inhibit proliferation at very low concentration of 0.001 nM, whereas in WM 1341 the inhibitory effect was seen at 10 nM concentration. 20,23(OH)2D3 had a similar effect. Cell cycle analysis showed G0/G1 arrest and apoptosis and detection of cleaved PARP protein indicated cells undergoing apoptosis. Both 20(OH)D3 and 20,23(OH)2D3 were similar in potency to 1,25(OH)2D3. In conclusion, these observations highlight 20(OH)D3 and 20,23(OH)2D3 as promising drugs for a future treatment of aggressive types of melanoma (e.g., VGP and metastatic melanomas), which show high VDR expression level.

Sources of Research Support: NIH grant R01AR052190.

Nothing to Disclose: ZJ, ACW, BZ, RCT, MNN, ATS

P2-29

AMPK Moderates the Actions of Glucocorticoids on Hepatic Glucose Metabolism *In Vivo* through P38 MAPK-Mediated GR Phosphorylation.

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Glucocorticoids play central roles in the regulation of energy metabolism, increasing circulating glucose and lipids, while AMPK is the master regulator of energy homeostasis, sensing energy depletion and stimulating fuel uptake and conservation. We previously reported that AMPK regulates glucocorticoid actions by phosphorylating the human glucocorticoid receptor (GR) at serine 211 through activation of p38 mitogen-activated protein kinase (MAPK). Here we further examined the *in vivo* effect of AMPK on glucocorticoid-stimulated hepatic glucose production and secretion. Activation of AMPK by injecting the AMP analogue 5-aminoimidazole-4-carboxamide-1-β-d-ribonucleoside (AICAR) in rats suppressed dexamethasone-induced elevation of circulating glucose levels and hepatic mRNA expression of the rate-limiting enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), while it reversed glucocorticoid-induced hepatic steatosis. In the same animals, AICAR induced phosphorylation of serine 232 of the liver GR (equivalent to serine 211 of the human GR), while injection of a lentivirus expressing shRNAs for AMPK α catalytic subunits or p38 MAPKα in mice abolished AICAR-mediated induction of hepatic PEPCK and G6Pase mRNA. Mechanistically, AICAR injection in rats decreased the attraction of transcriptional coregulators p300 and SNF2 to GR bound on GREs of the PEPCK and G6Pase gene promoters in the liver as a result of GR phosphorylation. Transcriptomic analysis of the rat liver suggested marked overlaps between the AMPK and glucocorticoid signaling pathways directed mostly from the former to the latter. In human circulating mononuclear cells, mRNA expression of the AMPK α catalytic subunit 1 correlated negatively with mRNA expression of the glucocorticoid-inducible leucine zipper protein (GILZ), which in turn correlated positively with the body mass index of the subjects. These results indicate that the AMPK-regulated energy control system moderates glucocorticoid action on hepatic glucose metabolism. Since stress-related chronically increased glucocorticoid concentrations have been associated with development of metabolic syndrome manifestations including diabetes mellitus type 2, selective activation of AMPK is a promising target for developing pharmacologic interventions to such chronic stress-mediated pathologies.

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Nothing to Disclose: NN, SMN, GL, NP, YHW, GPC, TK

P2-30

Regulation of the Human Oxytocin Promoter by 3-beta Diol.

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The hypothalamo-pituitary-adrenal (HPA) axis is activated in response to variety of stimuli that reach the paraventricular nucleus (PVN). HPA activity is modulated by testosterone (T) which inhibits HPA reactivity to stress. However, the neuroendocrine neurons of the PVN are devoid of androgen receptors. A potential pathway that may mediate the inhibitory actions of T may be via conversion of T into DHT and subsequently into 5 alpha androstane-3b, 17b diol (3-beta Diol), which binds and activates estrogen receptor beta (ER-beta) and inhibits stress reactivity in the PVN. A major group of cells in the PVN co-express oxytocin (OT) and ER-beta and are activated in response to ER-beta agonists. OT infusion in the PVN inhibits HPA reactivity and OT antagonist prevents the inhibiting actions of 3-beta Diol on stress response. Therefore, we hypothesized that androgen suppression of HPA reactivity is mediated by 3-beta Diol-induced activation of OT neurons via ER-beta. To begin to investigate this hypothesis, we co-transfected OT promoter-luciferase reporter construct (OT-luc) and ERbeta into N38 immortalized mouse hypothamic cells to examine the actions of 3-beta Diol on the OT-luc activity. We found that 3-beta Diol dose-dependently increases OT-luc activity when co-transfected with ER-beta, but not with ER-alpha. We also found that ER-alpha and ER-beta have opposing constitutive activities, where ER-alpha decreases while ER-beta increases OT-luc activity. These findings demonstrate that actions of androgens on OT neurons may be mediated by ER-beta, a novel pathway that may also be prevalent in other brain regions.

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Nothing to Disclose: RH, RJH

P2-31

Glucocorticoids Acting Via the Glucocorticoid Receptor Rapidly Induce Tissue Specific Expression of the Mouse MRAP Gene.

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Glucocorticoids play a major role in regulating metabolic homeostasis in the liver, skeletal muscle and adipose. To characterize additional hepatic target genes under glucocorticoid control we performed a gene microarray study using glucocorticoid-treated glucocorticoid receptor (GR)-null mice. Strongly induced previously characterized genes included phosphoenolpyruvate carboxykinase, serine dehydratase, lipin 1, metallothionine 1 and Cdkn1A. Novel genes included Ddit4, Fkbp5, Megf9 and Sult1d1, and were subsequently verified by real time PCR and other analyzes (1). Another interesting gene target was a gene called MRAP (melanocortin 2 receptor assessory protein), a protein essential for cell-surface trafficking of the melanocortin 2 receptor (MC2R). MRAP is highly expressed in the adrenal gland, adipose and areas of the brain. There was a six-fold higher level in hepatic MRAP mRNA levels in wildtype compared to GR-null mice three hours after treatment with the glucocorticoid dexamethasone. There was a nine-fold increase in hepatic MRAP mRNA levels six hours after injection of wildtype mice with dexamethasone compared to vehicle-treated control mice. Treatment of primary mouse hepatocytes with dexamethasone for six hours significantly increased mRNA levels of MRAP three fold, while co-treatment with 10mM RU486, a specific GR antagonist, blocked induction. MRAP mRNA was also increased by dexamethasone in mouse adrenal Y1 cell cultures, an effect also blocked by co-incubation with RU486. In summary, induction of the GPCR-accessory protein MRAP in the liver and adrenal gland by glucocorticoids suggest that MRAP may play a role in the response to systemic stress potentially via increased trafficking of the MC2R or other as yet unidentified GPCRs.

(1) Wong S et al., Endocrinology 2010; 151:185

Nothing to Disclose: TJC, SW, JN, KTC

P2-32

Effect of Hormonal Treatments on Expression of Abeta Degrading Enzymes in Primary Neuron Cultures and *In Vivo*.

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Amyloid beta (Abeta) is derived from a larger single-transmembrane protein, the amyloid precursor protein (APP), when it undergoes proteolytic cleavage by the activity of secretases through the amyloidogenic pathway. Normally Abeta is degraded by several enzymes which help to maintain a balance between production and clearance of Abeta. However, under certain circumstances there is an excess production and/or accumulation of Abeta which results in the formation of extracellular plaques in the brain of AD patients. Enzyme-mediated degradation of Abeta is an important step in APP processing to help maintain the level of Abeta in the cells. Some of the key enzymes that are implicated in Ab degradation are insulin-degrading enzyme (IDE), neprilysin (NEP), endothelin-converting enzymes 1 and 2 (ECE-1, ECE-2), angiotensin-converting enzyme (ACE) and transthyretin (TTR). Female sex steroid hormones, estrogen (E2) and progesterone (P4), have been shown to regulate Ab accumulation both in vitro and in vivo. The exact mechanism(s) involved in hormonal regulation of Abeta accumulation is unclear although several possibilities have been suggested. Studies have shown that E2 and P4 are capable of affecting the expression and activity of several Abeta degrading enymes in various types of tissues. In this study, we investigate the regulation of one or more of the Abeta degrading enzymes by E2 and P4 both in primary neuron cultures as well as in the rat brain. Our results demonstrate that some of the Abeta degrading enzymes are affected by either E2 or P4 or both at both RNA and protein level. Some of these regulations, especially of IDE, are seen not only in short-term in vitro cultures but also in short and long-term in vivo hormone treatment paradigms. We also show a correlation between the IDE levels and Abeta accumulation in rat brain. These results suggest a possible mechanism by which female steroid hormones, E2 and P4, could be affecting Abeta clearance.

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Nothing to Disclose: AJ, TEM, SL, LZ, CF, RDB, CJP

P2-33

Chemokines CCL11, CCL24, CCL 26 and CCL28 Expression by Retinal Pigment Epithelium Cells and Their up Regulation by Aldosterone.

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Purpose : The eosinophil/mast cell chemokine receptor CCR3 (c-c chemokine receptor type 3) is specifically expressed in choroidal neovascular endothelial cells in human with age-related macular degeneration. We analysed the expression of ligands for CCR3 by retinal pigment epithelium (RPE, a layer cells between choroids and photoreceptors) and their regulation by aldosterone in vitro.
Methods: Mineralocorticoid receptor (MCR) expression in human RPE cell was determined by RT- PCR and immunocytochemistry. Chemokines identification and the modification of their expression by aldosterone (incubation of cells with 100 nM aldosterone for 8h and 24 h) was analysed by transcriptional RNA gene array and *human antibody* proteins array.
Results. By gene array analysis, we showed that human RPE cells express in vitro several chemokines such as CCL11(eotaxin1), CCL24 (eotaxin 2), CCL 26 (eotaxin 3), CCL28 and monocyte chemoattractant protein1 receptor MCP-1 or CCR2 but not CCR3 and CCR5. These results were confirmed by protein array analysis. Interestingly, all these chemokines were identified as a ligand for c-c chemokine receptor type 3. We found that the secretion of CCL11, CCL24, CCL26 and CCL 28 by RPE was increased by aldosterone.
Conclusion: Considering the increased secretion of CCL11, CCL24, CCL26 and CCL 28 by RPE induced by aldosterone, it is suggested that the targeting of these chemokines in retina may be a therapeutic ways in future for age-related macular degeneration and uveitis.

Nothing to Disclose: NB, PM, AB, AT, MM

P2-34

Nuclear Receptor Signaling Atlas (NURSA): A Web Resource for the Nuclear Receptor and Coregulator Signaling Communities.

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Advances in the field of nuclear receptor and coregulator signaling rely both on the generation of novel data and, to an increasing extent in the post-genomic era, on the effective management of existing information. The NURSA website (www.nursa.org), originally developed (and currently used) to disseminate data generated by the NURSA, has developed into a comprehensive, freely-available resource for the entire community. It is currently among the most used of scientific websites, having a Google page rank of 6, the same as that of other well known scientific websites such as Oncomine and the Allen Brain Atlas. We believe this success is due both to an unprecedented depth and breadth of data about nuclear receptors (NRs), their co-regulators (CoRs), and ligands, the inclusion of innovative features such as a PubMed-indexed, peer-reviewed, fully citable electronic journal (*Nuclear Receptor Signaling*), and an exceptionally user friendly user interface. During the last year, we have continued the development of this resource by extending the depth of the data contained to include information about diseases and phenotypes associated with NRs and CoRs, molecular interactions between NRs, CoRs, and other molecules; by the addition of a blog; by the creation of two-way links between the print journal *Molecular Endocrinology* and the NURSA website; and by the addition of innovative and intuitive new search capabilities. We anticipate that with these and other features the NURSA website will continue to strengthen its position as the primary information resource in the nuclear receptor and coregulator field.

Nothing to Disclose: DLS, SAO, EMC, CMW, RBL, NJM

P2-35

Growth Inhibition of Androgen-Dependent and Androgen-Refractive Prostate Tumor by the Oncolytic Respiratory Syncytial Virus.

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Cancer therapy utilizing oncolytic viruses involves selective death of virus-infected cancer cells, while normal cells survive due to ability to restrict viral replication. We have identified the human respiratory syncytial virus (RSV) as a novel oncolytic virus against androgen-dependent and androgen-independent human prostate cancer cells *in vitro* and *in vivo* as xenograft tumors. RSV infectivity was markedly higher in LNCaP and PC-3 prostate cancer cells compared to non-tumorigenic RWPE-1 prostate cells. RSV susceptibility of cancer cells was caused by loss or reduction of innate antiviral survival response. RSV induced apoptosis in LNCaP and PC-3 cells, and mediated marked regression of LNCaP and PC-3 xenograft tumor burden in athymic nude mice. RSV-mediated cancer cell death occurred in a background of functional host immune response, since RSV dramatically inhibited growth of allograft tumors from murine RM1 prostate cancer cells in immune-competent C57BL/6J mice. Innate cellular defense against viral challenge, leading to antiviral gene induction, involves activation of the type-I interferon (IFNα/β)-regulated JAK-STAT pathway in addition to activation of nuclear factor-kappa B (NF-kB). We show that RSV-infected LNCaP cells did not induce type-I interferon (IFNα/β) signaling and IFNα/β-regulated activation of the downstream STAT-1 transcription factor, despite robust production of IFN and activation of NFkB. In contrast, RSV-infected PC-3 cells were muted for NF-kB activation, while still able to activate the JAK-STAT pathway. A neutralizing activity against IFN enhanced RSV infectivity and apoptotic death of RWPE-1 non-cancerous cells. These results hint at RSV's therapeutic potential in androgen-dependent and castration-resistant prostate cancer.

Sources of Research Support: NIH grants CA129246.

Nothing to Disclose: IE, T-HC, AS, IB, BC, SB

P2-36

Identification of a Small Molecule Inhibitor of Stat5a/b through Structure-Based Screen for Therapy Development for Prostate Cancer.

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Introduction: There are no effective treatments for metastatic or castration resistant prostate cancer. We have shown that transcription factor Stat5a/b, a member of the Stat gene family, is constitutively active in high-grade prostate cancer, but not in normal human prostate epithelium. In addition, Stat5a/b is active in 95% of clinical castration resistant prostate cancers (n = 198), and the expression of active Stat5a/b in primary prostate cancer predicts early disease recurrence. Importantly, Stat5a/b is critical for the viability of prostate cancer cells *in vitro* and for growth of subcutaneous or orthotopic prostate xenograft tumors in nude mice. We recently showed that Stat5a/b synergizes with androgen receptor (AR) in prostate cancer cells and that Stat5a/b promotes metastatic behavior of human prostate cancer cells in vitro and in vivo. Here, we hypothesize that Stat5a/b is a molecular target for rational drug design for prostate cancer.
Results: The novel Stat5a/b inhibitor IST5-002 specifically inhibited transcriptional activity of Stat5a/b at IC_{50} of 1.5 µM for Stat5a and 3.5 µM for Stat5b, but not of Stat3 or AR in prostate cancer cells in luciferase reporter gene assays. IST5-002 inhibited dimerization and nuclear translocation of Stat5a in prostate cancer cells. In electrophoretic mobility shift assay, IST5-002 inhibited binding of Stat5a/b to the Stat5 DNA consensus sequence with a higher efficacy for Stat5a than Stat5b. Furthermore, IST5-002 inhibited expression of Stat5a/b target gene CyclinD1 in LNCaP cells. Importantly, IST5-002 induced apoptosis of DU145, CWR22Rv1 and LNCaP human prostate cancer cells and inhibited prostate cancer xenograft tumor growth in nude mice. In long-term ex vivo organ cultures of clinical human prostate cancers, IST5-002 induced massive cell death of prostate cancer cells in tissue explant when compared to the control compound.
Conclusions: We have identified a small molecule Stat5a/b inhibitor IST5-002 for therapy development for prostate cancer. Future work will focus on chemical modifications of IST5-002 to achieve IC_{50} below 1 µM.

Sources of Research Support: ACS Research Scholarship Grant RSG-04-196-01-MGO; DoD Idea Development Grant W81XWH-07-1-0411; NIH R01 Grant 1R01CA113580-01A1.

Nothing to Disclose: ZYL, LG, SG, FS, AD, PM, ET, LG, NP, MTN

P2-37

Targeting the Putative Stem/Progenitor Populations in Benign and Malignant Prostate with Selective Estrogen Receptor β Modulator.

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Androgen ablation therapy, used to treat benign and malignant prostate disease, targets androgen sensitive cells but fails to affect androgen independent cells remaining in the epithelium (where the stem cells reside) that are implicated in disease etiology. Selective targeting of these cell populations remains a significant challenge to the field and new insight is essential for the development of novel therapies for prostate diseases.
This study reports a selective estrogen receptor β (ERβ) agonist that causes apoptosis in androgen independent basal cells (including stem cells) and in the stroma of benign human prostate specimens and cells. Using estrogen deficient mice with prostatic hypertrophy and hyperplasia, we show ERβ agonist action is mechanistically different to castration, being androgen independent and activating the extrinsic apoptosis pathway (involving activation of caspase-8 and stimulation of TNFα mediated signalling), whereas castration activates intrinsic, caspase-9 mediated apoptotic pathways. Using TNFα knock-out mice we showed a failure to respond to ERβ agonist, illustrating the requirement for TNFα signalling. Additionally, transient exposure to ERβ agonist has a prolonged effect on prostate recovery. Unlike castration where a full recovery occurs once androgens are restored, ERβ agonist causes altered secretory function, changes in ductal structure and a significant decline in the number of basal cells.
The agonist causes apoptosis in benign human prostate cell lines (RWPE-1, BPH-1), including a subpopulation of α2β1-integrinhi/CD133+ putative stem/progenitor cells. Two androgen insensitive, malignant human prostate cancer cell lines (DU145, PC3) also showed increased apoptosis following ERβ agonist treatment. In order to confirm the specific induction of apoptosis via TNFα, we utilised siRNA to show that the knock-down of these two proteins prevented the pro-apoptotic and anti-proliferative outcomes of ERβ agonist treatment.
In human tissues, ERβ agonist induces apoptosis in the stroma and epithelium of xenografted tissues from 4 BPH and 3 PCa (Gleason grade 7) patients. Short term exposure to ERβ-specific agonist provides new opportunities for the treatment of human prostate diseases, regulating apoptosis in stroma and epithelial stem cells of benign specimens and in malignant, androgen resistant, tumor cells.

Sources of Research Support: US Department of Defense NIA awards W81XWH-06-01-0018 (SJM) and W81XWH-07-1-0112 (PB), NHMRC Fellowship award 384104 (GPR), Cancer Council of Victoria Grant in Aid (SJM, GPR), Prostate Cancer Foundation of Australia Fellowship Award & Victorian Cancer agency (RAT) and the Peter and Lyndy White Foundation.

Nothing to Disclose: SH, SJM, PB, SLH, BN, MG, UC, RT, YW, RAT, GPR

P2-38

Antiandrogenic Effect of Novel Synthetic Compound DIMN on Hormone-Dependent Prostate Cancer Cells.

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Androgen signaling plays an important role in the development and progression of prostate cancers. Hormonal therapies, mainly with combinations of anti-androgens and androgen deprivation, are the mainstay treatment for prostate cancers. However, the emergence of androgen resistance limits their therapeutic usefulness. Here, we report that a synthetic compound DIMN shows a novel anti-androgenic action in prostate cancer cells. DIMN had an inhibitory activity on AR-mediated transcriptional activation in a dose-dependent manner. Thirty one derivatives of DIMN were further composed and tested for their anti-androgenic activity, and seven of them were selected for a strong inhibitory activity on AR-mediated transcriptional activation. Ligand competition assays revealed that [^{3}H]5-dihydroxytestosterone (DHT) binding to AR was inhibited to 50% at approximately 1~2 μM of DIMN and 10 μM of DIMN-d7, respectively. In addition, the growth of LNCaP cells was inhibited both by DIMN and DIMN-d7 in a dose-dependent manner. Furthermore, protein expression levels of the AR and the prostate-specific antigen (PSA) were downregulated by DIMN treatment in LNCaP cells. These data suggest that DIMN could be a useful chemopreventive and chemotherapeutic agent to delay the development and progression of prostate cancers.

Nothing to Disclose: C-HS, KL

P2-39

Antitumor Activity of Pasireotide (SOM230) Alone and in Combination with Everolimus (RAD001) in DU-145 Human Prostate Cancer Model.

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Pasireotide (SOM230) is a novel multi-receptor ligand somatostatin analogue with high affinity for somatostatin receptor subtypes sst1, 2, 3 and sst5. In prostate tumors, expression of sst1 and sst5 at the membrane and sst4 in the cytosol has been reported, while in a human prostate tumor model (DU-145), sst5 was present at the membrane and sst2 in the cytosol. A single injection of pasireotide LAR (8 and 80 mg/kg s.c.) resulted in a significant dose dependent reduction of tumors grown s.c. in male nude rats to 66% and 21% of control, respectively after 24 days. The mTOR inhibitor everolimus (5 mg/kg/week p.o.) reduced tumor size to 19% and the combination with 8 and 80 mg/kg pasireotide LAR resulted in a tumor size of 14% and 10% of control, respectively. Plasma levels of IGF-1 were reduced to 44% of control by either pasireotide LAR dose, were unaffected by everolimus, and the combination with 8 and 80 mg/kg of pasireotide LAR reduced plasma IGF-1 to 60% and 51% respectively. In a second experiment everolimus (5, 10, 15 mg/kg/2x/week p.o.) resulted in a dose-dependent increase of pAKT in tumor tissue determined by immunohistochemistry. This increase in pAKT was strongly reduced when everolimus and pasireotide LAR were co-administered. Pasireotide LAR alone had no effect on pS6, a substrate located downstream of mTOR, in contrast to everolimus, which strongly reduced pS6 levels. pIGF-1R and pIRS1, which are both upstream of mTOR, were not reduced by pasireotide LAR, despite the observed decrease in circulating IGF-1, but tended to be increased in the presence of everolimus. In conclusion, these studies show for the first time a significant dose dependent antitumor activity of pasireotide LAR alone in a human prostate tumor model. This effect can only partly be explained by reduced circulating IGF-1 and most likely involves additional endo-, auto- or paracrine factors. The reduction of the everolimus-induced increase in pAKT by pasireotide LAR may confer a therapeutic advantage on the combination of both compounds since one potential mechanism associated with the lack of sensitivity to mTOR inhibition is an increase in pAKT.

Disclosures: HAS: Employee, Novartis Pharmaceuticals. CL: Employee, Novartis Pharmaceuticals. PN: Employee, Novartis Pharmaceuticals.

P2-40

Development of New Compounds Specifically Targeting Prostate Cancer Bone Metastases.

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Introduction: Bone metastases are a major cause of morbidity for men with prostate cancer (PCa). Although PCa cells produce osteoblastic metastases, there is an initial and ongoing osteoclast (OC)-mediated osteolytic phase that is essential for PCa bone metastases. OC-mediated bone resorption is dependent upon OC secretion of bone acid phosphatase (BAP). PCa cells in bone secrete high levels of prostatic acid phosphatase (PAP) which differs from BAP in that PAP enzymatic activity is inhibited by tartrate and glyceric acid.
Hypothesis: PAP secreted by human PCa cells in bone acts similarly to OC-derived bone acid phosphatase to degrade bone matrix and enhance PCa bone-targeting and growth in bone. We developed new compounds consisting of glyceric acid or tartrate (known PAP inhibitors) conjugated to a bisphosphonate to inhibit PCa bone metastases.
Methods: PCa bone metastases derived from 7 patients were immunostained for androgen receptor (AR), prostate specific antigen (PSA) and PAP expression. The VCaP human PCa cell line derived from a vertebral metastases was inoculated intratibially into SCID mice (n=8) and bone lesions immunostained for AR, PSA, and PAP. Co-cultures of MC3T3 osteoblasts and VCaP cells were treated with tartrate and the effects on cell number, PAP and bone alkaline phosphatase (ALP) secretion measured by ELISA. Glyceric acid and tartaric acid were then conjugated with alendronate and the effects of these new conjugates on PAP enzymatic activity were compared to alendronate alone and tartrate alone.
Results: Human PCa bone lesions (7/7) had strongly positive expression of PAP, with little AR or PSA expression. 8/8 SCID mice inoculated with VCaP cells intratibially developed osteoblastic VCaP lesions and these similarly had high PAP expression and no PSA expression. Tartrate addition to co-cultures of VCaP and MC3T3 osteoblasts reduced MC3T3 cell numbers and ALP secretion. *In vitro* testing of the conjugates revealed that the glyceric acid-alendronate conjugate inhibited PAP enzymatic activity but the tartrate-alendronate conjugate and alendronate alone had no significant inhibitory activity.
Discussion: We present evidence that PAP secretion by PCa cells enhances their bone metastatic ability. We developed new bone-targeting agents that consist of alendronate conjugated to PAP-inhibitory small molecules for further development as oral agents to prevent and treat PCa bone metastases.

Nothing to Disclose: BMW, AK, VJ, SLR, SY, ACL

P2-41

STX2171, a 17β-Hydroxysteroid Dehydrogenase Type 3 (17β-HSD3) Inhibitor, Is Efficacious *In Vivo* in a Novel Hormone-Dependent Prostate Cancer Model.

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17β-Hydroxysteroid dehydrogenases (17β-HSDs) are a family of 15 enzymes responsible for the reduction / oxidation of steroids at the 17-position into active / inactive hormones. They vary in their substrate specificity, localization, and directional activity. 17β-HSD Type 3 (17β-HSD3), which has been seen to be expressed in prostate cancer, catalyzes the reduction of androstenedione (Adione) to testosterone (T), stimulating prostate tumor growth. 17β-HSD3 inhibitors may have a role in the treatment of hormone-dependent prostate cancer and also as male anti-fertility agents.
STX2171, a non-steroidal compound, was identified as a potent inhibitor of 17β-HSD3 (IC_{50} = 200nM). It is selective for 17β-HSD3 inhibition over 17β-HSD1 (estrone / E1 to estradiol / E2), and 17β-HSD2 (T to Adione and E2 to E1). In an *in vitro* proof of concept assay, STX2171 effectively inhibited Adione stimulation of an androgen receptor-positive LNCaP prostate cancer cell line stably transfected with 17β-HSD3, LNCaP[HSD3], and was non-androgenic with low cytotoxicity.

Castrated male MF-1 mice were inoculated s.c. with 1 x 10^7 LNCaP[HSD3] cells in Matrigel 24 h after an initial dose of testosterone propionate (100 µg TP / 50 µl s.c.; n = 50) or vehicle (4% EtOH in PG; n=10). After 4 weeks of daily TP dosing 25/50 mice developed tumors, whereas none developed in the vehicle-treated group. Of the tumor-bearing mice, 6 continued to be dosed with TP and 19 were switched to 100 µg Adione s.c. / 50 µl daily. After 1 week, 6 of the Adione-dosed mice were given vehicle, 7 were given STX2171 (20 mg/kg/day s.c.), and Adione dosing was discontinued in the remaining 6 mice. Tumor measurements and animal weights were recorded weekly, and after 4 further weeks of treatment, blood and tumors were taken at termination.
Both TP and Adione caused tumors to grow to ~600% of their initial volume at 4 weeks, whereas there was negligible growth of either the non-stimulated tumors or of Adione-stimulated tumors treated with STX2171. STX2171 significantly inhibited the formation of T in Adione-dosed animals.
In conclusion, a proof of concept tumor model has been developed to study the efficacy of 17β-HSD3 inhibitor compounds *in vivo*. STX2171, a selective, non-toxic, non-androgenic 17β-HSD3 inhibitor is efficacious in the model, inhibiting both Adione-stimulated tumor growth and lowering plasma T levels, indicating that 17β-HSD3 inhibitors may have application in the treatment of hormone-dependent prostate cancer.

Nothing to Disclose: JMD, PAF, HJT, JDH, FS, NV, BVLP, MJR, AP

P2-42

SHLP6: A Novel, Naturally Occuring, Mitochondrial Peptide Has Therapeutic and Prognostic Potential in Prostate Cancer.

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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 have 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. 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.
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.
Thus, our findings not only introduce the revolutionary concept that a previously unrecognized family of peptides is produced from the mitochondria, challenging existing paradigms about mitochondrial function, but also demonstrate that SHLP6 is altered in prostate cancer, and may serve as a novel therapeutic and diagnostic agent in this disease.

Nothing to Disclose: HN, PC, LJC

P2-43

In-Vitro Potency and Safety Index of Three Novel P450c17 17,20-Lyase Inhibitors.

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P450c17 is a single steroidogenic enzyme with two distinct activities, 17 α-hydroxylase (hydroxylase) and 17,20-lyase (lyase). Selective inhibition of P450c17 lyase activity is of therapeutic interest since it is a rate-limiting step in adrenal and gonadal androgen biosynthesis and its inhibition does not block glucocorticoid synthesis, as does inhibition of upstream hydroxylase. Selective lyase inhibitors may prove useful for the treatment of disorders such as polycystic ovary syndrome(PCOS) and castration resistant prostate cancer(CRPC) by lowering androgens while minimizing side effects related to reduced adrenal glucocorticoid production or off-target inhibition of liver enzymes, as found with other less selective P450c17 inhibitors such as ketoconazole, which is used off-label for treatment of CRPC.
The IC50, or concentration that provides 50% enzyme inhibition, data are presented below for a series of novel nonsteroidal compounds, and ketoconazole. The IC50 against hydroxylase and lyase were determined in rat testicular microsomes using their metabolic substrate (progesterone and 17 hydroxyprogesterone, respectively) and the IC50 against the liver enzymes 2C9, 2C19, and 3A4 were determined in pooled human liver microsomes using their metabolic substrates (diclofenac, omeprazole, and testosterone, respectively). Inhibitor selectivity is presented as the IC50 hydroxylase:lyase ratio and the most susceptible liver enzyme:lyase IC50 ratio provides a relative index of off-target liver effect. All IC50 values are expressed in µM.

Compound	Lyase	Hydroxylase	Hydroxylase:Lyase	2C9	2C19	3A4	Liver Enzyme: Lyase
VT-464	0.12	0.72	6.0	13	18	37	108
VT-478	0.29	2.7	9.3	29	27	61	93
VT-489	0.26	0.67	2.6	3.5	5.4	9.9	14
Ketoconazole	0.25	0.82	3.3	23	0.32	0.21	0.84

This series of novel metaloenzyme inhibitors exhibit in-vitro potency against P450c17 with a selectivity for lyase over hydroxylase, as well as up to 100 fold lyase selectivity over over off-target liver enzymes, compared to ketoconazole which exhibited little selectivity over the liver enzymes tested. Also, initial in-vivo results demonstrate an acceptable pharmacokinetic profile in rodents and a reduction in androgen dependent organ weight in male hamsters. These compounds may provide a therapeutic alternative to P450c17 inhibitors either approved or in development, and warrant further evaluation.

Disclosures: SWR: Employee, Viamet Pharmaceuticals, Inc., Viamet Pharmaceuticals, Inc. JRE: Employee, Viamet Pharmaceuticals, Inc. WJH: Employee, Viamet Pharmaceuticals, Inc. AJH: Employee, Viamet Pharmaceuticals, Inc. RS: Employee, Viamet Pharmaceuticals, Inc.

P2-44

An FGFR4 Transmembrane Polymorphism Promotes STAT3/5 Activation To Facilitate Breast Cancer Metastasis and Sustain Its Own Expression.

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Recent genome-wide association studies highlight the importance of Fibroblast Growth Factor Receptor 2 (FGFR2) as a risk factor for breast cancer. Since FGFR family members form strong heterodimers, understanding the contribution of other FGFRs will be critical in decoding FGF signals in this disease. In particular, FGFR4 has been implicated in membrane ruffling in breast cancer cells. Further, a glycine (G) to arginine (R) single nucleotide polymorphism (SNP) within the transmembrane domain of FGFR4 has been associated with breast cancer invasiveness and treatment resistance through mechanisms that remain to be elucidated and that serve as a focus for this study. Here we show that FGFR4 is frequently expressed in the heterozygous state for this codon 388 SNP in primary breast carcinomas. Interestingly, however, nearly 50% of metastatic carcinoma samples exhibited selective reduction of the FGFR4-G388 allele compared to matched primary tumors from the same patients. Over-expression of the wild-type FGFR4-G388 isoform in MDA-231 and MDA-459 breast cancer cell lines resulted in diminished STAT3/5 activation and reduced xenografting efficiency. In contrast, introduction of FGFR4-R388 enhanced STAT3/5 phosphorylation and promoted tumor growth in xenografts. In turn, we show that STAT3 binds the endogenous FGFR4 promoter to enhance transcription of FGFR4. These findings identify distinct signaling properties for FGFR4 isoforms whose functions serve not only to promote STAT3/5-mediated tumor progression but also to sustain their own FGFR4 drive.

Nothing to Disclose: XZ, LZ, SA, SE

P2-45

Tumorigenicity of Breast Cancer Cells Lacking the p38 Mitogen-Activated Protein Kinase.

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The p38 mitogen-activated protein kinase (p38 MAPK) is an important cell signaling molecule and its activity is frequently increased in apoptotic cells (1). We have generated cell lines with significantly reduced expression of the p38 MAPK (Min-p38 MAPK) and used these cells to investigate its role in tumorigenesis of breast cancer cells. MCF-7 cells were stably transfected with a plasmid producing small interfering RNA that inhibited the expression of p38 MAPK. Two cell lines were generated with no or low levels of p38 MAPK as determined by western blotting. Control cells were stably transfected with the same plasmid producing non-interfering RNA of the same size. The reduction in the p38 MAPK activity caused a significant increase in the expressions of the estrogen receptor-α (ERα) and the progesterone receptor, but eliminated the expression of the ERβ. The Min-p38 MAPK cells showed an enhanced overall growth response to 17β-estradiol (E2) treatment, whereas treatment with growth hormone plus epidermal growth factor was largely ineffective in stimulating growth of these cells compared to controls. Although the long-term net growth rate of the Min-p38 MAPK cells was increased in response to E2, their proliferation rate was not different from controls in short-term cultures. However, the Min-p38 MAPK cells did show a significant decreased rate of apoptosis after E2 treatment and a reduction in the basal phosphorylation of p53 tumor suppressor protein compared to controls. When the Min-p38 MAPK cells were xenografted into E2-treated athymic nude mice, their tumorigenicity was significantly enhanced compared to control cells. In summary, eliminating the activity of the p38 MAPK in breast cancer cells increased their growth rate in response to E2 and enhanced their tumorigenesis in immunodeficient mice. We did not find much increase in the rate of proliferation of the cells lacking the p38 MAPK but a significant decrease in apoptosis after E2 treatment. We conclude that the increased tumorigenicity of these cells was caused mainly through a decrease in apoptosis rate rather than increased proliferation. Further, we hypothesize that the lack of the p38 MAPK caused an imbalance to increase the ERα:ERβ ration and a reduction in the activity of the p53 tumor suppressor protein, a substrate for p38 MAPK (2), and that in combination these changes caused the reduction in the apoptosis of the Min-p38 MAPK cells.

(1) Xia Z et al., Science 1995; 270:1326
(2) Matsumoto et al., Med Mol Morphol 2006; 39:79

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Nothing to Disclose: RAM, EEM, MIE, SMM, GT

P2-46

The Melanoma Associated Antigen A (MAGE-A3) Engages Multiple Tumorigenic Pathways To Promote Breast Cancer Progression.

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We have previously reported that the melanoma-associated antigen A3 (MAGE-A3) represents a downstream target of Fibroblast Growth Factor Receptor (FGFR)-2 signaling. Further, we showed that loss of fibronectin-associated cell invasion relies on MAGE-A3. Consistent with these predictions, MAGE-A3 has become more widely recognized as a useful biomarker for breast cancer progression, correlating with tumor size, lymph nodal metastasis, as well as stage progression. To investigate the underlying basis for these effects, we stably introduced MAGE-A3 in endogenously deficient human breast carcinoma cells. This resulted in pronounced AKT activation, decreased p53 and its target p21, and enhanced Rb phosphorylation. Furthermore, MAGE-A3 expression significantly increased N-cadherin, but not E-cadherin, while enhancing the activity of the matrix metalloproteinase MMP9. Further, MAGE-A3 significantly induced estrogen receptor α (ERα) expression in ERα-deficient cell lines. Taken together, our findings uncover multiple mechanisms for MAGE-A3 in promoting several tumorigenic pathways. Consistent with its clinically-associated features, this nuclear antigen has the capacity to induce proliferative, survival, and metastatic signals to promote breast cancer progression.

Sources of Research Support: Canadian Institutes of Health Research.

Nothing to Disclose: WL, AG, SA, SE

P2-47

Insulin Could Contribute to Adenocarcinoma Progression by Stimulating Local Angiogenesis.

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Background
Deleterious effects on the progression of adenocarcinomas by the insulin analogue glargine have recently been suggested. Most actions of this insulin analogue have hitherto been explained by a direct stimulation of growth potential of neoplastic cells, and by its IGF-1 related properties. However, insulin-stimulated angiogenesis could be an additional factor involved in tumor progression and cancer associated clinical outcomes.
Methods
Insulin receptor (IR) and insulin like growth factor-1 receptor (IGF-1R) expression were examined by immunohistochemistry in 5 types of human adenocarcinomas (breast, colon, pancreas, lung and kidney (n=15 for each tumor type)). Expression of IRs on endothelial and epithelial cells within adenocarcinomas and in surrounding tissue was semi-quantitatively scored: 0=no, 1=minimal, 2=moderate, 3=strong IR expression.
Contribution of commercially available insulin compounds to angiogenesis was studied in-vitro by incubating human microvascular endothelial cells (hMVECs) with bFGF, TNF-α in the absence or presence of different insulin compounds (human insulin, lispro, detemir and glargine) at 10-9M, 10-8M and 10-6M for 7 days. Subsequently, capillary-like endothelial tube formation into 3D fibrin matrices was analyzed by phase-contrast microscopy.
Results
In all studied adenocarcinomas IR staining intensity was significantly increased on endothelium of tumoral microvessels (mean score=3) as compared to microvessels in non-tumoral stroma (mean score is 1; $p<0.001$). The pattern for epithelial staining revealed more intensive staining in the parenchyma for most tumor types compared to neoplastic epithelium. Staining for IGF-1R was weak, with no differences between tumoral and non-tumoral tissue.
Compared to stimulation with the combination of bFGF and TNF-α (control), co-incubation with all insulin compounds lead to significant increased capillary-like tube formation of hMVEC. The extent of capillary-like tube formation varied between insulin compounds, ranging from a 1.6 to 2.3 fold increase compared to control.
Conclusions
Common adenocarcinomas, which frequently afflict patients with type 2 diabetes, contain large numbers of neovessels. These vessels abundantly express IRs, independent of tumor type. Our results suggest that all tested insulin compounds may stimulate tumor growth by enhancing local angiogenesis. Future studies need to confirm the association between insulin therapy in type 2 diabetes and tumor progression.

Nothing to Disclose: KLR, FMHB, EMW, DJR, HRB, PK, CMvdL, TBT, JHvdT

P2-48

Upregulation of HER2/Neu by MMP-9 Is Associated with Decreased Apoptosis in Human Mammary Epithelial Cells (HMEC).

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Over expression and over activation of the receptor kinase HER2/neu in mammary cells is known to accelerate breast tumor formation. It is estimated that one out of every four breast cancer tumors are associated with over expression of HER2, which, in turn, is linked to a poorer prognosis. While the link between HER2/neu expression and a poor prognosis is well documented, few studies address how the rise in HER2/neu actually occurs in normal human mammary cells, and how, once increased, over expression of this protein directly influences the metastatic profile of these cells. We have previously shown that exposure of human mammary epithelial cells (HMEC) to matrix metalloproteinase 9 (MMP-9), alone or in combination with estrogen, significantly increases the surface expression of HER2/neu. We set out to determine whether alterations in HER2/neu expression, evoked by MMP-9, could translate into changes in the viability or behavior of these cells, especially those that would be consistent with metastatic characteristics. Cultured normal human mammary epithelial cells (HMECs) were treated with control vehicle or MMP-9 alone, or with MMP-9 in combination with estrogen. In parallel experiments cells were also exposed to dexamethasone (Dex) in addition to MMP-9, estrogen, or the combination. Following a 48-hour treatment duration, cells were harvested and stained for flow cytometry using fluorochrome-labeled anti-HER2 antibodies (for HER2/neu levels) or apoptosis detection reagents. Surface expression of HER2 was determined using a Guava PC cytometer and Guava Express software. Apoptosis levels were determined using a Guava PC, Guava Nexin software and a commercially available apoptosis assay kit. As we previously showed, exposure of HMECs to MMP-9 increased HER2/neu expression. This was blocked in the presence of an MMP-9 inhibitor. HMECs maintained in cultured and treated with control vehicle showed a basal apoptotic rate between 30-50%. Apoptosis level decreased significantly 15-20% in HMECs treated with MMP-9. This significant decrease in apoptosis was also shown for cells treated with MMP-9 in the presence of Dex. Our results suggest show that exposure of human mammary cells to MMP-9 leads to the over expression of HER2/neu and decreased apoptosis. Ongoing studies address whether the rise in HER2/neu, evoked by MMP-9 exposure, is sufficient to alter migratory behavior as well as promote pro-metastatic profiles of HMECs.

Sources of Research Support: HHMI Fellowship awarded to MF.

Nothing to Disclose: MF, SA

P2-49

Regulation of Tumor Suppressor Protein, p53, by Fisetin, a Phytoestrogen, in T47D Breast Cancer Cells.

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Phytoestrogens are nonsteroidal plant compounds with a structural similarity to estrogen and possess estrogenic activities (1). A phytoestrogen, fisetin (3,3',4'7-tetrahydroxyflavone) has been shown to inhibit cell proliferation and induce apoptosis of cancer cells, including cancers of the colon, cervix and prostate (2-4). We have previously shown (5-6) that presence of estradiol (E_2) in the growth medium causes proliferation of T47D breast cancer cells and an increase in the level of p53. For the present study, we have examined effects of fisetin on cell proliferation and p53 expression in T47D cells. The cells were grown in medium containing 5% fetal bovine serum. Upon confluency, the cells were grown in charcoal-stripped media for 6 days to deplete any endogenous steroids or effectors. The cells were then treated for 24 h with 10 - 70 µM of fisetin consistent with its use in published literature (2,7). The proteins in the cells were extracted, quantified and separated on SDS-PAGE and subjected to Western blot analysis for the detection of p53. Changes in T47D cell proliferation were quantified to reflect functional analysis.
Western blot analysis revealed that fisetin caused a decrease in the cellular p53 in a concentration dependent manner. However, cytolocalization of p53 upon treatment with estradiol and fisetin remained unaltered. Also, increasing concentrations of fisetin caused a 6 to 8 fold decrease in cell number while E_2 treatment caused significant increase in cell number. Thus, fisetin exerts anti-proliferative effects and lowers cellular levels of p53 in T47D cells. The levels of estrogen receptor (ERα) increased gradually in cells grown in charcoal-stripped media for 6-10 days. The presence of fisetin had a modest effect of down regulation of ERα as compared to control. These effects were sensitive to the presence of ICI. Our studies provide interesting leads to clearly delineate the possible mechanistic relationship among fisetin, estrogen receptor, and tumor suppressor proteins in breast cancer cells.

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Nothing to Disclose: ALS, AES, SD, VKM

P2-50

Urine Estrogens, Catecholestrogens and Phytoestrogens in BRCA1 Gene Mutations Carriers: Relation to Reactive Glycemia.

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The bearing of mutations of BRCA1 gene is associated with a combination of excessive aromatase activity or expression, predominantly estrogen receptor-negative phenotypes of tumors, and only scarce information about estrogens contents in body fluids. Here, isotope dilution capillary gas chromatography/mass spectrometry was used to study urinary excretion of estrogens (11 fractions), their catechol metabolites (4 fractions), and phytoestrogens (11 fractions) in 22 women (eleven with BCRA1 gene mutations and eleven without these mutations, mean age respectively 49.6±2.8 and 56.4±3.0, p=0.14). Surgery for breast cancer was performed in them correspondingly, on average, 4.2±0.6 and 5.9±0.3 years prior to the investigation. The carriers of mutations (including 3 premenopausal women) showed in comparison with non-carriers significantly higher urinary estradiol and estrone excretion and an increased 2-OH-E2 excretion. The tendency to the increment in 2-methoxyestrone excretion was revealed too. Among phytoestrogens, solely the increase in enterodiol excretion was significant (p=0.04). In the subgroup of untreated postmenopausal women, BCRA1 mutation carriers demonstrated a trend to increased estradiol and estrone excretion and to a higher sum of the mean levels of all estrogen metabolites tested. In mutation carriers, higher blood glucose 60min/0min and 120min/0min ratio in the course of oral glucose load was associated respectively with significantly lower estrone and relatively higher 4-hydroxyestradiol and 4-hydroxyestrone excretion. The treatment after the baseline laboratory investigation of six women (three in the BCRA1+ and three in the BCRA1- group) aged 58.7±4.4 years with the antidiabetic biguanide metformin at a dose of 1.0 to 1.5 g/day for 3 months was associated with decreases in the excretion rates of 4-hydroxyestradiol (p=0.07), 2-methoxyestradiol (p=0.051), and 16-epiestriol (p=0.09) and did not influence phytoestrogen excretion. The decrease in 2-methoxyestrogen excretion was more consistent in women without BCRA1 mutations than in BCRA1 mutation carriers. The data received suggest the possibility that described earlier aromatase complex activation in BCRA1 mutation carriers is combined with increases in estrogen metabolism into catecholestrogens and their inactivation by methoxylation, and that metformin may affect both of these pathways.

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Nothing to Disclose: LB, AK, MB, HA

P2-51

Progesterone Receptor Isoforms A/B Ratio Determines Hormone Requirement for Amphiregulin Gene Expression in a Novel Bi-Inducible Breast Cancer Cell Line.

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Progesterone receptor isoforms PRA and PRB differentially regulate expression of genes implicated in various signaling pathways most notably those implicated in the pathogenesis of breast cancer and metastasis. PRA/B ratio varies from 1 in normal human mammary cells to greater than 4 in highly aggressive breast cancers. To investigate the underlying mechanisms, we used human breast cancer cells MDA-MB-231 to establish a novel cell line harboring a bi-inducible promoter system, allowing selective and reversible induction of PRA and/or PRB. Of note, PRA and PRB expression level can be finely adjusted using non-steroid inducer ligands, in a dose-dependent manner, with undetectable basal expression in absence of both inducers. PRA or PRB expression is detected as early as 3h post specific inducer(s) exposure, reaching physiological PR levels after 24h and maintained up to 72h. Inducibly expressed PRA and PRB are functional by regulating synthetic as well as endogenous gene expression. This system allowed us to investigate the differential regulation of target gene transcription in various PRA/PRB expression ratio and hormonal conditions. Amphiregulin (AREG), an EGF-like growth factor previously identified as a ligand-activated PRB target, is involved in carcinogenesis. In our model, while PRB up-regulated AREG transcription in the presence of progesterone, PRA increased AREG transcript level irrespective of progesterone. Coexpression of PRA and PRB resulted in elevated basal AREG mRNA that was potentiated by progesterone, suggesting that altered PRA/PRB ratio dictates hormone requirement for AREG expression. AREG mRNA levels increased as early as 3h after PRA expression and remain elevated up to 48h. Actinomycin D abrogated this effect indicating that unliganded PRA regulates AREG at transcriptional level. In sum, we have generated an original cell-based system in which PRA/PRB expression is specifically controlled within the same cells allowing to gain new insights into the relative contribution of PR isoforms in breast cancer cell signaling and facilitating characterization of selective PR isoform antagonists. Using this unique model, we demonstrate for the first time that elevated PRA/PRB ratio increases AREG expression irrespective of hormone and thus may provide a continuous autocrine growth stimulus. Our results provide additional support for the aggressive phenotype of breast cancers with high PRA/PRB ratio.

Sources of Research Support: Inserm, University Paris-Sud, Doctoral Scholarship from Higher Education Commission, Pakistan (to JAK) and the Association pour la Recherche contre le Cancer (ARC).

Nothing to Disclose: JA-K, LA, CB, AG-M, ML, HL

P2-52

Selective Progesterone Receptor Modulator Ulipristal Differentially Regulates Expression and Activation of Multiple Signalling Pathways in Human Breast Cancer and Normal Human Mammary Epithelial Cells.

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Progesterone receptors (PRs) play important roles in breast health and function, and as an estrogen-responsive gene, the subtypes have pro-differentiating effects on breast tissue during normal development. PRs also mediate the increased proliferation of human mammary epithelial cells (HMEC) that occurs during pregnancy and dysregulation of PRs is a characteristic of breast cancers. Due to the complex activities of selective PR modulators (SPRMs), this class of molecules remains underexplored for efficacy and safety of individual compounds for use as contraceptives in young women, or women desirous of hormone therapy for other reasons. Using flow cytometry analysis and bioimaging, we determined, using human breast cancer MCF7 cells, that the SPRM Ulipristal affords protection against aberrant breast epithelial cell growth by slowing cell cycle kinetics without changes in viability or apoptosis, and by modifying the sub-cellular localization of steroid receptors, including PR. Similarly, a significant change in the HMEC cell cycle was observed. Changes in gene expression over time following Ulipristal treatment were evaluated using microarray and quantitative real-time PCR analyses. Phosphorylation of growth related signalling components was determined by Western analyses and single-cell cytometric assays, using site-specific immunoreagents. Following a single dose of Ulipristal (10^{-7}M), levels of phosphorylated p42/p44 mitogen activated ERK and JNK kinases were markedly decreased in HMEC and MCF7 cells, findings suggestive of non-PR-mediated or secondary effects. To test whether growth factor mediated proliferation was affected, breast cells were maintained in long-term culture +/- Ulipristal. For acute experiments, cells were sub-cultured and later dosed with epidermal growth factor (EGF, 10 or 100-ng/ml, 5-to-30 min). We demonstrated that long-term Ulipristal significantly decreased tyrosine phosphorylation of EGF receptor (EGFR) and EGF-activation in MCF7 cells compared to matched controls without the SPRM; no decrease was observed in long-term treated HMEC.
In summary, acute treatment with Ulipristal slows breast cancer and HMEC progression through the cell cycle, increases cells in G0/G1 and reduces growth. Long-term Ulipristal decreases EGF-mediated tyrosine-activation of EGFR and downstream signalling components in breast cancer cells but not HMEC. Findings are consistent with long-term use breast safety and suggest a potential for health benefit.

Sources of Research Support: U54 HD029990.

Nothing to Disclose: KH, LM, CR, RS-W, PLM

P2-53

Differential Effects of Progesterone and Medroxyprogesterone on Normal and Breast Cancer Cells through Glucocorticoid Receptor.

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An increase in breast cancer risk associated with combined postmenopausal hormone replacement therapy (HRT) has been reported. The risk associated with a combination of estradiol (E2) and progesterone (P) appears, however, to be lower than the risk observed with E2 and synthetic progestins. The specific steroidal properties of progesterone and synthetic progestins may account for these different effects. We thus were interested to investigate the effects of E2+P or E2+medroxyprogesterone acetate (MPA), the synthetic progestin preferentially used in the USA.
We first performed microarrays analysis in primary cultures of normal luminal human epithelial breast cells (HBE) expressing cytokeratine 18. Gene Ontology revealed that genes controlling cellular function and cellular movement were more significantly altered under E2+P treatment, whereas genes involved in cellular metabolism and cell death were predominately modified under E2+MPA treatment. The results suggested, therefore, a different effect of each treatment on gene regulation.
Cellular and molecular aspects of proliferation and differentiation effect were also studied in HBE, MCF-7 and T47-D cells. In T47-D cells, E2+MPA and E2+P treatments, both displayed similar effects on cellular proliferation, and fatty acid synthetase transcription level regulation. In MCF-7 cells, MPA decreased the E2 proliferative effect whereas P had no effect. In contrast, the same treatments in HBE cells resulted in opposite effects on proliferation and differentiation. To explain these differences we studied the glucocorticoid receptor (GR) content of the cells. GR is abundant in HBE cells and in epithelial cells from biopsies of normal breast tissues and in MCF-7 cells but absent in T47-D cells. Using an anti-glucocorticoid and RNA interference strategy for GR, we were able to confirm that the proliferative effects of MPA were mediated through the GR according to cell phenotype. We also observed that Dexamethasone had a mild proliferative activity on HBE cells and an antiproliferative effect in MCF-7 cells.
In conclusions we have demonstrated that P and a synthetic progestin due to their steroidal properties can behave in an opposite manner on normal and breast cancer cells on proliferation and differentiation which can account for their possible different impact on breast cancer risk.

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Nothing to Disclose: AC, LD, DC, MV, NM, MC, PF, MDDB, AG

P2-54

Differential Expression of Fibroblast Growth Factor Family Members in a Progestin-Dependent BT-474 Human Breast Cancer Cell Xenograft Model.

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During normal embryonic development of tissues and organs members of the fibroblast growth factor (FGF) family have important angiogenic functions. FGFs also play an important role in the development and progression of different types of cancer, though their role in the pathogenesis of breast disease remains controversial. Many breast cancers are hormone-responsive and depend on estrogens (E) and progestins (P) for growth. Recent clinical trials suggest an increased risk of breast cancer in post-menopausal women receiving combined E-P hormone replacement therapy (e.g. WHI study) compared with women receiving E alone. However, the molecular mechanism through which P increases breast cancer risk remains unknown. We recently established an in vivo xenograft model and demonstrated that natural and synthetic P increase the progression of human breast cancers and that such progression is dependent on expression of VEGF by tumor cells (Liang et al. Cancer Res. 2007, 67:9929). In this model, tumors initially grow for about 6-8 days following tumor cell injection (pre-regression phase), but then begin to regress (regression phase). On implanting a progestin pellet on day 8-10, tumors once again begin to progress (progression phase). Using this model we sought to determine distribution of FGF family members, FGF 2, 4 and 8, since these factors have been linked with angiogenesis and tumor progression in other models. Tumor tissues were collected during various phases and soon after implantation of progestin pellets. Our results show that FGF 2 (expressed in stromal and epithelial cells) and FGF 8 (expressed in tumor epithelial cells) were significantly reduced following P administration, when tumors began to progress. FGF 4 (expressed in epithelial cells) did not change during the entire course of the experiment. The basis for loss of FGF 2 and FGF 8 is currently not known but could represent down-regulation of secondary angiogenic factors since P induces the secretion of massive amounts of VEGF sufficient for tumor progression in this model. Alternatively, loss of FGF 2 and FGF 8 during tumor progression could also be indicative of increased cellular autonomy, allowing cell growth without the need for autocrine or paracrine signaling from these two factors. It also remains to be established whether maintenance of FGF 4 is essential for tumor progression, and if so whether FGF 4 could be a potential target through which P-driven breast tumors might be controlled.

(1)Liang et al. Cancer Res. 2007, 67:9929

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Nothing to Disclose: FRL, CB-W, YL, SMH

P2-55

Apigenin Inhibits Progestin-Induced VEGF at Both mRNA and Protein Secretion Level in T47-D Human Breast Cancer Cells.

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Clinical trials indicate that the risk of breast cancer is higher in post-menopausal women on estrogen plus progestin therapy compared with women taking estrogen alone or placebo. Progestins are necessary to prevent the proliferative effects of estrogen in the uterus, which increases the risk for endometrial cancer. Current evidence suggests that progestins may promote breast cancer by stimulating the elaboration of vascular endothelial growth factor (VEGF), a potent angiogenic growth factor that in vivo is essential for progestin-dependent breast cancer progression [Liang et al. Cancer Res.2007, 67:9929-36]. Consequently, identifying compounds with the capacity to block progestin-dependent VEGF induction in breast tumor cells would allow post-menopausal women to continue to benefit from estrogen plus progestin therapy without the increased risk of breast cancer. In this study we investigated the chemopreventive and therapeutic potential of apigenin, a low molecular weight flavone found in fruits and vegetables and a compound with significant anti-angiogenic potential, to inhibit progestin-induced VEGF secretion from human breast cancer cells. We treated T47-D human breast cancer cells grown in medium containing charcoal-stripped serum with 10 nM natural or synthetic progestins for 18 h ± apigenin (100 mM), or the synthetic anti-progestin RU-486 (1 mM). Media was collected and assayed for VEGF by ELISA. Using RT-PCR we also determined the effect of treatment with different levels of apigenin (1-100μM) on MPA-induced mRNA expression of various genes associated with progestin-accelerated breast cancer development. We found that apigenin inhibited MPA-induced VEGF at both the mRNA and protein levels. RT-PCR analysis showed that MPA alone or in combination with apigenin (100μM) down-regulated VEGFR-1 (flt) and VEGFR-2 (flk) mRNA expression. These observations suggest that apigenin could block progestin-dependent angiogenesis in vivo and could potentially block breast cancer development and/or progression, though this remains to be tested. In conclusion, this study provides evidence that apigenin inhibits MPA-induced VEGF in T47-D human breast cancer cells, indicating that this naturally-occurring flavone possesses considerable chemopreventive potential through anti-angiogenic mechanisms. Our findings suggest that apigenin could reduce the risk of progestin-accelerated breast cancer when administered to post-menopausal women on estrogen plus progestin therapy.

(1) Liang et al. Cancer Res. 2007, 67:9929-36

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Nothing to Disclose: BM, IB, SMH

P2-56

The Early Effects of Aromatase Inhibitors on Bone Metabolism and Bone Density in Postmenopausal Women with Breast Cancer: An Interim Analysis.

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Aromatase Inhibitors (AIs) are effective for secondary prevention of postmenopausal breast cancer and considered first-line treatment of hormone-sensitive breast cancer. Reduction in estradiol (E_2) levels by these medications leads to substantial bone loss, as reported in clinical trials. Antiresorptive medications prevent bone loss, but they have frequent side effects and expense. Thus, treating all women receiving AIs with bisphosphonates might not be the most prudent approach. We hypothesized that women who demonstrate high bone turnover in the first 3 to 6-months on treatment, will have greater bone loss. The current study evaluated women with normal or moderately low bone mass during their first year of treatment with anastrozole or letrozole to determine if early changes in bone turnover markers correlate with bone loss at one year. We present an interim analysis of the first 12 women who have completed the study. Hormones inclusing E_2 and sex hormone binding-globulin (SHBG) and bone turnover markers including type-1 procollagen peptide (P1NP), urine N-terminal collagen crosslinks (UNTX) and serum C-telopeptide collagen crosslinks (sCTX) were measured at baseline, 1, 3, 6 and 12 months. Bone Mineral Density (BMD) was measured at baseline, 6 and 12 months at the Lumbar spine (LS), Femoral Nexk (FN) and Total Hip (TH) using a Lunar DXA (Prodigy). L-spine and Total hip BMD decreased significantly by 2% and 1.4% ($p<0.05$ respectively) at 6- months and by 3.4% and 2.0% ($p<.01$ and $p<.05$, respectively, at 12 months). E_2 levels decreased from BL values (P=.03), as did SHBG although not significant. sCTX and PINP increased significantly from BL to 12 months, ($p \leq .001$, p=.003, respectively), and there was a trend for increase of UNTx (p=.096). Subjects with highest E2 levels had lowest sCTX (p for trend = .06). Vitamin D increased from a BL of 33 to 42 ng/ml at 12 months due to supplementation. The greater the increase in vitamn D at 6-months the less the increase in UNTX over time (p=.009), and the less the decline of BMD at the total hip (p for trend = .09).

We conclude that in women receiving AIs for breast cancer significant spine and hip bone loss occurs in the first year of therapy. The data suggests an interaction between bone turnover markers, vitamin D and BMD; however, further analysis of these parameters in the complete study group is necessary to determine if these relationships remain and thus can identify those women at highest risk of bone loss.

Sources of Research Support: Connecticut Breast Health Initiative, Inc.

Nothing to Disclose: PT, FARM, PF, RF, ST

P2-57

Molecular Predictors of Response to Aromatase Inhibitors in Breast Cancer.

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BACKGROUND: Aromatase inhibitors (AI), which block estrogen formation, are now considered the most effective class of drugs in the endocrine treatment of breast cancer. However, the response rate is only between 50-60%.
OBJECTIVE: To identify novel molecular predictors of response to AI.
METHODS: Frozen or paraffin-embedded primary breast carcinomas from 84 women (1989-1995) who subsequently developed advanced or recurrent breast cancer and were treated with AI were analyzed using Applied Biosystems Human Genome Survey Microarray V2.0. There were a total of 41 responders and 43 nonresponders. Sixty-nine patients were determined to be ERα-positive and 15 patients were found to be ERα-negative via immunohistochemistry. Microarray analysis was validated by real-time PCR on select genes.
RESULTS: A total of 17686 genes were found to be expressed in the samples analyzed. Of these, 202 genes were differentially expressed in responders versus nonresponders with a false detection rate (FDR) ≤ 25% and p-value ≤ 0.005. ESR1 was the top gene and its mRNA was found to have the highest fold change difference between responders versus nonresponders (x 3.87). Genes with known association with breast cancer such as GREB1, MYB, KRT19, SLC39A6, HPN, FOXA1, and CA12 were also found to have higher expression profiles in AI responders. Initial validation with real-time PCR of a number of novel genes revealed significant fold change differences in the mRNA expression between ERα-positive versus ERα-negative groups (MAPT- x6.1; SUSD3- x6.4) and our responder versus nonresponder groups (MAPT- x2.4; SUSD3- x4.3).
CONCLUSIONS: Differences in genetic signatures between ERα-positive/ERα-negative and AI responders/nonresponders exist. The novel genes identified from our microarray analysis could potentially be employed to predict AI responsiveness in breast cancer patients. Additional work will need to be performed to explore the in-vivo function of these novel genes and their role in breast cancer and AI responsiveness.

Sources of Research Support: NIH grant CA67167.

Nothing to Disclose: IM, SR, SH, SB

P2-58

The Relation of Parity with the Serum Prolactin Levels in Women with Breast Nodular Disease.

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Prolactin is synthesized in the anterior pituitary and in secondary sites: adipose tissue, brain, endometrium, and the immune system. For a long time it was accepted that prolactin was important only in gestation and puerperium, but other important roles have already been established for its secretion as the synthesis and secretion of prolactin, together with the expression of its receptor, both in the normal breast tissue and in breast carcinoma. Multiple lines of evidence support an inverse association between parity and prolactin levels, with no relation to the number of pregnancies. Also shows the decrease of prolactin secretion with elderly and menopause.
In this study, we analyzed 99 women with breast nodular disease between ages 18 and 80 years relatively to their prolactin levels, parity and age. Prolactin was measured by Immulite immunometric assays with reference interval 1,9-25 ng\\ml. A t-test was used to compare mean prolactin levels for the nulliparous and parous groups, as so as for the women with age above or less than 50 years. All the women with age above than 50 years and 50% of the parous women were postmenopausal.
During their medical evaluation, subjects answered a questionnaire, went through an excisional\\ core biopsy or through a fine needle aspiration punction(FNAP) in order to ascertain nodule malignancy, serum prolactin was collected before the breast clinical examination or nodule procedure(biopsy\\ FNAP). In menacme women, the collection was made at the beginning of the menstrual cycle. All analyses were performed with the SPSS Software, version 16. The women had the medium prolactin levels compared by the t-test from two independent samples and were separated in groups: nulliparous or parous; age above 50 years or less . Differences were considered statistically significant at the $a=0.05$ level, and 95% confidence intervals.
We observed that parity and menopause causes prolactin levels reduction. Prolactin may be one of the many hormones responsible for the link between reproductive risk factors and breast cancer. Although breast cancer risk increases after pregnancy, parity is associated with long- term risk reduction , what is probably mediated by prolactin levels.
In conclusion, the inverse association between parity and prolactin levels, should be also remembered in cases of discrete hyperprolactinemia with the objective to avoid expensive and unnecessary diagnostics and treatments.

Nothing to Disclose: MF, MH, AR, AV, CR, RA, AV

P2-59

Neonatal Treatment with Bisphenol A Decreased Cell Proliferation and GnRH Gene Expression in Adult Rat Ovary.

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Bisphenol A, (BPA), a component of polycarbonate plastics, epoxy resins and polystyrene found in many common products, is a xenoestrogen that alters several functions in different species, including rats and humans. Previously we described the effects of this endocrine disruptor on the hypothalamic-pituitary-gonadal axis in female rats (1). Following this research line, in this study we analyzed the effects of neonatal exposure to BPA on granulosa and theca cell proliferation, as well as GnRH gene expression, in ovaries of adult rats.
Female Sprague-Dawley rats were injected sc, daily from postnatal day (PND)1 to 10 with BPA: 500 µg/50 µl oil (H), or 50 µg/50 µl oil (L), or solvent as control (C). In adults (PND120-160), euthanized by decapitation in the morning of estrus following Institutional Ethical Standards, one ovary of each animal was immediately fixed in 5% neutral buffered formaldehyde for histological examination. Cell proliferation in early preovulatory follicles was determined in ovarian sections by immunohystochemistry of the proliferating cell nuclear antigen (PCNA) in theca (TC) and granulosa (GC) cells, as described in (2). Results were expressed as PCNA positive cells/total cells. In the second ovary, GnRH mRNA levels were determined using real-time PCR as described in (3). Results were expressed as means ± SE and considered significant when $p<0.05$. Data were analyzed by one-way analysis of variance.
Animals treated with high-dose BPA showed a decrease of GC [C: 51.42±3.45 (n=5); L: 39.75±6.92 (n=5); H: 17.20± 3.54 (n=5), $p<0.001$] and TC [C: 42.46±3.58 (n=5); L 38.59±9.49 (n=5); H: 13.40± 4.15 (n=5), $p<0.02$] proliferation. A dramatic reduction in GnRH mRNA expression was found even with the lower dose [Arbitrary Units; C: 0.8136±0.1279 (n=5); L: 0.0862± 0.0333 (n=4); H: 0.0043± 0.0021(n=4), $p<0.001$].
Neonatal exposure to BPA decreased ovarian granulosa and theca cell proliferation, as well as GnRH mRNA expression, in ovaries of adult rats. These results show that exposure to BPA during the neonatal period irreversibly alters ovarian physiology in adulthood, long after the exposure to the agent ceased. Exposure to BPA during ontogeny could contribute to the development of many reproductive disorders of increased incidence in humans.

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Nothing to Disclose: NSB, MOF, VARLL, CL

P2-60

Epigenetic Regulation of NADPH Oxidase 4 (NOX4) by 17Beta-Estradiol (E2) and Bisphenol A (BPA) in Prostate Cells.

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A connection between elevated reactive oxygen species (ROS) and prostate carcinogenesis has become increasingly apparent. In this regard, we previously reported chronic exposure of rats to low dose E2 increased Nox4 expression in prostate epithelial cells along with DNA damage and protein adduct formation, hallmarks of oxidative stress, and subsequent development of pre-neoplastic lesions (1). In this study, two non-tumorigenic rat immortalized prostate epithelial cell lines, NPR152 and NBE-1, were used to ascertain whether estrogens regulate Nox4 expression via an epigenetic mechanism. These cell lines were found to express low levels of Nox4 and have a hypermethylated 5'-CpG island. Following a 3 day-treatment of these cells with E2 (1 & 10 nM) or BPA (10 &100 nM) the 5'-CGI was significantly demethylated along with an increase in Nox4 expression. Co-treatment of E2- or BPA-treated NPP152 cells with fulvestrant, an estrogen receptor antagonist, abrogated the action of E2 but not that of BPA, suggesting the two estrogens may differ in their mode of action in Nox4 regulation. Gel shift assays and deletion mutant experiments demonstrated that CG 6-8 within the 5'-CGI played a role in gene transcription. In silico analysis suggest this region harbors an E2F1 cis-element. Collectively, our data suggest that Nox4 transcription is under epigenetic regulation by estrogens, possibly mediated via hypermethylation of a key transcriptional factor binding site. They further support a link between estrogens/estrogen mimics and ROS production as an etiological factor for some prostatic disorders.

(1)Tam et al 2007 Am J Pathol 171:1334

Sources of Research Support: NIH Grant ES016817; NIH Grant ES006096; NIH Grant ES015584; NIH Training grant CA15776; NIH Training grant CA112532.

Nothing to Disclose: LMM, WYT, XZ, SMH

P2-61

In Utero Exposure to Bisphenol A Alters Social Behaviors and Methylation Levels of Esr1.

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The widespread environmental estrogenic compound, bisphenol A (BPA), accumulates in the blood of embryos and adults. Exposure to endocrine-active compounds (EAC) alters normal hormonal function, affects neural organization and ultimately may modify behavior and can lead to neurological disorders. BPA affects the developing brain both via its role as a DNA hypomethylator and an EAC. Loss of DNA methylation can lead to dysregulated gene expression and downstream effects on behavior. We hypothesize that *in utero* BPA exposure causes persistent, epigenetically tractable alterations in the brain and these changes influence social behaviors, many of which are sexually-dimorphic. Here, we present data on several sexually dimorphic social and perseveration behaviors that are influenced by BPA exposure. Female C57BL/6J mice were placed on phytoestrogen reduced chow with or without 5mg/kg BPA addition. This yields circulating BPA equivalent to levels noted in humans. A week later females were mated, at birth, pups were fostered to control dams to limit BPA's effect to gestation. At day 21, offspring were tested for social behaviors. Control juvenile males spent more time with a conspecific, while females spent less time with a conspecific and more time exploring an adjacent empty arena. *In utero* BPA specifically decreased the time males spent with a conspecific. During social play, control females spent more time sitting side by side and engaging in anogenital sniffing than control males. BPA males, but not females, increased time spent in side by side sitting and in sniffing behavior. Thus, in three sexually dimorphic behaviors, BPA exposure alters typical social interactions in males making them more female-like.
To identify potential mechanisms underlying these behaviors, we measured the methylation status of several candidate genes following *in utero* BPA. In BPA males, estrogen receptor alpha (Esr1) was significantly hypomethylated as compared to control males. Preliminary results suggest that this effect may be male-specific since BPA females do not show reduced Esr1 methylation.
In humans, males are at least 4 times more likely than females to develop autism spectrum disorders (ASD). ASD is characterized by perseveration and disrupted social behaviors. We hypothesize that hypomethylation of Esr1 in BPA exposed males is related to the behavioral modifications we noted. In future studies we will use this model to explore potential effects of BPA exposure to ASD.

Sources of Research Support: NIH R01 MH086711 and Autism Speaks Grant #4802.

Nothing to Disclose: JTW, KHC, JJC, EFR

P2-62

Bisphenol A Effect on Bone Marrow-Derived Mesenchymal Stem Cell Differentiation.

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Introduction: The industrial chemical bisphenol A (BPA) is a known endocrine disruptor. Sensitivity of fetal uterine cells to BPA in animals implies that vulnerability of endometrial cells may depend on their developmental stage at the time of BPA exposure. We recently showed that human bone marrow-derived mesenchymal stem cells (MSC) differentiate to endometrial stromal phenotype. Here we studied the effect of BPA exposure on human MSC - model for early developmental stage endometrial stem/progenitor cells.
Materials & Methods: MSC were exposed to BPA in a dose-dependent manner (1, 10, 10mM, 1, 10, 100µM) for 48hrs. BrdU proliferation and TUNEL apoptosis assays were performed. To assess BPA effects on MSC differentiation and if exposure timing is critical, we exposed MSC to BPA doses as above for short term (48 hrs) and long term (14 days), before or during culture in endometrial-stroma differentiating medium (with 8-Br-cAMP). Specificity of BPA effects on endometrial lineage differentiation was determined by comparing to adipogenic and osteogenic differentiation. Endometrial differentiation markers PRL and IGFBP1 were assessed by QPCR and ELISA after 14 days of cAMP. Estrogen receptor (ESR) mRNA was studied. Analysis of MSC-specific markers CD90,105,73, and HOXA9-11, Wnt5a and 7a transcripts is ongoing.
Results: Short-term BPA treatment showed significantly decreased BrdU incorporation, hence decreased DNA synthesis in MSC in a dose-dependent manner. There was no difference in apoptosis between BPA-treated and untreated cells. QPCR revealed increased PRL and IGFBP1 mRNA in MSC treated long-term with cAMP+BPA, while BPA alone had no effect. cAMP+100nM BPA significantly up-regulated PRL and IGFBP1 mRNA and protein, compared to cAMP-only, suggesting a synergistic activity between cAMP and BPA at high doses. There was a significant increase in PRL and IGFBP1 secretion by MSC after 100nM and/or 1uM BPA+cAMP. PRL and IGFBP1 mRNA and protein decreased in MSC under higher doses (10, 100µM) of BPA, potentially due to decreased survival in prolonged culture. Differentiation of MSC in osteogenic and adipogenic media was not significantly affected by BPA. 1 and 10µM BPA significantly increased ESR1 expression in MSC, while same doses decreased cAMP-induced expression of ESR1.
Conclusions: BPA affects MSC DNA synthesis, but not cell death. Differentiation to endometrial lineage was compromised, as accessed by PRL and IGFBP1. BPA-ESR interaction needs further studies.

Sources of Research Support: California Institute for Regenerative Medicine SEED grant RS1-00207-1 (LCG).

Nothing to Disclose: LA, ZZ, LCG

P2-63

Low-Doses of BPA and Estrogen Increase Ventricular Tachycardia and Fibrillation Following Ischemia-Reperfusion in the Female Heart.

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There is broad human exposure to the ubiquitous estrogenic disruptor bisphenol A (BPA). The effects of environmentally-relevant concentrations of BPA on the heart are poorly understood. In earlier studies, we report that rapid exposure to low-dose BPA alters myocyte Ca cycling and promotes arrhythmogenic triggered activities in myocytes from female, but not male rat hearts. The pro-arrhythmic effect of BPA is particularly prominent in the presence of physiological level of 17β-estradiol (E2). In the present study we investigated the acute effects of BPA and E2 on ventricular arrhythmias and infarction following ischemia-reperfusion in rat whole hearts.
Ischemia-reperfusion (IR) injury of the heart is a major contributing factor to arrhythmias, infarction, and ventricular dysfunction post myocardial infarction (MI). Rat hearts were subjected to 20 minutes of global ischemia, and arrhythmias upon reperfusion were analyzed using surface electrocardiography. In female, but not male hearts, acute exposure to BPA combined with E2 (both at 1 nM) resulted in a significant increase in the duration of sustained ventricular arrhythmia, from 306 sec in control to 670 sec ($P < 0.05$). While estrogens increased the durations of both ventricular tachycardia and fibrillation, their effect on ventricular fibrillation (VF) was particularly pronounced. BPA plus E2 increased the duration of VF from 27 sec to 126 sec and the fraction of VF over total duration of sustained ventricular arrhythmia from 9% to 27% ($P < 0.05$). The pro-arrhythmic effects of estrogens were not blocked by MPP or PHTPP (ERα and ERβ selective blocker, respectively) alone but were abolished by MPP combined with PHTPP, suggesting the involvements of both ERα and ERβ signaling. In contrast to their deleterious effects on arrhythmogenesis, BPA combined with E2 had a protective effect on infarction following IR. In female hearts, exposure to estrogens reduced infarction size from 13.2% of the whole heart in control to 5.9%, agreeing with previously described beneficial effect of estrogen on cardiac infarction.
In conclusion, our results show that rapid exposure to low-doses of BPA and E2 markedly increase both the duration and severity of ventricular arrhythmia following ischemia in female hearts. These pro-arrhythmic effects may contribute to the higher susceptibility to arrhythmias and sudden cardiac death reported in subpopulations of women.

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Nothing to Disclose: SY, WS, SMB, H-SW

P2-64

Effect of Bisphenol A on the Comprehensive DNA Recognition Profile of Estrogen-Related Receptorγ and Estrogen Receptorα in Breast Cancer Cells.

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Interaction of transcription factors, including the estrogen receptor (ER) sub-family, at DNA sites plays a critical role in regulating breast cancer gene expression and mediating tamoxifen resistance, but the ability to translate this knowledge towards tumor responsiveness and transcription-based therapies has yet to be achieved. The plasticizer bisphenol A (BPA), found in common plastics, toys, and bottles, induces the expression of genes associated with aggressive breast cancer (1). Estrogen-related receptor γ (ERRγ) is an orphan receptor that is constitutively active in the absence of ligand and is over-expressed in tamoxifen-resistant invasive lobular breast cancer cells (2). BPA is a recently-identified high-affinity ligand for ERRγ but binds ERα with lower affinity, and the effect of BPA on receptor-DNA binding specificity is unknown (3). ERRγ binds several other ligands, including 4-hydroxytamoxifen (OHT) and diethylstilbestrol (DES), and both OHT and DES can down-regulate ERRγ activation by inducing a conformational change in the receptor. BPA competes with OHT for ERRγ binding and maintains the receptor in an active conformation. As proof-of-principle, we are comparing the effect of BPA to current therapies for breast cancer, such as OHT, on the DNA interactions of ERRγ and ERα. We hypothesize that the affinity and specificity of receptor binding to DNA sites is differentially affected by BPA and OHT in breast cancer cell lines. We have developed a DNA microarray technology, known as cognate site identifier (CSI) arrays, which displays all permutations (over 8 million DNA combinations) of the receptor DNA binding site. A CSI microarray containing every variation of a 10 to 12 base pair DNA sequence within duplex DNA has been custom-designed to analyze the DNA interactions of ERα and ERRγ. The array output is an interactive data display, known as the sequence specificity landscape (SSL), which provides a quantitative profile of the comprehensive binding characteristics of the receptor-DNA interactions for every DNA sequence. We are titrating the amount of receptor using cell lysates from breast cancer cell lines to determine the lowest level of detection for the DNA binding profiles. The ultimate goal of these studies is to develop a miniaturized, high throughput assay for primary cells from breast cancer patients that can be used by physicians for immediate tumor characterization of the entire DNA-binding cellular proteome.

(1) Dairkee SH et al., Cancer Res 2008; 68:2076
(2) Riggins RB et al., Cancer Res 2008; 68:8908
(3) Matsushima A et al., J Biochem 2007; 142:517

Sources of Research Support: Department of Defense Concept Award W81XWH-09-1-0581 awarded to MSO.

Disclosures: MSO: Part-time employee, VistaMotif LLC; Founder, Illumavista Biosciences LLC. CLW: Part-time employee, VistaMotif LLC; Founder, Illumavista Biosciences LLC.
Nothing to Disclose: JDS

P2-65
Bottle Feeding and Serum Bisphenol A Level in Infants.

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Objectives: Exposure to endocrine disrupting chemicals (EDCs), especially in embryonic, fetal, early postnatal and pubertal periods, gives rise to a variety of adverse health outcomes. Bisphenol A (BPA) is a well-known EDC widely found in plastic products including food and water containers, baby bottles and linings of metal cans. The objectives of this study were to determine serum BPA levels in infants and to investigate the effects of bottle feeding on serum BPA level.
Methods: Serum BPA levels of normal healthy infants age 4 to 16 months (n=60) were assayed with a competitive ELISA.
Results: Even though the levels are quite low, BPA was detected in every sample (71.05 ± 79.89 pg/mL). Serum BPA level of bottle-feeding (n=30) infants were significantly higher than that of breast-milk-feeding infants (n=30) (96.58 ± 102.36 vs. 45.53 ± 34.05 pg/mL, P = 0.014). There was no significant difference in serum BPA level between male (n=31) and female (n=29) infants (62.45 ± 63.86 vs. 80.25 ± 94.39 pg/mL, P = 0.393). There were no significant associations between BPA level and age, body weight and birth weight.
Conclusions: The wide-spread exposure to BPA even in infancy is supposed. Bottle-feeding has an effect on the serum BPA level in infants. The efforts to reduce and prevent the exposure to BPA in infancy are required. Small sample size is the limitation of this study. Larger size group is necessary to confirm our findings.

Nothing to Disclose: YJR, KHL, YJO, WJL, HSK

P2-66

4-Vinylcyclohexene Diepoxide (VCD)-Induced Fibroadenomas: A Novel Rat Model of Mammary Tumorigenesis.

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Fibroadenomas are common breast lesions that, while benign, account for over 50% of breast biopsies and are associated with a slightly increased risk of breast cancer. Their pathogenesis, however, is not well defined. Fibroadenomas also occur spontaneously in female Sprague Dawley (SD) rats but with a late onset, occurring around 14 months of age when rats are in a state of persistent estrus, and are hormone responsive as early ovariectomy (OVX) prevents their later occurrence. 4-Vinylcyclohexene diepoxide (VCD) is an occupational chemical that selectively targets primordial and primary follicles in the ovary and accelerates ovarian senescence in rodents (1), leading to an early onset of persistent estrus, followed by premature ovarian failure. We hypothesized that mammary tumor incidence would thus be altered in VCD-treated female SD rats, having an earlier onset due to premature induction of persistent estrus and/or a decreased incidence due to premature ovarian failure, analogous to OVX rats. To test this postulate, 28-day old female SD rats were dosed ip for 25 days with vehicle or VCD (80 mg/kg/d or 160 mg/kg/d; n=18-29/group) and monitored to PND 604 for mammary tumor onset and incidence. Mammary tumor onset was accelerated (PND 219 vs. 394) and incidence was increased (84% vs. 38% at PND 604; p=0.035) in VCD-treated animals in a dose-dependent manner relative to vehicle-treated, age-matched controls. Development of mammary tumors, which were fibroadenomas on histologic evaluation, was independent of plasma prolactin levels and changes in ovarian function, assessed by time spent in persistent estrus and ambient 17β-estradiol. These data suggest that VCD may be acting as a tumor enhancer when administered to rats during terminal end bud epithelial differentiation (PND 21-55), a period when the breast is most susceptible to carcinogens. Consistent with this hypothesis, administration of VCD (80 mg/kg or 160 mg/kg) to 3-month female SD rats, after terminal end bud differentiation did not alter the incidence or onset of mammary tumor development as compared to vehicle-treated controls. Since VCD is known to target c-kit signaling in the ovary (2), this may represent a possible mechanism for VCD's effect in the breast, as c-kit is known to regulate differentiation and growth of mammary epithelium (3). VCD may serve as a useful tool for examining the physiology of branching morphogenesis in the breast, as well as the pathophysiology of fibroadenomas.

(1) Flaws et al., Reprod Toxicol 1994; 8:509
(2) Fernandez et al., Biol Reprod 2008; 2:318
(3) Yared et al., Breast J 2004; 10:223

Sources of Research Support: NIH Grant AT003614 awarded to JLF.

Nothing to Disclose: LEW, ALL, JBF, PBH, DGB, JLF

P2-67

Alterations in Mammary Gland Morphology and the IGF-Estrogen Axis May Contribute to Increased Mammary Tumor Susceptibility in Fetal Alcohol-Exposed Offspring.

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In utero exposure to toxins can alter fetal programming, leading to an increased risk of diseases such as breast cancer later in life. The CDC reports that 1 in 12 women drink during pregnancy, while 1 in 30 women admit to binge drinking. Therefore the possibility that fetal alcohol exposure increases breast cancer risk could represent a significant public health concern. We have previously reported that fetal alcohol exposure decreases tumor latency in response to the carcinogen N-nitroso-N-methylurea (NMU) and causes more malignant, estrogen-receptor negative mammary tumors with decreased IGFBP-5 expression relative to tumors of control animals. This study examined changes in mammary gland development and underlying hormonal mechanisms that may contribute to the increased susceptibility of the mammary gland to NMU-induced carcinogenesis in alcohol-exposed offspring. Pregnant Sprague-Dawley rats were fed either a liquid diet containing 6.7% (approximately 35% of calories) ethanol (AF), an isocaloric liquid diet (PF), or ad libitum (AL) rat chow. Offspring were euthanized at postnatal day 20, 40, and 80 (n=11 pups per group at each time point). Pups were injected with 50 mg/kg bromodeoxyuracil (Brdu) 4 hrs and 2 hrs before study termination. Mammary glands, liver, and trunk blood were collected. While there was no difference in mammary gland density at any time point, 20d mammary glands of AF animals tended to have more terminal end buds. In addition, AF animals displayed significantly more Brdu stained cells than either control group which did not differ from each other at 20d, indicating that the alcohol-exposed gland is more proliferative at this stage in development. To investigate the mechanism behind the increased proliferation we examined hepatic IGF-I mRNA expression and found that AF animals had increased hepatic mRNA expression at 20d and 40d of life. In addition, mammary glands from AF animals exhibited increased aromatase expression at these time points, suggesting that local production of estradiol may be increased, contributing to the increase in cell proliferation. Collectively, these data suggest that fetal alcohol exposure induces alterations in the estrogen/IGF-I axis during mammary development which may contribute to an increased susceptibility to mammary tumorigenesis.

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Nothing to Disclose: TPP, CC-G, WSC

P2-68

Epigenetic Response of the Human Breast Epithelial Cells to Xenoestrogens.

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Xenoestrogens are part of a group of agents termed endocrine disruptors because of their capacity to perturb normal hormonal actions and it has been suggested that they may contribute to the development of hormone-dependent cancers, such as breast and endometrial cancers. Bisphenol A (BPA) is polymerized to manufacture polycarbonate plastic and epoxy resins. Human exposure occurs when BPA leaches from plastic-lined food and beverage cans.
In the present work we are aiming to determine if BPA has carcinogenic properties by using an *in vitro* system. For this purpose the human breast epithelial cells MCF-10F were treated with 10^{-3}M to 10^{-8}M BPA continuously for two weeks. We have found that the cells treated with 10^{-3}M and 10^{-4}M BPA died indicating that these concentrations were toxic. The cells treated with 10^{-5}M to 10^{-8}M BPA were evaluated for formation of solid masses in collagen and invasion capacity, both phenotypes that are indicators of cell transformation. MCF-10F treated with 10^{-5}M, 10^{-6}M, 10^{-7}M and 10^{-8}M BPA formed a high percentage of solid masses (27%, 20%, 42.4% and 31.8% respectively. Ten passages after BPA treatments, the invasive capacities of the cells were evaluated using Boyden chambers. The cells that invaded through the Matrigel membrane were counted under a light microscope, and values of chemo-invasion were expressed as the number of cells that migrated to the lower chamber. Cells treated with BPA showed an increased invasion capacity that was higher for lower doses.

Expression and DNA methylation analysis were performed with the cells treated with 10^{-5}M and 10^{-6}M BPA. We found that these cells showed an increased expression of *BRCA1*, *BARD1*, *CtIP*, *RAD51* and *BRCC3*, all genes involved in DNA repair, and downregulation of *PDCD5* and *BCL2L11*, both involved in apoptosis. We found that upregulation of *CtIP* was related to hypomethylation of the promoter/exon1 of this gene. Furthermore, BPA induced silencing of *BCL2L11* by hypermethylation. One mechanism by which estrogen (E_2), and probably BPA, initiates breast cancer is by generating reactive oxidative species (ROS) that can produce a variety of DNA modifications that, if not countered by DNA repair, can lead to cell transformation.
This is the first demonstration that BPA induces neoplastic transformation of breast epithelial cells and that aberrant DNA methylation of genes involved in DNA repair and apoptosis could be involved in the initiation of the neoplastic process.

Sources of Research Support: Grants NIH-ES-12771 and R21-ES-15894 from the NIH.

Nothing to Disclose: SVF, YH, KES, JR

P2-69

A Model System for Assessment of Endocrine Disruptors of Endometrial Cancer.

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Problem: Metastatic and recurrent endometrial cancers present therapeutic challenges; systemic treatments generally have limited efficacy. The lack of experimental model systems as well as inconsistencies in reproducibility, hormonal milieu and sustained growth with primary endometrial cells remain barriers to research. Our lab has exploited well-characterized endometrial cancer cells to model an innovative system for finding and testing new therapeutic agents.
Methods: ECC-1 cells are epithelial derivatives of a well-differentiated endometrial cancer possessing functional steroid response elements and typical steroid coactivators. We created ECC-1 cells with a stably integrated progesterone response element (PRE) linked to a TK promoter driving a firefly luciferase reporter. PRE comprises palindromic repeats of hexamers flanking 3 nucleotides and is recognized by the receptors for progesterone, androgen, and glucocorticoids. Based on this common recognition sequence, we hypothesized that this novel cell model could be used to report activities of steroid hormones and inhibitors. Conditions for cell plating, growth, treatment and assay were optimized for use with a high throughput automated system. Cells were grown in stripped serum medium with 1 nM 17β estradiol to allow for maintenance of progesterone receptors. Treatment with vehicle (ethanol), progesterone, cortisol, testosterone, or tamoxifen alone or in combination with mifepristone was carried out for 24 hours in 96 well plates. Luciferase activity was assessed using a Synergy plate reader.
Results: Dose response curves showed that the cells were responsive to progesterone and cortisol and inhibited in a predictable manner by mifepristone. Likewise, the ER antagonist, tamoxifen, did not alter activity, as expected. Surprisingly, however, testosterone was ineffective in this assay. These data will be validated through expression analysis of steroid receptors and endogenous target genes.
Implications: We have developed a novel system to allow rapid readout of steroid receptor activity in uterine cells compatible with high throughput applications with potential to identify small molecule endocrine disruptors of importance in endometrial cancer proliferation. These tools enable mechanistic studies of factors that modulate steroid receptor function and will have broad utility for understanding epigenetic and epidemiological risks and for development of targeted therapy for endometrial cancer.

Nothing to Disclose: NMS, ETA, ACE

P2-70

Reproductive Disruption by Estrogenic Wastewater Effluents.

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The reproductive potential of native fishes may be compromised in stream reaches of western states where large volumes of treated wastewater are discharged into relatively small-sized streams. We investigated the impact of City of Boulder wastewater treatment plant (WWTP) effluent on fish reproduction. This effluent contains endocrine-active compounds including nonylphenol, bisphenol A, and synthetic and natural reproductive steroids. We have identified female biased sex ratios, gonadal intersex, asynchronous ovarian development, elevated vitellogenin, and other forms of reproductive disruption in feral white suckers (Catostomus commersoni) collected downstream of WWTP effluent but not at reference sites (1,2). Analysis of museum specimens collected between 50 and 100 years ago from these sites reveals no evidence of reproductive disruption. We conducted on-site controlled exposure experiments using a mobile flow-through laboratory, exposing adult male fathead minnows (Pimephales promelas) to either WWTP effluent, reference water from Boulder Creek upstream of the WWTP, or mixtures of reference water and WWTP effluent (containing an average of 29 ng/L estradiol-equivalents of estrogenicity) for up to 56 days. Exposure to diluted WWTP effluent significantly elevated vitellogenin and suppressed male sex characters after 7 days of exposure. A similar study of possible effects of wastewater effluent from a small mountain community on juvenile brown trout (Salmo trutta) showed no increase in vitellogenin. A re-evaluation conducted in 2008 following a major upgrading of the Boulder WWTP revealed diminished responses to wastewater effluent in adult male fathead minnows.

(1) Woodling, JD et al., Comp Biochem Physiol Pt C 2006; 144, 10
(2) Vajda, AM et al., Environ Sci Technol 2008; 42: 3407

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Nothing to Disclose: AMV, ALB, JDW, LBB, DON

P2-71

In Utero Exposure to Di-(2-Ethylhexyl) Phthalate Affects the Adrenal Potassium Channel Expression Leading to Reduced Circulating Aldosterone Levels in the Adult.

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In utero exposure to the phthalate plasticizer DEHP induces reduction of testosterone production in the adult rat. We previously reported that DEHP decreases the mineralocorticoid receptor (MR; NR3C2) in Leydig cells as well as aldosterone serum levels. MR activation in Leydig cells increases androgen production, and therefore a receptor/ligand decrease could mediate the antiandrogenic effects of DEHP. In search of the mechanism underlying the effect of *in utero* DEHP on adrenal aldosterone formation in adult animals, we observed that DEHP decreases the expression of angiotensin II receptors despite the presence of normal serum levels of the main aldosterone stimulants, angiotensin II and potassium. To further understand the long-term effects of DEHP, we examined global gene expression in adult (postnatal day 60) adrenals. *In utero* exposure to DEHP resulted in the up-regulation of genes associated with aldosterone production and also *de novo* cholesterol synthesis, a paradoxical finding given the reduced aldosterone levels. Clustering of genes affected by DEHP into those shown to be up-regulated when zona glomerulosa cells are stimulated by angiotensin II or potassium revealed that *in utero* exposure to DEHP triggers a potassium-like response in the adult. Detailed gene array data analysis followed by confirmation using quantitative real time -PCR identified the potassium channels Kcnk5 and Kcnn2 as novel targets of DEHP exposure. It is our hypothesis that a deregulation in potassium channel gene expression can lead to a chronic activation of the adrenal zona glomerulosa, in turn leading to reduction of angiotensin II receptor expression and inhibition of aldosterone production.

Sources of Research Support: NIH grants ES01349 & AG021092.

Nothing to Disclose: DBM-A, MC, BRZ, VP

P2-72

Effects of Extremely Low Doses of Chlorobenzenes on Hormone-Mediated Aggressive Behavior in Rat Model System.

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Introduction. With the development of chemical industry, the environment is disturbed by more and more specific groups of persistent, organic pollutants (POP). Some of these agents have the ability to interfere with or disrupt certain endocrine systems (EDC). Homeostatic regulation plays a vital role in survival and competition for various resources. Laws and by-laws concerning the handling of chemicals are based primarily on data about exposures to higher or even toxic levels.
Aim. Our present investigation was aimed at demonstrating the incidental effects of discrete, and often legally uncontrolled, rarely examined, extremely low doses of chlorobenzenes (ClB) as POP with EDC abilities. This study focusses on anxiety-related neurobehavioral and endocrine effects of long-term and discrete ClB exposures via food ingestion (a typical route of exposure).
Methods. Male Wistar rats were chronically administered with a ClB mixture for 30, 60 or 90 days. 0.1 or 1 µg/bwkg/day doses were applied. Aggressive behavior with offensive elements eg. lateral threats, chasing, menacing posture was recorded during a modified resident-intruder test. Smaller male Wistar rats as intruders were introduced to ClB-exposed animals. Serum arginine-vasopressine (AVP) and oxytocin (OXT) as aggression-related, and adrenocorticotrophic hormone (ACTH) as stress-related hormones of sacrificed animals were measured. Liver transferase enzyme activities were determined to evaluate the toxicity levels of applied exposures. Neurohypophyseal tissue cultures were prepared and basal and serotonin (5-HT)- or norepinephrine (NE)–stimulated AVP/OXT release were measured from the culturing media.
Results. Overt signs of toxicity (in terms of bw-loss and increased transferase levels) were not found in the POP exposed animals. However, serum AVP/OXT levels were found elevated in a dose/time depending manner. Tissue cultures showed modulations in both the basal and 5-HT- and NE-stimulated AVP/OXT release after ClB exposures. The POP exposed rats revealed higher levels of aggressivity in resident-intruder tests.**Conclusion.** Subtoxic environmental chemical expositions may influence aggression-related hormonal secretions and attached behavioral patterns. In human populations, chronic subtoxic POP/EDC exposition may be hypothesized to get involved in some psychiatric disorders with modulated aggressiveness.

Nothing to Disclose: ZV, GN, ML, MR, AJ, JJ, MG

P2-73

Ameliorative Effects of Progesterone Supplementation on Delayed Behavioral Development in Rat Pups Exposed to PCB Perinatally.

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Anecdotal reports have appeared suggesting that children of women treated with progesterone to maintain pregnancy exhibit acceleration of behavioral development. The mode of action is unknown, but progesterone is currently being considered for use as a supplement in treatment of traumatic brain injuries, bolstering its likely central affect. Our previous work has shown the lipophilic and ubiquitous environmental contaminant polychlorinated biphenyl (PCB) to disrupt neuroendocrine and learning/behavioral development through placental and lactational exposure of rat offspring, perhaps through modification of neurochemical activity. PCB has also been reported to reduce circulating progesterone levels. Thus, in the present study we examined the effect of gestational progesterone administration on behavioral development in their offspring. Female Sprague-Dawley rats were mated to males of the same strain. Progesterone supplementation was given via intramuscular injection (0.2 mg in sesame oil) beginning on day 1 of pregnancy and continued twice a week throughout pregnancy in half of the pregnant rats. Controls received only sesame oil. Rats receiving PCB were given a mixture of a non-coplanar (PCB 47) and a coplanar congener (PCB 77), and from the first day of pregnancy through lactation dams were fed either standard ground chow or chow to which PCB 47/77 mixture was added at 1.25, 12.5 or 25 ppm. Behavioral analysis of the rat pups was performed on PND 9 through PND 15 and PND 28-30. Ultrasonic vocalizations (USV) were monitored with regard to rate of calling and sound frequency. The ability to complete a syntactic grooming chain and number of chains performed per minute were determined, along with open field behavior and additional motor tests. Gestational progesterone supplementation to mothers of pups not exposed to PCB enhanced motor development of the pups as compared to controls. The depression of same skills caused in PCB exposed pups was improved by progesterone regardless of amount of PCB exposure. Progesterone treatment also resulted in USV profiles in PCB exposed rats that were more similar to those of controls than profiles from animals without progesterone. The results of the present study support the idea that progesterone supplementation may counteract the deleterious effects of PCB in neurobehavior, and may be applied to reverse PCB disruption during pregnancy.

Sources of Research Support: Bowling Green State University Graduate College; Department of Biological Sciences.

Nothing to Disclose: CLB, VE, TS, ML, KA, BR, HCC, LAM

P2-74

Diet-Induced Obesity Alters the Estrous Cyclicity in Female and Gonadotropin Levels in Male C57BL/6 Mice.

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Obesity is a well-known risk factor for many disorders including insulin resistance, type 2 diabetes, cardiovascular disease, hypertension, dyslipidemia and reproductive disorders. With respect to reproduction, obese males have increased incidence of erectile dysfunction and/or low sperm count. Obesity in women is associated with oligo-ovulation or anovulation, a characteristic of Polycystic Ovary Syndrome. Although there are numerous reports on the effects of obesity on the fertility of men and women, consistent data in animal models, particularly, in mice is lacking. Therefore, we began with studying the effect of diet-induced obesity (DIO) on reproductive hormones of the male and female C57Black mice. We fed 13 week old normal cycling C57Black female mice on 60 % high fat diet (HFD) (n=10) and normal chow (NC) (n=10) for a period of 12 weeks. Similarly, male mice (11 week old) were fed on HFD and NC (n=12/group). Female as well as male mice on HFD gained weight during the study period of 12 weeks. Vaginal lavage was performed daily for 21 days to determine the pattern of estrous cycles. The female mice on HFD exhibited an increase in the total number of days at estrus stage; 7.0 for HFD vs 4.8 for the NC mice after 6 weeks, and 8.6 days vs 5.8 days after 12 weeks on HFD. Additionally, after 12 weeks, the HFD female mice correspondingly exhibited significantly reduced number of proestrus days, 1.9 compared to 3.6 for the NC mice, thereby, reflecting reduced number of cycles compared to the NC mice. However, these irregularities in the estrous cycles and reduced number of estrous cycles were not reflected in the basal (at diestrus stage) gonadotropin, LH and FSH levels at 6 or 12 weeks. Further analysis for gonadotropin levels at different stages of estrous cycle including the proestrus, estrus and, analysis of ovarian histology might explain the observed defects in estrus cycles and will provide further insight into the effects of HFD in female mice. In male mice, the FSH levels were significantly reduced by 20% at 6 weeks and 45% after 12 weeks on HFD as compared to the NC males with no significant changes in the corresponding LH levels of the two groups. Taken together, our findings suggest that diet induced obesity can lead to reproductive defects in male as well as female mice. Further studies are warranted to identify the specific molecular defects leading to these differences.

Sources of Research Support: NIH grants U54HD12303 and R01HD047400.

Nothing to Disclose: SS, NJW

P2-75

Immune Effects of DDE in Murine Diet-Induced Obesity Model.

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DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene) is a metabolic breakdown product of the petsticide DDT. While DDT was banned in the US in 1973, DDE persists in the environment. It accumulates in adipose tissues of animals and humans and acts as an androgen receptor antagonist in the reproductive system. Studies in our laboratory showed that DDE induces DNA fragmentation and increased expression of pro-apoptotic proteins in murine thymocytes. Since androgens also induce thymocyte apoptosis, DDE may act as an androgen receptor agonist in immune cells. Androgens have immunoprotective effects and explain in part the female predominance of many autoimmune diseases. In murine models of lupus, increased levels of monocyte chemoattractant protein-1 (MCP-1) and osteopontin (OPN) are found in kidneys. We saw that DDE treatment of normal male C57BL/6 mice causes increased kidney expression of MCP-1 and OPN, indicating that DDE may counteract the immunoprotective effects of androgens. We observed altered immune cell populations in DDE-treated mice, with greater percentages of immature CD4+CD8+ cells (12.5% vs. 3.9%, $P < 0.01$), $CD4^+$ T helper (17.2% vs. 10.9%, $P < 0.0001$), and $CD8^+$ T cytotoxic cells (10.1% vs. 6.6%, $P < 0.05$) in spleens of DDE-treated mice. Since DDE accumulates in adipose tissue we hypothesized that effects of DDE will be more pronounced in obese mice. We used males of the C57BL/6J diet-induced model of obesity. From the time of weaning, mice were fed either the study diet (60 kcal% fat) or control diet (10 kcal% fat). At 6 weeks of age, both diet groups were divided into DDE and vehicle groups. DDE (200 mg per kg body wt) was given by oral gavage twice, 2 days apart. The vehicle group received an equal volume of oil. Mice were maintained for 6 months. Mice fed the high fat study diet were at least 50% heavier, regardless of DDE treatment status. Trace proteinuria was observed in most mice and was independent of diet and treatment. We did not observe glucosuria in any mice. Upon sacrifice, blood serum and kidneys were obtained for MCP-1 and OPN determination and spleens and thymuses were used for immune cell population determination. Percentages of total T cells (CD3+), T helper (CD4+), T cytotoxic (CD8+), and of B cells were measured. These data should help to determine whether high fat diets and/or obesity enhance the ability of DDE to impact the immune system, with possible ramifications for humans and autoimmune disease.

Nothing to Disclose: DJH, RTM, SMV

P2-76

Neonatal Genistein Exposure Permanently Disrupts Female Reproductive Tract Gene Expression and Tissue Architecture during Pregnancy.

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The phytoestrogen genistein is found in soy products including soy-based infant formulas. Approximately 25% of US infants consume these products and have high circulating levels of genistein (1-5 uM). Previous studies demonstrate that mice exposed neonatally to genistein are completely infertile. Because preimplantation development and implantation rely on precise hormonal regulation of reproductive tract tissue architecture and patterns of gene expression, we tested the hypothesis that neonatal genistein treatment disrupts these characteristics. Oviductal histology revealed disorganization of the muscle layers and regions of excessive epithelial proliferation following genistein treatment. The uteri had increased extracellular matrix in the muscle and stromal compartments, an overall lack of glandular epithelium, and abnormal appearing luminal epithelial cells. To identify specific genes and gene pathways that were responsible for these abnormalities, microarray analysis was performed on oviductal and uterine mRNA from control and genistein-treated mice during early pregnancy. Of the >40,000 genes on the array, the major gene ontology categories altered by genistein treatment in both tissues were cancer, cell signaling, and development. In the oviduct, there were 1,126 genes significantly altered on day 2 of pregnancy; these included the transcription factors *Pitx1*, *Nkx3.1* and *Wnt7a*, genes important for development of reproductive tissues. In the uterus, there were 682 genes altered on day 2 and 1,514 genes altered on day 4 of pregnancy. These genes included the transcription factor *Foxa2*, which is important for epithelial cell polarity and migration. Few genes were altered in both tissues; however, *Six1*, a homeobox transcription factor important in muscle development, was highly upregulated in both tissues and may explain the disorganized muscle phenotype. Several genes involved in estrogen and/or progesterone signaling, such as *Aqp5* and *Spink3*, were repressed in the uterus. Taken together, these data suggest that exposure to genistein during female reproductive tract development causes infertility by permanently altering the expression of genes that control muscle development, endometrial gland formation, and epithelial morphology, and by changing critical cellular responses to hormonal signals of pregnancy. These findings may have implications for the use of soy-based infant formula and subsequent female reproductive health.

Nothing to Disclose: WNJ, SMW, EP-B, CJW

P2-77

Adult Male Rats Exposed to Dietary Soy Isoflavones in the Perinatal Period Exhibit Elevated Serum Adiponectin Concentrations with Implication for Steroid Hormone Secretion by Leydig Cells.

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Activation of the ERα during early development is thought to play a critical role in the accretion of adipose tissue. Indeed, estrogen treatment can reduce adipose tissue mass by up to 90% in rodents by activating estrogen receptor-α (ERα). On the other hand, there is evidence indicating that the serum concentrations of adiponectin, an adipokine, are increased after a reduction in adiposity and improved glucose and lipid metabolism. The use of soy-based diets by the population is ostensibly for their health beneficial effects. However, soybeans contain the isoflavones genistein and diadzein, which mimic the effects of estrogen on adipogenesis and lipogenesis. Testicular Leydig cells produce the male sex steroid hormone testosterone (T), which maintains the male phenotype. Interestingly, Leydig cells possess binding sites for both estrogen and adiponectin. The present study was designed to characterize the effects of soy-based diets on serum concentrations of adiponectin and investigate adiponectin action in Leydig cells. Time-bred Long Evans dams were fed diets containing 0, 5, 50 or 1000 ppm of soy isoflavones from gestational day 12 until day 21 postpartum. Weanling male rats were fed soy-free diets until sacrifice. As positive control, diethylstilbestrol (DES) was fed by gavage at 2 µg/kg/body weight. At 90 days, fasting blood glucose levels in rats (n = 10) exposed to the 5, 50 and 1000 ppm diets were similar (170 ± 20, 165 ± 8, and 160 ± 13 mg/ml) to control (140 ± 9) but were increased in the DES group (196 ± 17) ($P < 0.05$). However, adiponectin protein expression levels were higher in epididymal adipose tissue from animals exposed to soy isoflavones than in control rats ($P < 0.05$). Enhanced adipose tissue expression of adiponectin protein were related to elevated serum adiponectin concentrations, which were 35% and 55% greater in the 50 ppm diet and DES groups (22.4 ± 1.6 and 25.6 ± 3.2 µg/ml) than in control (16.5 ± 0.9) ($P < 0.05$). In separate experiments (n = 3), incubation of immature Leydig cells isolated from 35 day-old male rats in culture medium (DMEM/F12) containing recombinant adiponectin (0, 1, 10, and 100 µg/ml) caused a decrease in T production (control 180 ± 28 vs 150 ± 18, 140 ± 22 and 130 ± 15 ng/10^6 cells • 24 h) ($P < 0.05$). These results demonstrate that soy-based diets may affect serum adiponectin concentrations and that metabolic changes due to phytoestrogen action have implication for Leydig cell steroid hormone secretion.

Sources of Research Support: Animal Health and Disease Research and the Boshell Diabetes and Metabolic Diseases Research Programs of the College of Veterinary Medicine at Auburn University.

Nothing to Disclose: TT, DS, EPP, RJ, BTA

P2-78

Comparison of H295R Based Steroidogenesis Assay and Primary Leydig Cell Steroidogenesis Assay To Identify Endocrine Disruptors.

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The steroidogenesis assay is to detect any substance that would disrupt estrogen and/or androgen gonadal steroid hormone production. H295R cell-based steroidogenesis assay has been suggested as a possible alternative to minced testis assays proposed by US EPA. Primary Leydig cell steroidognesis assay is in vitro method for assessing the androgenic or anti-androgenic effects of substances in using the isolated and cultured Leydig cells. This study investigated characteristics between H295R cell-based steroidogenesis assay and primary Leydig cell steroidogenesis assay to identify endocrine disruptors.
The qualifying experiments of H295R cell-based steroidogenesis assay were performed by exposing with a known inducer, forskolin(1uM and 10uM) and a known inhibitor, prochloraz (0.3uM, 3uM). The results were analyzed by qualified performance parameters such as fold induction. So we found that our assay systems worked properly.
To compare characteristics of two assays, we performed experiments with known sex hormone inhibitors, ketoconazole, prochloraz and bisphenol A and a known inducer, forskolin. H295R cells were exposed to ketoconazole(10^{-9}M~10^{-4}M) for 48 hours. Ketoconazole reduced testosterone production at concentratons greater than 10^{-9}M. The reduction of testosterone production at 10^{-6}M was approximately 5-fold that of the control levels. For primary Leydig cell steroidogenesis assay, isolated and cultured Leydig cells were exposed to ketoconazole(10^{-10}M~10^{-5}M) for 48 hours. Ketoconazole reduced testosterone production at concentratons greater than 10^{-7}M. The reduction of testosterone production at 10^{-7}M was approximately 2-fold that of the control levels. Forskolin(10^{-10}M ~ 10^{-4}M) increased testosterone production in H295R cell based steroidogenesis assay. Its maximum increase for testosterone production by forskolin was approximately 3.5-fold at 10^{-4}M. In primary Leydig cell steroidogenesis assay, its maximum increase for testosterone production were approximately 2-fold at 10^{-5}M. Testosterone production by prochloraz and bisphenol A in primary Leydig cells was similar to that of H295R cells.
From the above results, we found that H295R cell based steroidogenesis assay was excellent in term of dose-responsive reactivity in comparison with primary Leydig cell steroidogenesis assay.

Nothing to Disclose: TSK, IHK, KKJ, HSN, SKH, SYH, MSD, HJY, TSK

P2-79
Effects of Cadmium on Rat Leydig Cell Steroidogenesis.

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Cadmium has potentially adverse effects on male reproduction via Leydig cells. Thirty 90-day-old Sprague Dawley rats were exposed to cadmium with doses of 0 (control), 5 and 50 mg/L/day in drinking water for one month. One month after exposure, rats were sacrificed and testes were removed to measure testicular testosterone by RIA. Testicular testosterone levels were significantly reduced in 5 and 50 mg/L groups with 69.6 ± 15.5 (5 mg/L group) and 62.8 ± 5.1 ng/mg (50 mg/L dose group) compared to control (156.2 ± 16.6 ng/mg) after one month exposure. Immature (ILCs) and adult Leydig cells (ALCs) were purified from 35- and 90-day-old rat testes, respectively. Leydig cells were treated with different concentrations of CdCl2. CdCl2 at ≥10^{-8} M significantly inhibited testosterone production in ILCs, while 10^{-5} M or above of CdCl2 was required to significantly inhibit testosterone production in ALCs. Therefore, rat ILCs were more sensitive to the inhibition by cadmium than ALCs.

Nothing to Disclose: HYZ, HL, BHZ, CMS, YHC, RSG

P2-80

Effects of Endocrine Disruptors on Steroidogenic Enzymes Gene Expression and on Aromatase Activity in Two Human Cell Lines.

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Endocrine disruptors are chemicals that can alter functions of the endocrine system by several ways. In the present study, we have evaluated the effects of several endocrine disruptors and of some of their metabolites on the last step of steroidogenesis in two cell line models. Two enzymes are implicated in this step: aromatase (CYP19A1), which plays a central role by catalyzing the irreversible conversion of androgens to estrogens, and 17β-hydroxysteroid dehydrogenase (HSD17B), which catalyses the conversion of inactive sexual hormones to active ones in different steroidogenic organs. We have screened the selected chemicals for their ability to modulate the expression of steroidogenic enzymes and aromatase activity in the human choriocarcinoma JEG-3 cell line and in the human adrenocortical H295R cell line after both short (4 h) and long exposure (24 h). All chemicals were tested at concentrations that did not cause cytotoxicity after 24h of exposure, as tested by the MTT viability assay. Gene expression profile was assessed by real-time RT-PCR and aromatase activity was assessed by the method of tritiated water.
Treatments of JEG-3 cells by atrazine, methoxychlor and the vinclozolin metabolite M2 resulted in an increase of CYP19A1 mRNA levels. In contrast, Bisphenol-A and the methoxychlor metabolite HPTE down-regulated the expression of CYP19A1 mRNA. Methoxychlor and HPTE decreased the amount of HSD17B1 mRNAs. In H295R cells, atrazine up-regulated CYP19A1 mRNA expression, HPTE increased HSD17B1 mRNA levels and methoxychlor increased HSD17B1 and HSD17B5 mRNA levels. Concerning the aromatase activity, treatment of H295R cells by atrazine induced aromatase activity. Furthermore, exposure of these cells to either bisphenol A or HPTE inhibited aromatase activity. In JEG-3 cells, which display higher basal aromatase expression than H295R cells, the pattern of aromatase activity regulation was similar but less pronounced.
Taken together, these initial results contribute to a better characterization of the action of endocrine disrupting chemicals and their metabolites on the steroidogenic pathways, which needs to be considered when assessing their effects on human health.

Nothing to Disclose: NQ, SD, RB, EL

P2-81

2,3,7,8-Tetrachlorodibenzo-Para-Dioxin Increases Aromatase (CYP19) mRNA Stability in MCF-7 Cells.

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Dioxins are industrial pollutants that can be bio-accumulative in our food chain. Humans can be exposed to this class of pollutant through contaminated food, air, drinking water, etc. Displaying both pro- and anti-estrogenic properties, these pollutants are also known as endocrine disruptors. The link between breast cancer and TCDD exposure has not been resolved, although TCDD is classified as 'known human carcinogen'. Estrogen is a documented risk factor for breast carcinogenesis. In the present study, effect of 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD) on estrogen synthesis was investigated in the breast cancer cells MCF-7. Our results showed that TCDD increased the aromatase activity in a time- and dose-dependent manner. Real-time PCR and western blot analysis verified the induced expression by about 3 fold; however, gene reporter assay revealed that the promoter activity of exons I.3 and II was not elevated. Further investigation indicated that TCDD slowed down the CYP19 mRNA degradation with concurrent activation of ERK. The ERK inhibitor U0126 could reverse the extended stability of the transcripts. In summary this study demonstrated that TCDD might induce a post-transcriptional regulatory mechanism of gene expression in breast cancer cells.

Nothing to Disclose: HH, MYC, LKL

P2-82

Hepatic Enzyme Inducers Increase Thyroxine (T_4) Catabolism in Human and Rat Hepatocytes.

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Nuclear receptor agonists such as 3-methylcholantrene (3-MC), phenobarbital (PB), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), and, pregnenolone-16α-carbonitrile (PCN) decrease serum thyroxine (T_4) concentrations in rats. It appears that this decrease occurs through the induction of hepatic metabolizing enzymes resulting in an enhanced catabolism of T_4. There is some evidence that this decrease in serum T_4 occurs in humans, but the mechanism by which this happens is unclear. Using primary rat and human hepatocytes, we compared the effects of aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), and pregnane X receptor (PXR) agonists on T_4 catabolism. 48 hours after plating, fresh sandwich-cultured hepatocytes from male human or Sprague-Dawley rats were treated with the following: CAR agonists, PB (1000uM) or PCB 153 (30uM); AhR agonist, 3-MC (5uM). 5uM rifampicin (Rif) and 10uM PCN were used as PXR agonists for human and rat hepatocytes, respectively. Control and treated cells were exposed to a final concentration of 0.1% DMSO. After 72 hours, media were removed and replaced with 0.05uM (rat median serum concentration) or 0.1 uM [I^{125}]-T_4 (human median serum concentration). After 24 hours, media were collected for analysis. T_4 and its metabolites were separated on an UPLC from which fractions were collected and quantified on a gamma counter. The predominant metabolite found in the media of control and treated hepatocytes was T_4-glucuronide (T_4G). Initial studies show a 660-fold higher activity in T_4G formation in unexposed rat hepatocytes as compared to unexposed human hepatocytes (0.002- vs. 0.000003 pmol T_4G/min/mg protein). In rat hepatocytes, there was no significant increase in T_4G formation with PB or PCN treatment compared to control; 3-MC and PCB 153 treatment increases T_4G formation by 4 to 5-fold. In contrast, initial studies with human hepatocytes show significant increases in T_4G formation following PB, Rif, 3-MC, and PCB 153 treatment (100-, 17-, 10-, and 10-fold, respectively). These results highlight the utility of hepatocytes to evaluate the potential species differences in the effects of thyroid hormone disruptors. (This abstract of a proposed presentation, does not necessarily reflect USEPA or NIH policy.)

Nothing to Disclose: VMR, MJD

P2-83

Gold Nanoparticles Alter Progesterone and Estradiol-17 Beta Accumulation *In Vitro* in a Rat Ovary Culture Model.

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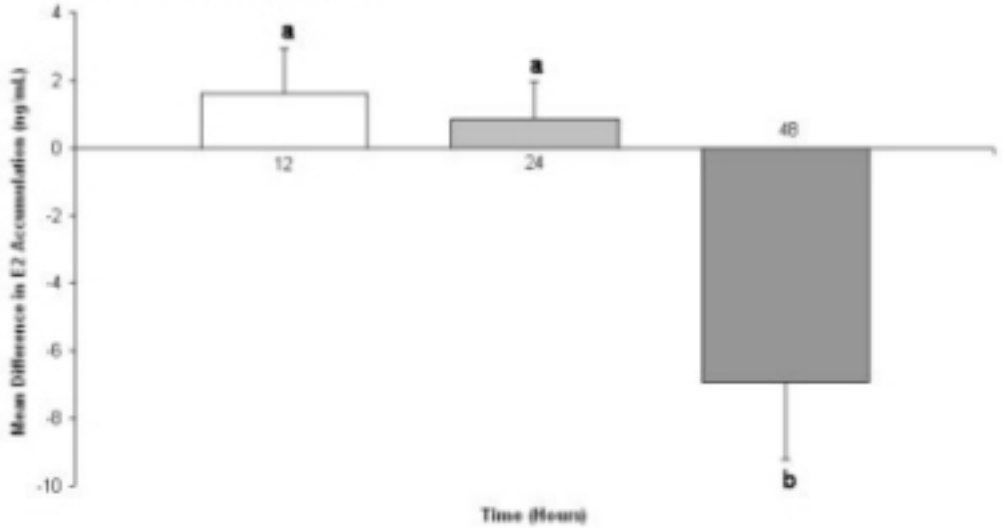
Engineered nanoparticles have been investigated for potential application of their unique physicochemical properties due to their inherent size. Recently, nanogold has gained considerable attention for medicinal drug delivery, bio-imaging, and diagnostic purposes. However, research exploring the potential untoward effects of nanoparticles on female reproductive function is almost nonexistent. We have demonstrated previously that 10nm nanogold ($2.85x10^{10}$ particles/mL) is able to enter rat ovarian granulosa cells, alter mitochondrial morphology, and modulate estrogen accumulation *in vitro* (1). The objective of the present study was to evaluate potential time- and dose-dependent effects of gold nanoparticles (10nm) on ovarian progesterone (P4) and estradiol-17 beta (E2) accumulation via a cultured whole-ovary approach and radioimmunoassay. We hypothesized that gold nanoparticles would perturb ovarian P4 and E2 accumulation in culture and ultimately diminish sex steroid production long term. Ovaries from each rat remained paired; thus, one ovary was cultured under control conditions and the other under treatment conditions ($2.85x10^{4}$ particles/mL, $2.85x10^{7}$ particles/mL, and $2.85x10^{10}$ particles/mL medium) for three different time periods (12, 24, and 48 hours). One-way ANOVA and *post-hoc* Tukey's test results revealed a highly significant decrease in P4 accumulation in nanogold-treated versus control cultures at 48 hours *(n=9; p<0.001; not shown)* compared to that at 12 and 24 hours of incubation. Similarly, a significant decrease in E2 accumulation *(Fig. 1; mean difference between control & nanogold-treated ovaries +/- 1 SEM)* at 48 hours *(n=9; p<0.05)* compared to that at 12 and 24 hours was apparent.

Figure 1. Estradiol-17 beta



These results suggest that gold nanoparticles exhibit a time-dependent inhibition of ovarian steroid hormone secretion. We are currently investigating, at a molecular level, the mechanism of action of this inhibition. Collectively, our studies point to the ovary as a target of nanoparticle action, and from a public health standpoint will aid in determining the etiology of possible female reproductive pathologies upon nanogold exposure.

(1) Stelzer R and Hutz RJ, J Reprod Dev 2009; 55:685-90

Sources of Research Support: University of Wisconsin-Milwaukee College of Letters and Sciences; UWM Department of Biological Science's Ruth Walker Grant-in-aid; UWM NIEHS Children's Environmental Health Sciences Core Center Pilot Proposal Program (Grant:PRJ32IR).

Nothing to Disclose: JKL, MJC, RK, RJH

P2-84

Use of Non-Invasive MRI/MRS Techniques To Assess Recovery in Muscle Tissue.

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We attempted to develop a noninvasive MRI/MRS technique that can track metabolite markers for muscle disuse and atrophy. The right sciatic nerve of C57bl/6 mice was injured, followed by recovery for 21 days. Nerve crush was chosen instead of denervation of the sciatic nerve to provide us the opportunity to measure for recovery. The recovery was assessed by measuring wet weight for the gastrocnemius, plantaris, and soleus muscle, which revealed significant recovery in the third week post-injury. Localized Magnetic Resonance Spectroscopy (MRS) measured the time water molecules took to change from a uniform orientation in alignment with the magnetic field to a random orientation. The time from order to random orientation was influenced by the nature of the microenvironment that revealed tissue quality on the lateral gastrocnemius. Several metabolites were quantitated post-nerve crush and statistical differences were obtained for creatine and taurine. As early as day 2, the total water intensity showed significant increases that persisted to day 21. Glycogen levels were monitored using Chemical Exchange Saturation Transfer (CEST), and it too showed statistically significance differences for the early muscle wasting at day 2 that continued till day 21. Time course analyses suggested a correlation between these analytes and disease progression leading to recovery. To check the validity of the MRS glycogen measurement, we biochemically determined the glycogen concentrations for tissue lysates as a function of time. We further confirmed the activity of genes related to the glycogen synthesis pathway (GYS1, GYS2, GSK3b, Pygm) in the nerve crush and control tissues for days 1, 3, 8, and 15 of recovery. The gene activity for GYS1, GYS2, GSK3b, and Pygm confirmed the irreconcilable differences between the biochemical measurements and the results of the MRS technique. We also analyzed gene expression related to the mobilization of taurine to validate the MRS. The change in mRNA levels of Slc6a6, taurine transmembrane transporter activity, indicated the MRS measured changes in taurine levels is consistent with these gene changes. Therefore that data suggest that ^{1}H-MRS could be used as a means for detecting taurine and may pose limitations for other metabolic markers in determining the recovery of muscle tissue in longitudinal, clinical studies after atrophic insult.

Sources of Research Support: Musculoskeletal Research, Lilly Research Laboratories, Indianapolis, IN, 46285, USA; Department of Biology, Depauw University, Greencastle, IN, 46135, USA.

Disclosures: VK: Chief Scientific Officer, Lilly USA, LLC.
Nothing to Disclose: AYS, BCY, AK

P2-85

Prognostic Factors in Prolactin Pituitary Tumors: Clinical, Histological and Molecular Data from a Series of 94 Patients with a Long Postoperative Follow-Up.

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Context and Objective: Predicting pituitary tumor behavior remains a challenge. This multiparameter investigation aimed to identify markers for recurrence and progression in prolactin tumors.
Design: From a cohort of patients treated for prolactin tumors by surgery, we retrospectively studied clinical data, tumor characteristics, clinical outcome and the expression of 9 genes by q-RTPCR.
Results: This study included 94 patients (62 females and 32 men), with long post-operative follow-up periods (mean: 138 ± 46 months), 54.3% of patients had a macro or giant adenoma. Tumors were classified into 3 pathological groups based on their radiological and histological characteristics (non-invasive: 61, invasive: 22, aggressive-invasive: 11). Immediately after surgery, 60 patients (63.8%) went into remission (PRL level normalization). Persistently elevated prolactin levels (36.2%) were associated with increasing age, male sex, high preoperative prolactin levels, large tumor size on univariate analysis ($p=1x10^{-6}$ to $7x10^{-10}$) and invasion and pathological classification on univariate ($p= 5x10^{-20}$ and $3x10^{-18}$) and multivariate ($p=8x10^{-10}$ and $3x10^{-8}$) analysis. During follow-up, 19 patients (20%) had tumors that recurred or progressed under dopamine agonist treatment. Invasion and pathological classification were associated with recurrence or progression on univariate analysis. Seven genes (*ADAMTS6*, *CRMP1*, *PTTG*, *ASK*, *CCNB1, AURKB, CENPE)* were associated with tumor recurrence or progression and 5 of these (*ADAMTS6*, *CRMP1*, *ASK*, *CCNB1, CENPE)* were associated with the pathological classification. Malignancy was suspected in 11 aggressive-invasive tumors, with or without metastasis.
Conclusion: This study indentifies both the clinical and histological factors that relate to prolactin tumor recurrence or progression. Molecular markers then enhance the diagnostic and prognostic value of these factors. Altogether, our results could influence the management of patients with pituitary tumors.

Sources of Research Support: Grants from the Ministere de la Sante (Programme Hospitalier de Recherche Clinique National n°27-43), research contracts from the Institut National de la Sante et de la Recherche Medicale, Ligue Contre le Cancer Rhone-Alpes, and the NEURODIS foundation (www.fondation.neurodis.oro).

Nothing to Disclose: GR, AW, ED, CA, TB, DF-B, EJ, MJ, JL, JT

P2-86

The Pathogenic Role of AIP Mutations – The Importance of the Integration of Clinical, Experimental and Modeling Data on Advising Patients and Their Families with Pituitary Adenoma Predisposition.

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Introduction: Mutations in the Aryl hydrocarbon receptor-Interacting Protein (AIP) gene predispose patients to pituitary adenomas. While the majority of the identified mutations result in a truncated protein, which is likely to lead to a loss of biological activity, we need to understand the possible pathogenic role of promoter, silent and missense mutations in the AIP gene in order to appropriately advise patients and their families about predictive genetic testing and regular screening. Therefore, in the current study we have systematically examined all the AIP variants identified to date in the literature.
Methods: An in vitro luciferase assay was used to study promoter activity and regulation, while patient RNA and minigene constructs were utilized to study splicing abnormalities. A quantitative β-galactosidase assay was performed on all missense mutations to study AIP-phosphodiesterase-4A5 (PDE4A5) protein-interactions. A model of AIP is provided based on the crystal structure of the closest related protein FKBP52. The novel MutPred program was used to predict the effect of missense mutations.
Results: Of the known 48 AIP mutations, 34 mutations result in a truncated protein or no protein (12 nonsense mutations, 11 frameshifts, 6 splice-site, 4 large deletions and 1 promoter mutation). An in-frame insertion mutation on exon 6 disrupted the anti-proliferative effect of AIP, while no functional data are available for the 4 in-frame deletions; 10 missense changes were identified.
The luciferase assay showed reduced basal promoter activity of the mutant promoter in GH3 cells. The silent mutation c.249G>T created a new splice-site leading to alternative splicing and the generation of a frameshift and premature stop-codon. The c.807C>T silent mutation led to decreased AIP expression.
The PDE4A5 binding assay correlated with the clinical data. It showed complete loss of binding for the C238Y and R271W mutations, reduced binding for the K103R, K241E and R304Q mutations, but normal binding for R16H, V49M, I257V and A299V variants. In silico analysis correlated well with the clinical and experimental data.
Conclusions: In summary, with the help of a combined clinical experimental and modeling approach, this study is the first to confidently predict the pathogenic role of missense AIP variants. This is important for counseling affected patients and their families, and to indicate or avoid complex investigations.

Disclosures: ABG: Speaker, Novartis Pharmaceuticals, Ipsen; Advisory Group Member, Novartis Pharmaceuticals, Ipsen.
Nothing to Disclose: GT, GBB, SI, HC, MK

P2-87

Developing Biomarkers of Pituitary Tumor Progression and Recurrence.

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Pituitary tumors are the most common brain tumor occurring in 10-20% of autopsied subjects. Clinically, patients present with hormone imbalances, headaches and visual disturbances progressing to blindness. Surgery remains the treatment of choice but tumors are often locally invasive and recur. The genetic and molecular mechanisms underlying the pathogenesis of these tumors are unknown and few markers predict aggressiveness or recurrence. Previously, we used microarray expression analysis comparing human pituitary gonadotrope tumors and normal pituitaries to identify novel factors that are differentially expressed. We demonstrated the roles of BRINP3, Eps8 and Reprimo on proliferation, invasion, and/or survival to impact on tumor development and/or progression. To examine the genetic events that promote human pituitary tumor expansion and invasion, microarrays were performed on 7 aggressive (AGG) gonadotrope tumors (defined as >2cm with cavernous sinus invasion and/or recurrent tumors) compared to non-aggressive (NON) gonadotrope tumors. A one-way ANOVA test was applied to the three experimental groups as well as used to compute p-values for the pair-wise comparisons for these classes. A false discover rate (FDR) of 5% (Benjamini – Hochberg method) was then calculated to control for multiple testing. Based upon this analysis, a molecular discovery set of 98 transcripts representing 64 genes were differentially expressed between AGG and NON at an FDR<0.05. Preliminary studies of quantitative RT-PCR on several putative candidates and housekeeping genes shown not to change across the experimental groups demonstrated that FAM84B and ID3 showed differential expression based on tumor phenotype similar to the arrays, supporting the strategy for the screening/selection process. Thus this genetic strategy has identified a subset of candidate biomarkers to be tested prospectively to predict aggressiveness and recurrence of human pituitary tumors.

Sources of Research Support: VA Merit Review to MEW and UCD Cancer Center and Genomics Core.

Nothing to Disclose: AK, MX, ME, MG, BK-D, KL, MEW

P2-88

Surgical Approaches in 84 Patients with Insulinomas in Multiple Endocrine Neoplasia Type 1 (MEN 1).

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Introduction: Management of insulinomas in the setting of Multiple Endocrine Neoplasia type 1 (MEN1) remains controversial.
Goal: 1) to evaluate long-term results of various surgical approaches in the control of hypoglycemia in insulinoma-MEN 1 patients 2) to characterize other parameters involved in the therapeutic decision – making process (WHO status, post-operative morbidity, functioning and non functioning duodenopancreatic recurrences).
Methodology and patients: All 84 consecutive patients with insulinoma and MEN1 from 30 french centers of the GTE were retrospectively studied. They had clinical and biochemical diagnosis of insulinoma and MEN 1 syndrome according to the standardized definitions. Results are classified as a function of surgery: total pancreatectomy (TP), cephalic duodenopancreatectomy (CDP), splenopancreatectomy (SPC), or enucleation alone (E).
Definitive cure of insulinoma was defined as absence of recurrence of symptomatic hypoglycaemia during the follow-up. Recurrences were classified in 3 subgroups: functioning syndrome (hypoglycemia or non hypoglycaemia) and pancreatic tumor. Permanent postoperative adverse effects were recorded.
Results: 84 patients (29 males and 55 females) aged 33 ± 16 years were enrolled. Among patients who beneficed from surgery, respectively TP, CDP, SPC, E alone in 3, 9, 50, and 16 cases, malignant insulinomas were diagnosed in 0, 3 (33%), 3 (6%), 0 of patients. Other duodeno-pancreatic functioning NET were diagnosed in 1 (33%), 3 (33%), 10 (20%), 2 (12%) patients. Median follow-up in each surgical subgroup was respectively 3, 11, 11 and 13 years.
Definitive cure of hypoglycaemia was observed in 62/78 patients including 3 (100%), 4 (44%), 45 (90%) and 10 (62%) of patients in the same therapeutic groups. Recurrent non hypoglycaemia-functioning syndromes were observed respectively in 0 (0%), 1 (11%), 16 (32%), 4 (25%) of patients. Recurrent pancreatic tumor was respectively observed in 0 (0%), 4 (44%), 19 (38%) and 8 (50%). Permanent post-operative adverse effects were respectively observed in 3 (100%), 3 (33%), 20 (40%), 0 (0%) of patients. One post-operative death was observed after TP.
Conclusions: SPC offers the best chance of definitive cure of hypoglycaemia syndrome in MEN1 patients. Due to a lower morbidity, enucleation alone appears as an acceptable compromise. Recurrent non hypoglycaemia-functioning syndromes and/or pancreatic tumors were observed in 25 to 50% of cases after SPC or E alone.

Nothing to Disclose: DV, CC-B, AM, MB-g, PN, NL-B, LG, PB, AT, PC, PL, IG, EM, AB, FP , FB-C, PC, PG, EB

P2-89

Molecular Characterization of a Large *MEN1* Gene Deletion from a Saudi Family with Multiple Endocrine Neoplasia Type 1 (MEN1) and Phenotype-Genotype Correlation.

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The common features of multiple endocrine neoplasia (MEN1) include primary hyperparathyroidism secondary to parathyroid hyperplasia in most patients, pituitary adenomas in up to 30-40% of patients, and pancreatic tumors in 35-70% of cases. Other features may include carcinoid tumors and silent adrenal adenomas. A phenotype-genotype correlation has not been established in MEN 1. We encountered a large Saudi family (two parents and10 siblings) with MEN1 in whom, malignant pancreatic tumors were present in the father and 3 of his children who had clinical disease, and were the presenting features in 2 of them. In the father with malignant large gastrinoma, insulin was also co-secreted and resulted in severe hypoglycemia. An initially nonfunctional malignant pancreatic neuroendocrine tumor removed from another subject, recurred as liver metastasis with high gastrin and serotonin levels. Adrenal adenomas were also seen in all 4 subjects with clinically proven disease, and in one subject the adenoma produced cortisol, resulting in florid Cushing's syndrome and diabetic ketoacidosis (DKA). All subjects had evidence of prolactinomas, and in one subject aggressive GH secreting adenoma developed later.
Conventional mutation screening of patients' peripheral blood DNA did not reveal any mutation of *MEN1* gene, but copy number analysis by MLPA and array CGH demonstrated a monoallelic deletion of 5 kb genomic DNA involving *MEN1* gene promoter, exon 1, and 2. This germline deletion was also found in four other siblings who did not have full clinical disease but had biochemical abnormalities. The expression of menin, the *MEN1* gene product, was not detected in pancreatic tumors by immunohistochemistry. Loss of heterozygosity (LOH) analysis using microsatellite markers indicated a large somatic deletion of at least 280 kb in the tumor DNA spanning the *MEN1* gene locus. These data are consistent with the role of *MEN1* as a tumor suppressor gene. The aggressive tumor behavior is likely due to the complete loss of *MEN1* gene function. The large somatic deletion of *MEN1* locus could affect the function of other genes in the region and may contribute to the aggressive phenotype of the disease. Gene copy number analysis should be used to detect large deletions in suspected patients in whom *MEN1* mutation was not detected by conventional mutation screening.

Nothing to Disclose: HR, BM, MZ, EB, RAA-R, NK, MA-H, DM, MHA-G, HA-H, YS

P2-90

Exon 1 and 2 Menin Gene Deletion in a Large Italian Family.

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Familial pituitary adenoma is frequently associated with germinal mutations of several genes, including menin gene. MEN1 syndrome is an autosomic dominant disease, characterized by parathyroid adenomas, endocrine gastroenteropancreatic tumors, and pituitary adenomas, due to inactivating mutations of the *MenI* gene (11q13). MEN1 mutations are scattered within and around the menin open reading frame and are mainly represented by single nucleotide polymorphisms (SNPs), and small deletions/insertions, which can be detected by genomic DNA direct sequencing. However, heterozygous wide deletions in the menin gene cannot be detected by direct sequencing and other techniques have to be employed to characterize the genetic base of some syndromic families. We employed MLPA and a Real-time PCR application, the TaqMan Copy Number Assay, to evaluate a family in which we failed to identify MEN1 SNPs or deletions/insertions by direct sequencing, despite a clear clinical picture of MEN1 syndrome. By MLPA and directly evaluating the number of genomic copies by quantitative PCR, we identified a wide deletion of the MEN1 gene involving exon 1 and of exon 2 in 3 affected family members, but not in the other 7 family members, that are, so far, clinically silent. We also evaluated the presence of the same genetic alteration in a group of 10 unaffected subject, without family history of endocrine tumors, and none of them displayed exon 1 and 2 deletion. Therefore, this new approach allowed us to correctly diagnose 3 MEN1 patients that were, so far, considered as MEN1 phenocopies. More importantly, we were able to exclude the presence of any MEN1 genetic alteration in the unaffected family members. These results further underline the importance of the new biotechnology approaches in the diagnosis of genetically determined endocrine diseases.

Nothing to Disclose: MCZ, MT, CF, MB, FT, MRA, AB, ECDU

P2-91
Analysis of p27Kip1 Gene in Men1 Tumors.

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Context: The majority of the patients with multiple endocrine neoplasia type 1 (MEN1) harbors an inactivating mutation in the MEN1 gene and are predisposed to a large spectrum of tumors, mainly in the pituitary, parathyroids and endocrine pancreas. Loss of heterozygosity (LOH) at 11q13 is observed in the MEN1 tumors, leading to a complete inactivation of the MEN1 tumor suppressor gene. Recently, rare mutations in another tumor gene, p27Kip1, have been identified in patients with MEN1-like phenotype but carrying no MEN1 mutation.
Objective: In this study we aimed to analyze the somatic status of the p27Kip1 gene in MEN1 tumors.
Tumors: Ten tumors (including parathyroids, pancreas and carcinoids) were investigated in the current study.
Methods: The two exons of the p27Kip1 gene were analyzed by PCR amplification and automatic sequencing.
Results: We analyzed so far the somatic status of ten tumors from MEN1-positive patients. All tumors presented 11q13-LOH, indicating inactivation of the MEN1 tumor suppressor gene. No somatic p27Kip1 mutation was indentified in the tumors. The genotyping of p27Kip1-V109G polymorphism in the leukocyte and tumor DNA reveled somatic loss of p27Kip1 in one parathyroid tumor.
Conclusions: Rare mutations of the p27Kip1 gene have been recently reported in MEN1-negative patients. Our study showed that somatic loss of p27Kip1 may occur in tumors from MEN1-mutated patients. We are currently expanding the p27Kip1 analysis to a larger number of MEN1 tumors.

Nothing to Disclose: MBM, RAT, VCL, DML, SPT

P2-92

Process to the Diagnosis of Multiple Endocrine Neoplasia Type 1 in Japanese Patients.

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Background: MEN1, an autosomal dominantly transmitted hereditary endocrine tumor syndrome, develops a variety of lesions and can often progress without symptoms. Therefore most of patients are not diagnosed early and correctly.
Purpose: The purpose is to find factors for earlier diagnosis of MEN1 in Japan.
Method: We have established a study group of MEN named "MEN Consortium of Japan". Anonymized clinical data of patients are registered to the database. As of January 2010, 25 major institutions have joined this Consortium and clinical data of over 400 patients with MEN1 have been registered. In the present study, using this database, interval between appearance of the initial symptom(s) and the final diagnosis of endocrine disorders has been analyzed.
Results: Forty three percent of patients were asymptomatic at the initial diagnosis. They were diagnosed of having MEN1-related endocrine disorders through health screening, screening of family members of the affected patients, or hypercalcemia was found by chance when they visited hospitals for another purposes. Frequent symptoms appeared before diagnosis included urinary calculi (20.9%), peptic ulcer (18.3%), hypoglycemia (10.3%) and amenorrhea (7.6%). Among patients with urinary calculi, 36% were diagnosed as having hyperparathyroidism at the time of initial attack. However, another 36% of patients took more than 10 years from the appearance of initial symptom of urinary calculi to the diagnosis of hyperparathyroidism. Similarly, diagnosis of hyperparathyroidism was made in 37.5% of patients with peptic ulcer at the same time when they was diagnosed as having peptic ulcer, but 32% of patients took more than 10 years to reach the diagnosis of hyperparathyroidism. Amenorrhea was the initial symptom in 19 patients. Among those, 5 patients (26%) had not been diagnosed of having pituitary tumor (prolactinoma or nonfunctioning tumor) for more than 10 years.
Considerations and conclusion: There was a long interval between the appearance of the symptom(s) and the diagnosis of MEN1-related endocrine tumors. Common symptoms seen in MEN1 may be diagnosed and treated by urologist, gastroenterologist, and gynecologist. There might be insufficient acknowledgement of MEN1 and related endocrine disorders among those medical practitioners.

Nothing to Disclose: MY, SK, SU, SS, TO, TI, HK, MY, SH, AS, AS

P2-93

Multiple Endocrine Neoplasia Type 1 (MEN-1), the Hadassah-Hebrew University Medical Center Experience.

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Introduction:
MEN-1 is an autosomal dominant genetic disorder with a prevalence of 2-4 per 100,000. The main manifestations are parathyroid (PT), gastro-enteropancreatic (GEP) and pituitary tumors, but may affect other organ systems as well. MEN-1 is associated with significant morbidity and mortality with up to 50% dying before the age of 50. Treating MEN-1 affected subjects presents a unique diagnostic and therapeutic challenge.
Aims:
To present our experience with MEN-1 affected patients, including clinical and genetic information.
Methods:
Clinical data was obtained for patients followed at Hadassah Medical Center between the years 2003-2009. Genetic analysis was carried out in the laboratory of Prof. A Calender, France, and the NIH, USA, and included direct sequencing and quantitative multiplex short fragment PCR of exons 2-10 of the menin gene. Clinical diagnosis of MEN-1 was defined as the presence of at least 2 out of the 3 main manifestations of MEN-1 (PT, GEP, pituitary).
Results:
Our cohort included 25 subjects from 20 families. Clinical information was available on 22. Average age at presentation was 36 years (range 17-75). Initial presentation was hyperparathyroidism in 12 (55%), GEP tumor in 6 (27%) and pituitary tumor in 4 (18%). During evaluation and follow up, 19 (86%) developed hyperparathyroidism, 15 (68%) GEP tumors (7 non-secreting , 6 gastrinomas, 2 insulinomas) and 12 (55%) developed pituitary tumors (9 prolactin, 2 ACTH and 1 null). Two patients had metastatic carcinoid tumor, and 1 thymic carcinoid. Genetic testing was performed in 18 subjects. MEN-1 mutations were found in 12 (67%), and a genetic variant of unknown significance in one. Eleven (50%) patients underwent abdominal surgery for resection of tumor, 7 are treated with somatostatin analogs and 2 patients underwent peptide receptor radionuclide therapy (PRRT). Eight patients underwent parathyroid surgery, 6 treated with cabergoline for prolactinoma and 3 underwent pituitary surgery. Twenty two subjects are alive (age 45.4±10 years), whereas 3 died of metastatic GEP tumors at the ages 41, 45 and 56.
Conclusions:
MEN-1 is a complex genetic disorder. Hyperparathyroidism is the most common and earliest manifestation. GEP tumors cause most of the morbidity and mortality associated with MEN-1. Menin mutations or variants were found in 72% of patients with clinically defined MEN-1.

Nothing to Disclose: SK, BG, DB, MF, DJG

P2-94

Succinate Dehydrogenase (SDH) Mutations in Patients with “Wild-Type” (Non-KIT, Non-PDGFRA-Mutated) Gastrointestinal Sarcomas.

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Pediatric gastrointestinal stromal tumors (GIST) are rare mesenchymal neoplasms of the gastrointestinal tract arising from the interstitial cells of Cajal. The majority of GIST tumors are associated with activating mutations of the KIT or PDGFRA proto-oncogenes. However, 85% of pediatric GIST patients do not have these mutations, therefore termed wild-type (1). Carney-Stratakis syndrome (CSS), first described in 2002, is characterized by the presence of paragangliomas and GIST that are inherited in an autosomal dominant manner with incomplete penetrance (2). Germline mutations of genes encoding subunits B, C, and D (SDHx) of the mitochondrial enzyme succinate dehydrogenase (SDH) are present in the majority of patients with CSS (3). SDH is a component of the mitochondrial electron transport chain and mutations in this gene cause increased susceptibility to cancer, suggesting its role as a tumor suppressor. The frequency of patients who present with a GIST and SDHx mutations in the absence of a coexisting paraganglioma, or family history suggestive of paraganglioma, is still under investigation. In this study, we investigated 37 patients with wild-type GIST for mutations in SDHB, -C, and D; 5 out of 37 patients with wild-type GIST were found to have mutations of the SDHB (4) and SDHC (1) genes. A frequent, possibly functioning polymorphism of SDHD gene (G12S) was also found in two patients. Data are pending in 11 additional patients. As part of this investigation, we also screened wild-type GISTs for SDHB expression and mitochondrial Complex II activity; deficient SDHB expression and absent mitochondrial Complex II activity was seen frequently among wild-type GISTs, even in the absence of SDHB mutations. We conclude that SDHx mutations are present frequently in pediatric and young adult patients with wild-type GIST; deficient regulation of SDHx gene expression and function is also present, regardless of the presence of mutations. We recommend that genetic testing for an underlying mutation in SDHx be performed in all pediatric patients with wild-type GIST; adult patients with wild-type GIST may at least be screened clinically for the presence of paragangliomas and/or pheochromocytomas.

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Nothing to Disclose: MBL, SYK, KJ, ERB, MR, TH, FF, AH, KP, JAC, JG, PR, AG-R, LH, CAS

P2-95

Chronic Complications of Peptide Receptor Radionuclide Therapy (PRRT) – A Single Center Experience.

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Peptide receptor radionuclide therapy (PPRT), is an established treatment for gastro-entero-pancreatic neuroendocrine tumors (GEPNET). PRRT has been associated with several complications including bone marrow suppression and renal toxicity.
We retrospectively analyzed our data of 90 patients with different GEPNETs who received PRRT. CBC and renal function tests were performed prior to, and every 2 weeks following each treatment cycle. Only patients with complications that persisted for over 8 weeks were included. Three women who developed premature ovarian failure (POF) were assessed for pituitary gonadal axis function. Adverse event grading was based on CTCAE version 3 NIH/NCI grading system.
Of 90 patients, 13 were excluded due to lack of follow-up. 29 (37%) developed late hematologic complications: mean age 58 years, 17 men and 12 women, 19 (66%) PNET and 10 (34%) carcinoids, 55% received Y^{90} DOTATOC, 28% received Lu^{177} DOTANOC/TATE and 17% received both isotopes, mean cumulative dose 531±265 mCi. 13 (44%) developed anemia (G1-2), 21 (72%) developed leucopenia (G1-3), and 18 (62%) developed thrombocytopenia (G1-4). During follow-up (range 1-60 months), of patients with anemia 30% improved and 70% remained unchanged, 67% of leucopenia improved and 33% remained unchanged, and 44% of thrombocytopenia improved while 44% remained unchanged and only 7% (n=1) worsened. 12 (41%) had monocytopenia, 13 (45%) bicytopenia and 4 (14%) pancytopenia. There were a total of 8 complications secondary to hematologic toxicity: bleeding - 2, infections- 2, blood transfusions-4. Three women (mean age 41) who had prior regular menses developed POF. A total of 6 patients (~8%) developed renal impairment: mean age 66±8 yrs, 2 (33%) had carcinoid and (66%) PNET. 5 had prior renal compromise or risk factors. 3(50%) received Y^{90}DOTATOC 1(17%) received Lu^{177} DOTANOC/TATE and 2 (33%) received both, with a mean cumulative dose of 747±547 mCi. Only one patient had worsening of renal function 8-12 weeks from the last treatment but during follow up ranging between 2-24 months (median 16.5 month) 5 of 6 patients had further deterioration of their renal function. Only one patient required dialysis.
In patients with GEPNETs PRRT is a safe treatment. The major side effects in our cohort were bone marrow suppression that stabalized or improved in the long-term. Young women may be at risk for POF. Deterioration in renal function was uncommon and usually appeared late after treatment.

Nothing to Disclose: MF, DB, JM-B, YK, BG, AS, DJG

P2-96

Bronchopulmonary Carcinoids: Long-Term Outcome in a Clinicopathologic and MIB-1 Labeling Study of a Single-Institution Series of Italian Patients.

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Background*:* The histological distinction between typical and atypical bronchopulmonary carcinoids is based on mitotic activity and presence of necrosis, but at the individual patient level, none of these features gives a reliable prediction of the clinicopathologic outcome.
Aim of the study: To evaluate the expression of the proliferating antigen MIB-1 as a potentially better indicator to classify carcinoids into two clearly distinct histological and clinical groups.
Patients and Methods*:* The long-term postsurgical outcome of a single-institution series of 86 radically treated bronchopulmonary carcinoids was correlated with the tumor characteristics assessed by combining conventional histology with a panel of immunohistochemical markers exploring cell differentiation (chromogranin, NSE) and cell turnover (MIB1). Tumor features were all obtained by serial sections of paraffin-embedded samples stained with H&E and with immunohistochemistry for Mib1 and independently assessed by two pathologists.
Results*:* 59 (68.6%) carcinoids were assessed as typical (TC) and 27 (31,4%) as atypical (AC). The mean follow-up was of 8.3 years (range: 0-18; median: 8). All cases expressed neuroendocrine markers. At univariate analysis, tumor recurrence (n=24):12/59 TC (20.3%), 12/27 AC (44.4%) correlated significantly with the carcinoid histotype (P = 0.035), tumor dimension (P=0.011), mitotic index (P = 0.017), Mib1 expression (P = 0.020), and synchronous node metastasis (P = 0.050). All these potential prognostic variables were tested with Cox multivariate analysis that only confirmed Mib1 expression (P=0.009) as independent predictor of disease recurrence. The best cutoff for Mib1 expression (calculated by receiver operating characteristic curves) discriminating recurrent versus non recurrent tumors was 4% (sensitivity 79.2%; specificity 84.7%; area under curve 0.86). By stratifying the patients according to this cut-off, significant differences emerged in the patients' disease-free survival (log-rank test P< 0.0001).
Conclusions: Mib1 expression was found to accurately separate carcinoid tumors into two well-distinct histo-prognostic categories with important clinical implications (identify high recurrence risk patients for whom adjuvant chemotherapy could be proposed). MIB1 score seems to be a better predictor of survival and recurrence than the mitotic count combined with the evaluation of the presence of necrosis (as used in the last WHO classification).

Nothing to Disclose: DM, CAB, PM, AM, SP, GT, FG

P2-97

Incorporation of Biochemical, Anatomic and Perfusion Data to Improve the Localization of Occult, Sporadic Insulinoma by Selective Intra-Arterial Calcium Stimulation with Hepatic Venous Sampling.

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Background: Sporadic insulinoma is the commonest cause of endogenous hyperinsulinemic hypoglycemia in adults. However invasive procedures for pre-operative localization are required in approximately 25% of patients seen at our institution. The Selective Intra-arterial Calcium Stimulation Test (SACST) is a technique that provides functional as well as anatomic information to better regionalize these occult tumors. However, the literature has ignored the impact of variant arterial anatomy and regional perfusion on the interpretation of SACST.
Objective: To incorporate pancreatic arterial anatomy, biochemical data and regional perfusion information from SACST to improve the localization of occult, sporadic insulinoma.
Methods: We undertook a retrospective review of 38 patients with surgically-confirmed occult, sporadic insulinoma (out of a total of 190 patients seen) who underwent SACST in the period from January 1996 to December 2009. The results obtained from SACST were reviewed in a step-wise fashion by an interventional radiologist blinded to the surgical / pathology data. Initially, biochemical data was used to predict location. Subsequently, arterial anatomy and then regional perfusion data were also utilized. Once the location of insulinoma was predicted (left or right of the superior mesenteric vein or more specific anatomic localization), the prediction at each step was compared to actual localization.
Results: 38 patients underwent 41 SACST. The test was repeated in 3 patients due to initial uninterpretable results. Of the 38 interpretable tests, the biochemical results were positive in one arterial distribution in 73.7% and in 2 distributions in 26.3%. The median maximal rise in insulin concentration over baseline was 15-fold (range: 2.1 to 142-fold). Celiac and superior mesenteric arterial anatomy was aberrant in 40.6% and 37.5% respectively. Significant variation and overlap in regional perfusion was observed. Incorporating anatomic and perfusion data with biochemical data improved sensitivity over biochemical data alone from 76.3% to 86.8%. Arteriography alone demonstrated a sensitivity of 55.3% but in combination with anatomic, biochemical and regional perfusion data, this increased to 94.7%.
Conclusions: Careful review of the pancreatic arterial anatomy and regional perfusion is critical for correct interpretation of the biochemical results of SACST, particularly in the presence of anatomic variants or overlap in regional perfusion.

Nothing to Disclose: SMT, JCA, GBT, CSG, RVL, FJS, AV

P2-98

BLyS Role in the Diagnosis and Prognosis of Neuroendocrine Tumors: Preliminary Results.

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Background.
BLyS is a member of a tumor necrosis factor superfamily shown to be important for B-cell development, maturation and survival. Recent data also indicate that it may regulate the survival and maintenance of malignant cells in hematological and non-hematological cancer patients.
Aim of the study.
To analyze the possible pathogenetic, diagnostic and prognostic role of BLyS in neuroendocrine tumors (NET).
Patients and Methods.
Forty-five consecutive unselected patients (23 F; mean age 59.5±13 yrs) with a diagnosis of NET: 13 pancreatic (8 functional); 14 pulmonary carcinoids (10 atypical); and 18 gastro-enteric (4 gastric, 10 duodenum, 4 colon) were enrolled in the study. BLyS serum levels were analyzed by ELISA (sensitivity: 3.38 pg/ml) and Chromogranin A (CgA) by routine radioimmunometric methods (CGA-RIACT; range 19.4-98 ng/ml). At the time of analysis 26 patients were in therapy with somatostatin analogs and 18 patients presented metastases. We then analyzed one or more follow-up sera in 14 cases. Patients were compared to 77 age and sex-matched blood donors (BDs). Statistical analyses were performed using non parametric paired and non-paired t-test for data comparisons and Spearman rank test for correlations (significant p value if <0.05).
Results.
All NET patients presented more elevated BLyS levels than BDs (1285.8±610.2 pg/ml vs 660±240 pg/ml.; $p<0.0001$). Patients with stable or remission condition (n. 20) presented significant lower BLyS levels than the 25 patients with disease progression (977.4±295 pg/ml versus 1532.5±686.4 pg/ml; $p=0.0009$). No significant correlation was found between BLyS levels and CgA serum levels or Ki67 values in the histological specimens. No differences were found in BLyS serum levels with respect to organ origins and presence or absence of metastases. Of note, BLyS levels during the follow-up (mean 4.8±2.3 months) of 14 NET patients demonstrated a significant increase in the 6 progression patients (from 1345.5±549.5 pg/ml to 2057.5±990.1 pg/ml; $p=0.031$), while the values remained constant in the 8 stable/remission cases (from 1263.4±921.3 pg/ml to 1132.6±561.8 pg/ml; p=ns).
Conclusions.
Elevated BLyS levels characterized patients with NET and BLyS appear as a new potential marker in the diagnostic and prognostic process of such tumors. Data are preliminary, but interesting and very new; a confirmation study on larger series is in course.

Nothing to Disclose: MF, CP, NB, EP, CF, FC, DV, ET, SDV, GDM, SP, FG

P2-99

Somatostatin Analog-Specific Regulation of sstr2A Receptor Phosphorylation and Trafficking.

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The somatostatin 2A receptor (sstr2A) mediates many of the neuroendocrine and neuromodulatory actions of somatostatin (SS) and is the target for the SS analogs currently used to treat neuroendocrine tumors. Until recently, all clinically utilized SS analogs were thought to act as full agonists at sstr2A. However, we found that SOM230 (Pasireotide) and KE108 both exhibit biased agonism when signaling through this receptor (1). Thus, these compounds behave as agonists to mimic some of the actions of SS and as antagonists to block others (1). Further, both SOM230 and KE108 are less effective at inducing receptor internalization than SS (2), suggesting that they exhibit functional selectivity for receptor regulation as well as signaling. Because the therapeutic effectiveness of SS analogs depends on maintaining the level and the functionality of their target receptors, we examined the molecular mechanisms involved in analog-specific regulation of sstr2A by determining the effects of SS and SOM230 on receptor trafficking, site-specific receptor phosphorylation and receptor-arrestin association. Stimulation of sstr2A-expressing cells with a maximal concentration of SS or SOM230 led to rapid receptor endocytosis in both cases. However receptor recycling occurred more quickly following SOM230 than after SS treatment. Consistent with these kinetics, the extent of receptor internalization at steady state was substantially greater with SS than with SOM230. Using our newly developed phospho-site specific antibodies (3, Ghosh et al unpublished) we showed that SOM230 was markedly less effective than SS at stimulating sstr2A phosphorylation at multiple G-protein-coupled receptor kinase sites. Although both SS and SOM230 caused rapid association of the receptor with arrestin, the receptor-arrestin complex was less stable with SOM230 than with SS14, consistent with the reduced level of receptor phosphorylation. These studies clearly demonstrate that regulation of sstr2A by SOM230 differs markedly from that by the native hormone and indicate that the reduced effectiveness of SOM230 to induce receptor phosphorylation and internalization may help explain the clinical effectiveness of this analog. Thus, identification of new biased compounds defective in sstr2A regulation provides a promising strategy for the development of more effective therapeutic agents for sstr2A expressing neuroendocrine tumors.

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Nothing to Disclose: YJK, MG, AS

P2-100

Anti-Tumor Activity of the Tumor-Vascular-Disrupting Agent ASA404 (Vadimezan) in Endocrine Tumor Models.

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Vascular disrupting agents (VDAs) differ from angiogenesis inhibitors by attacking established tumor blood vessels rather than preventing growth of new ones. We investigated effects of the Tumor-VDA ASA404 against neuroendocrine tumors of the gastroenteropancreatic system (GEP-NETs) and adrenocortical carcinoma (ACC) 24 hours after treatment of BON and NCIh295 tumor bearing mice with ASA404 (A), paclitaxel (P) or the combined administration (A+P). A significant decrease in cell proliferation (Ki 67 index, %) was detectable for BON tumors after all treatments (A: 51.6±0.7, P: 55.2±0.8 and A+P: 47.9±0.6; each $p<0.0001$ vs. controls 60.2±0.8), for NCIh295 tumors only after P treatment (A: 46.6±1.5, $p=0.87$; P: 43.4±1.4, $p<0.05$ and A+P: 45.8±0.9, $p=0.4$; vs. controls 46.88±1). BON tumors treated with A or A+P exhibited extensive necrotic areas not apparent in the control or P groups. TUNEL and CD31 staining showed that A or A+P treatments resulted furthermore in a significant increase of apoptotic cells (%) in BON tumor (A: 28.8±2.7, A+P: 34.3±3.6; each $p<0.0001$ vs. controls 9.2±1.3 and P: 10.1±1.5) and a significant loss of microvessels compared with controls and P treatment (A: 7.3±0.8, $p=0.0003$ and A+P: 5.42±0.4, $p<0.0001$ vs. controls 11.5±0.64 and vs. P: 12.6±1.1). Interestingly, no significant effects on tumor morphology, apoptosis or microvessel density were detectable in NCIh295 tumors. To evaluate the mechanisms which cause these differences we initiated further *in vitro* analyses. As TNFα -signaling is assumed to mediate parts of ASA 404 induced effects we investigated induction of apoptosis and detected a 4-fold higher rate of apoptosis in BON cells after TNFα treatment compared with NCIh295 cells (basal: 100%±2; BON: 823.1%±34.5 vs. NCIh295: 244.2%±12.4; $p=0.007$). Thus, while ASA404 (vadimezan) treatment holds promise in the treatment of GEP-NETs, the utilized tumor models might help to delineate molecular mechanisms involved in VDA induced anti-tumor activity.

Nothing to Disclose: CH, RF, AO, TM, FB

P2-101

Glucagon-Like Peptide-1 Compared with Somatostatin Receptor2 Targeting in Malignant Insulinoma.

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Introduction/Background
In vitro data have demonstrated that benign human insulinoma cells exhibit a high density of glucagon-like peptide-1 (GLP-1) receptors. GLP-1 receptor targeting was, therefore, performed in vivo using [Lys40(Ahx-DOPA-^{111}In)NH2]exendin-4 and found to be a promising tool to localize small benign insulinomas in humans.
In contrast, malignant insulinomas are often characterized by a high expression of somatostatin subtype 2 (sst_2) receptors that result in positive Ocetroscan®. It is currently unknown whether GLP-receptor targeting is useful in malignant insulinomas.
Aim:
To prospectively evaluate GLP-1- and sst_2 receptor targeting in patients with endogenous hyperinsulinemic hypoglycemia suspicious for having a malignant insulinoma.
Methods:
Ten patients (6 males, 4 females, age 63 ± 9 years) were included after positive fasting test and positive dual-phase CT imaging. All patients underwent imaging with ^{111}In-DTPA-exendin-4 (GLP-1 analogue) plus sst_2 imaging (6 patients) and/or histological assessment of their tissue samples including GLP-1 and sst_2 receptor quantification using in vitro autoradiography (7 patients).
Results:.
All ten patients had a malignant insulinoma confirmed by histology. GLP-1 receptor scintigraphy was positive in 4/10 patients, whereas sst_2 receptor targeting (Octreoscan® and/or in vitro autoradiography) was positive in 7/10 patients. At least one of the two receptors was expressed in the tumor tissue of each patient.
Conclusion:
GLP-1 receptor imaging can detect a subgroup of malignant insulinomas (~ 40%) while the remaining GLP-1 receptor negative cases can all be identified by Octreoscan®. Thus, one of the two imaging methods is always positive in a malignant type of insulinoma.

Nothing to Disclose: EC, DW, MC, TK, HM, PJE, MB, AP, JCR

P2-102

Secretion of Insulin, C-Peptide, Proinsulin, Chromogranin A, Pancreatic Polypeptide, and Neuron Specific Enolase in Response to Local Arterial Calcium Administration in Patients with Insulinoma.

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Objective: To test whether insulin, C-peptide, proinsulin, chromogranin A (CgA), neuron specific enolase (NSE), and pancreatic polypeptide (PP) are released from insulinoma cells by calcium stimulation during the selective arterial calcium stimulation and hepatic venous sampling test (ASVS).
Design: Prospective case series.
Methods: We determined insulin, C-peptide, proinsulin, CgA, NSE and PP in blood samples obtained during the ASVS test in 19 patients with insulinoma and 6 individuals without insulinoma.
Results: After calcium injection into the artery supplying the insulinoma, a significant 8-fold increase in insulin (range 2.3-117; $p<0.001$), a 3.8-fold increase in C-peptide (range 1.7-32.4; $p<0.001$), a 1.9-fold increase in proinsulin (0.7-5.3, $p<0.001$) and a 1.5-fold increase in PP (range 0.8-4.5; $p=0.017$) was detectable. PP also increased 2.4-fold after injection into the control artery of insulinoma patients (range 0.8-7.9; $p=0.016$) and 2-fold in healthy subjects (0.6-4.8; $p=0.014$). No significant increase of insulin, C-peptide, proinsulin, CgA and NSE concentrations was found after calcium injection into the control artery and in healthy subjects.
Conclusions: Whereas insulin, C-peptide, and proinsulin may be stimulated by calcium injection into an artery supplying the insulinoma, CgA and NSE are not secreted in response to calcium by insulinoma cells. PP may be released by both healthy islet and insulinoma cells after local arterial calcium stimulation.

Nothing to Disclose: PW, RK, TP, GAS, CS

P2-103

The Treatment of Neuroendocrine Tumours (NETs) with Long-Acting Somatostatin Analogues: Preliminary Data in a Single Centre Experience with Lanreotide Autogel.

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The treatment of neuroendocrine tumours (NETs) is often carried out with somatostatin analogues (SSA), and their long-lasting release formulations allows a single administration by month.
The aim of this retrospective study was to evaluate the efficacy, safety and tolerability of lanreotide autogel given to NET patients observed in our Institute between 2005 and 2008.
Patients with metastatic NETs and disease progression were given lanreotide autogel 120 mg/month by deep subcutaneous injection. Before entering into the study, any anticancer treatment was withdrawn and a disease restaging was performed. The efficacy was evaluated by the relief of disease symptoms, behaviour of tumour markers and response rate. Safety and tolerability were evaluated by assessing the onset of adverse events and treatment feasibility.Twenty-three patients (13 males), median age 62 years (range 32 -87) were considered for the study. NET diagnosis was: 11 carcinoids, 7 non-functioning tumours, 2 gastrinomas and 3 tumours with unknown primitive site. The primary site was bowel (30.4%), pancreas (34.8%) and lung (21.7%). Fourteen patients (60.9%) showed flushing and diarrhoea which improved by 85.7% and 55.6% respectively, bronchoconstrinction and abdominal pain also ameliorated. Eleven patients (47.8%) with carcinoids had abnormal CgA and 5-HIAA serum levels which normalized after 12 months of treatment. There were 2 PR (8.7%) and 15 SD (65.3%); six patients (26.0%) progressed, and 4 patients died, not for cancer reasons. No patient complained from any local adverse reaction; tolerability was satisfactory.
The results of this study confirm that lanreotide autogel 120 mg/month is effective and well tolerated. The long-acting formulations are extremely useful in clinical practice for the management of long-term anticancer treatments.

Nothing to Disclose: AB, AF, DM, FL, LT, MM, APL, RMP, CAR, SDC, SMC, AP, LDM

P2-104

Presentation and Outcome of Pancreatico-Duodenal Tumours in Multiple Endocrine Neoplasia Type 1 (MEN1) Syndrome.

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Introduction: multiple endocrine neoplasia type 1 (MEN 1) is a rare autosomal dominant condition characterized by the development of parathyroid, pancreaticoduodenal endocrine and pituitaty tumors. Pancreatic-duodenal endocrine tumors (PDETs) are a frequent manifestation of MEN1 with a prevalence ranging from 30 to 75% and represent a major cause of death in one-third of patients. There is still a debate on their management, mainly on the optimal surgical strategy, due to their multicentricity and high recurrence rate.
Objective: to assess presentation, timing of diagnosis and outcome of (PDETs) in a monoinstitutional series of MEN1 patients.
Methods: prospective collected data of a cohort MEN1 patients observed at the internal medicine and surgical units of the University of Verona (Italy) were retrospectively analyzed.
Results: Thirty-one patients had PDETs, 16 (52%) of them led to MEN1 diagnosis. Among this latter group 15 out of 16 (94%) had already primary hyperparathyroidism (PHPT), asymptomatic in 60% of cases. Twenty patients (64.5%) underwent surgery, 19 of them with curative and in 1 with debulking intent, with no mortality. Among the 19 patients resected with radical intent, 13 (68%) patients are disease-free [7 out 8 non-functioning (NF)-PDETs, 4 out 4 insulinomas and 2 out 7 ZES]. Eight patients with NF-PDETs $\leq$20 mm, treated with conservative approach, showed stable disease at follow-up.
Conclusions: PDETs can frequently be the manifestation that leads to MEN1 diagnosis, since PHPT, although already present, is often not recognized or considered as sporadic. Therefore the coexistence of PDETs should be looked for in all cases of patients with PHPT, even if asymptomatic, mainly in young age. One the other hand if biochemical screening for MEN1 was performed in all patients with PDETs, an increased number of sporadic PDETs should be diagnosed as MEN1. Surgery may be curative in the majority of NF-PETs and insulinomas, but not in ZES. A conservative approach can be safely reserved to patients with NF-PDETs $\leq$ 20 mm.

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Nothing to Disclose: MVD, LB, MT, AS, GF, MF

P2-105

Effects of Testosterone Undecanoate Administered with Dutasteride or Placebo in Female to Male (FtM) Transsexual Subjects.

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The relative role of T and dihydrotestosterone (DHT) on different target organs is still largely unknown. The aim of our study was to investigate the effects of T and its metabolite DHT on metabolic parameters, body composition and muscle strength in FtM transsexuals. These subjects represent an interesting model to study the effects of testosterone and its metabolites on different physiological functions.
Twelve long-term ovariectomized FtM subjects received TU 1000 mg i.m. at week 0, 6, 18, 30, 42 with placebo (n=6; group A) or dutasteride 5 mg/day (n=6; group B), orally, in a randomized, double-blind, placebo-controlled trial. At week 0 and 54 the following measurements were performed in all subjects: isokinetic knee extension and flexion peak torque (PT-IKE and PT-IKF) and handgrip strength, body composition by DEXA, anthropometric, biochemical, hematological and hormonal measurements.

	Group A		Group B	
	Baseline	week 54	Baseline	week 54
TT (ng/ml)	1.2 +/- 1.1	3.8 +/- 2.5*	1.7 +/- 1.7	6.7 +/-
HDL-c (mg/dl)	75.2 +/- 22.0	61.7 +/- 19.3*	62.1 +/- 25.1	48.7 +/
Hematocrit (%)	40.7 +/- 2.1	45.0 +/- 2.9*	41.3 +/- 1.9	43.6 +/
HOMA-IR	1.84 +/- 0.64	1.24 +/- 0.81	0.99 +/- 0.45	1.26 +/
BMD hip (g/cm2)	1.0 +/- 0.1	1.0 +/- 0.1	1.0 +/- 0.1	1.0 +/-
Body weight (kg)	65.1 +/- 13.2	65.4 +/- 15.6	63.8 +/- 9.9	66.1 +/
Waist circ. (cm)	89.4 +/- 12.4	84.8 +/- 15.2	87.7 +/- 8.7	83.5 +/
Fat mass (kg)	17.1 +/- 12.6	14.7 +/- 12.2	15.9 +/- 7.7	15.7 +/
Lean mass (kg)	43.9 +/- 5.5	45.5 +/- 4.4	46.4 +/- 3.4	48.2 +/
Grip strength (kg)	32.5 +/- 4.7	35.0 +/- 4.7*	37.5 +/- 3.6	37.5 +/
Leg strength (Nm) PT-IKF	52.7 +/- 8.9	57.1 +/- 10.3	57.8 +/- 6.8	64.3 +/
Leg strength (Nm) PT-IKE	114.7 +/- 23.4	114.2 +/- 33.8	122.9 +/- 29.0	125.6 +

* = P < 0.05 vs baseline

These results demonstrate that T treatment in FtM subjects improves physical strength, tends to increase lean mass and to decrease waist circumference with no effects on insulin resistance. HDL-c levels significantly decrease while hematocrit increases. The addition of dutasteride does not affect T effects on these end-points. These results are particularly important in view of the development of selective androgen receptor modulators.

Disclosures: FS: Employee, Bayer Schering Pharma.
Nothing to Disclose: FA, FDM, VM, CM, CP, AMP, GP, MCM

P2-106

Administration of Testosterone to Elderly Hypogonadal Men with Crohn's Disease Improves Their Crohn's Disease Activity Index: A Pilot Study.

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Objective: Both elevated and depressed testosterone (T) levels have been reported in Crohn's disease (CD). In this study, we assessed effects of T administration on CD.
Design: 13 men with CD, aged 45-67 years, had subnormal plasma T (mean ±SD = 9.0 ± 1.4 nmol/L) (N>14.0); they were compared to a group of 110 men of similar age with sexual and urological problems whose plasma T was also subnormal: 10.4± 1.4 nmol/L. All received treatment with parenteral testosterone undecanoate for 24 months. The Crohn's Disease Activity Index (CDAI) was assessed as an indicator of the severity of the disease every 3 months.
Methods: Levels of T and C-reactive protein (CRP) were compared between the 13 men with CD and the other men in this study. Values of CDAI and CRP were followed up.
Results: C-reactive protein (CRP) levels were 22.7 mg/dL (95% confidence interval of the mean [CI]: 14.9-34.3) in 13 men with CD versus 3.5 (2.9-4.1) in 107 control men (*P*=0.001). T tended also to be lower in CD men *vs.* controls (2.6 ± 0.4 ng/mL; 95% CI: 2.3-2.9; vs. 2.9 ± 0.4; 95% CI: 2.8-3.0; *P*= 0.02). Upon normalization of serum T, CDAI and CRP level declined significantly. Hemoglobin / hematocrit increased significantly.
Conclusions: Upon normalization of plasma T the CDAI and CRP levels declined in hypogonadal patients with CD. The mechanism of this improvement may be through immunosuppressive effects of testosterone, reducing chronic inflammation of the intestinal wall in CD.

Disclosures: LJGG: Speaker, Bayer Schering Pharma. FS: Employee, Bayer Schering Pharma.
Nothing to Disclose: AH, WK, EJG

P2-107

Different Testosterone (T) Therapies in Female to Male (FtM) Transsexuals: Effect on Body Weight and Composition, and Metabolism.

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Introduction: Hyperandrogenism in women is clustered with features of the metabolic syndrome. T therapy of FtM transsexuals before sex-reversal surgery is an hyperandrogenic condition in which androgen levels reach men physiological concentrations. **Materials and methods**: We analyzed the effects of 1 year of treatment with Testoviron Depot (T.D. i.m.: 100 mg/7-15 days), testosterone-gel (T-gel: 50 mg/die), and testosterone undecanoate (T.U. i.m.: 1000 mg at 0, 6 wks and then every 12 wks) on body weight and composition and on the main metabolic parameters in 33 FtM transsexuals prior to surgery.

	T.D. (n:14)		T-gel (n:12)		T.U. (n:7)	
	Basal	After	Basal	After	Basal	After
BMI (kg/m2)	22,4±3,02	23,5±3,05a	24,4±5,25	24,9±4,66	24,1±5,16	23,5±2,51
Fat mass (kg)	14,2±2,73 (4)	13.0±2,56(4)	21,1±9,94	20,4±7,83	18,7±11,4	15,1±5,18
Lean mass (kg)	38,5±2,71(4)	43,0±3,02(4)b	45,4±6,06	48,6±5,79c	45,0±6,69	49,7±4,81c
Fast. glucose (mg/dl)	87,4±7,97	81,0±7,15a	84,5±11,5	79,2±11,9	83,1±5,27	81,4±9,45
Fast. insulin (µU/mL)	6,61±1,82	4,50±1,57	5,22±1,84	7,02±2,13	8,41±4,47	8,10±2,78
HOMA-IR	1,39±0,33	0,86±0,35	1,03±0,34	1,40±0,65	1,81±1,07	1,61±0,57
Tot. Cholesterol (mg/dl)	174±25	179±33	168±34	173±42	161±27	168±34
HDL-c. (mg/dl)	68,6±15	56,3±8,96b	67,1±13,4	58,3±15,8	58,6±12,2	53,7±9,39
LDL-c. (mg/dl)	92,7±28,6	110±28,5a	90,3±36,0	100±43	87,7±31,2	103±30,6a
Triglycerides (mg/dl)	60,4±23,5	67,6±27,4	60,4±21,5	89,0±35,6a	73,9±26,9	54,7±14,1
Testosterone (ng/ml)	0,62±0,35	7,30±3,69c	0,42±0,13	5,74±2,94 c	0,60±0,15	5,94±0,93 c

a p<0,05, b p<0,01, c p<0,001

Results: BMI increased only in the T.D. (p=0.02). A gain in total lean mass was found in all groups (T.D. p=0.002; T-gel p=0.0001; T.U. p=0.007). Glucose levels did not change except for the T.D (p=0.03). No effect on insulin sensitivity index was reported. No change in total cholesterol level was shown, while plasma HDL-c levels declined in T.D (p=0.003) and LDL-c concentrations increased in the T.D. (p=0.02) and T.U. (p=0.04). Triglycerides levels increased in the T-gel (p=0.04). **Conclusion:** All T therapies in FtM transsexuals induce changes in body composition with an increase in total lean mass. All T formulations are responsible of mild changes in lipid profile but none has effect on insulin sensitivity after 1 year of administration.

Nothing to Disclose: CP, AC, SC, GP, MCM

P2-108

The Analysis of Testosterone by LC-MS/MS (A Comparison with Immunoassay).

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LC-MS/MS is rapidly getting adopted in the analysis of steroids in biological matrices. Current steroid level detection uses immunoassays which encounter problems with poor antibody specificity in combination with abundant cross-reacting.
Steroid analysis is critical for research into a number of common endocrine disorders and also applied as biomarker for numbers of diseases. Major challenges of steroids analysis include sensitivity, selectivity, and large sample volume. Using liquid chromatography mass spectrometer to analyze steroids at low level has been becoming a new trend to overcome these challenges.
Testosterone, the major androgenic hormone in humans, is commonly measured for its excess or deficiency. The concentration of testosterone in women, children and men undergoing anti-androgen therapy are typically less than 50 ng/dL (500 pg/mL).
A simple one step liquid-liquid extraction method was developed using 200 uL serum with 10 pg/mL detection limit and less than 10% CV. The method has been verified for robustness in different laboratories. Good correlation was observed between the results obtained from this method and those from twenty samples analyzed by immunoassay.
A robust and sensitive procedure for the quantitation of testosterone by LC-MS/MS will allow for economical fast throughput analysis of human serum samples to aid in early and accurate clinical research. This approach provides a viable alternative to immunoassay.

Nothing to Disclose: JM, H-FL, BF, JS

P2-109

Validation of Assays for Isolating and Measuring Androgens in Stool.

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Polycystic ovary syndrome (PCOS) is a common medical problem characterized by excessive androgens. Much of the existing research has focused on androgen levels based on systemic measurements. To date, there is limited information on stool androgen content in this condition. In the current study, we validate a technique for extracting and evaluating fecal androgens in humans.
Methods: Female subjects with PCOS (NIH criteria) and healthy controls were recruited from a tertiary care medical center. Subjects were excluded if they recently received oral antibiotics. Eligible subjects were asked to provide a stool sample which was immediately kept at -70ºC. The stool was thawed and 0.5g of stool was used to extract sterols using a methanol extraction procedure. After extraction, free testosterone was measured in 2 ways. The first was via Radioimmunoassay kit (RIA) (DSL 4900) and the second was measured by liquid chromatograph mass spectrometry (LC-MSMS, Quest diagnostics). The RIA for free testosterone in stool was then compared to LC-MSMS via regression analysis to validate that RIA was measuring true testosterone and that the tests were equally directional.
Results: After exclusion criteria, 43 subjects provided stool for analysis (n=23 controls, n=20 PCOS). The average age for the group of 43 was 32.9±7.0 years and BMI was 29.5±6.1. The groups were not compared since the subjects were part of a larger study (still underpowered) for examining stool testosterone in PCOS and controls. However, to validate the ability to quantify free testosterone in stool, RIA and LC-MSMS were examined. The average testosterone for RIA was 45.4±32.6 pg/mL. LC-MSMS confirmed that there was true testosterone in the extract at an average level of 190.9±137.2 ng/dL. Comparing the two techniques, there was a good linear correlation between the two tests in the total group ($R^2=0.33$, $P<0.001$) suggesting both tests were directional. In addition, there was a significant correlation within the control group ($R^2=0.46$, $P<0.001$) and the PCOS groups ($R^2=0.25$, $P=0.02$) individually as well.
Conclusion: In this study we found presence of testosterone in steroids isolated from stool in both PCOS and healthy controls by RIA and LC-MSMS. The measurement by RIA and LC-MSMS appear to be significantly directional in regression analysis. The RIA technique needs further exploration as to reason for reduced levels compared to LC-MSMS.

Disclosures: NC: Employee, Quest Diagnostics.
Nothing to Disclose: RM, SW, WS, MP, WM, VP, RR, KC, CC, RA, MP

P2-110

Case Report: Clinical and Radiologic Features of Hypopituitarism Due to Adenohypophysitis in a Case with Transient Hepatitis of Possible Viral Etiology.

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Introduction: Hypopituitarism secondary to hypophysitis is rare. Etiologic diagnosis is often presumptive based on clinical and radiologic findings. Lymphocytic hypophysitis is generally due to autoimmunity occurring in association with other autoimmune disorders. Lymphocytic hypophysitis and subacute thyroiditis have been described in a patient with viral aseptic meningitis(1). Lymphocytic hypophysitis has been produced in animal models after injection of viral antigen(3,4). In case of suspected viral etiology, it is unclear if the inflammation is directly due to viral infiltration or an autoimmune process. We report a case of hypopituitarism due to hypophysitis, following a viral illness associated with transient hepatitis.
Case Report: A 48 year old male presented with 8 weeks history of worsening mild occipital headaches, hot and cold sweats, fatigue, joint stiffness and generalized malaise. Initial laboratory tests showed leucopenia, mild thrombocytopenia and elevated liver enzymes. Viral serology was negative for hepatitis A, B and C, lyme disease, Parvovirus, HIV and Cyptomegalovirus, with evidence of a remote infection with Epstein Barr virus. Free T4 was 0.34 [0.89-1.80] ng/ml with free T3 of 2.4 [2.3-4.2] pg/ml and thyroid stimulating hormone of 0.17 [0.35-5.50] uIU/l with a negative thyroperoxidase antibody. Serum cortisol was <0.5 [4.3-22.4]ug/dl with an ACTH level of 25 [<46] pg/ml. The rest of the anterior pituitary hormone panel was normal. Magnetic resonance imaging of the sella showed homogenous enhancement of the anterior pituitary, a very slight thickening and enhancement of the stalk with preservation of the posterior bright spot. A diagnosis of central hypocortisolism and central hypothyroidism secondary to adenohypophysitis due to a viral illness associated with hepatitis was made and patient's symptoms improved significantly with steroid and thyroxine replacement.
Conclusion: To our knowledge, this is the second report of adenohypophysitis and hypopituitarism following a presumed viral infection with elevated liver enzymes and neutropenia. Diagnosis was based on clinical, laboratory and radiologic findings. It is unclear if hypophysitis is due to the direct viral infiltration of the pituitary or triggered autoimmunity(2). Case reports of hypophysistis following injection of rubella and Adenovirus antigen in animal models support a role for viral etiology for autoimmune endocrine organ damage.

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2.Suspected lymphocytic hypophysitis in a man. Elham Reda. The new Zeland Medical Journal, October 2007, Vol 120 No 1264
3. Adenovirus-mediated gene transfer in the ovine pituitary gland is associated with hypophysitis. J R E Davis, R F T McMahon, P R Lowenstein, M G Castro, G A Lincoln, A S McNeilly. J of Endocrinology 2002, 173, 265 271
4.Induction of an organ-specific autoimmune disease, lymphocytic hypophysitis, in hamsters by recombinant rubella virus glycoprotein and prevention of disease by neonatal thymectomy. Yoon JW, Choi DS, Liang HC, Baek HS, Ko IY, Jun HS, Gillam S. J Virol. 1992 Feb;66(2):1210-4.

Nothing to Disclose: LA, MH

P2-111

Pediatric Endocrinologists' Practices in Screening for Celiac Disease among Type 1 Diabetic Patients.

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Background: Celiac disease (CD) and type 1 diabetes (T1D) share common genetic origins. Undiagnosed CD leads to short term morbidities e.g., poor growth, weight loss, and difficult glycemic control in T1D. Long term morbidities include anemia, osteoporosis and small bowel malignancy. Therefore,appropriate screening guidelines for CD are needed in patients with T1D.
Methods: Five hundred and thirty two (532) consecutive charts were reviewed from patients with Type 1 diabetes treated in Pediatric Endocrinology at SCH from 2007-2009. We collected data related to initial and repeat screenings for CD, its frequency, expressed symptoms, and biopsy results. Descriptive statistics were tabulated and analyzed.
Results: Of the 493 patients (93% of the cohort) who underwent celiac screening within ~3 months of T1D diagnosis, 25 (5%) were serologically positive on initial testing. Of those serologically positive patients, 15 underwent intestinal biopsy for celiac disease. Eleven patients were biopsy positive and 4 negative. Most of the biopsy-proven patients (73%) were said to be asymptomatic. The only signs observed in the symptomatic group were increased frequency of reported hypoglycemic episodes and weight loss. The majority of biopsy-confirmed CD patients were identified within the first 3 years following the diagnosis of T1D. However, some CD patients were detected 5-10 years after the diagnosis of T1D.
Conclusion: Based on these data from a single center, it appears that patients with T1D should be screened soon after diagnosis and regularly thereafter, regardless of overt gastroenterologic symptoms of CD. This will allow early dietary intervention and may reduce morbidities in this high-risk population.

Nothing to Disclose: LG, DEC, PF, GRF, PMK, PWS

P2-112

Association between Plasma Selenium Concentration and Total IGF-1 in Older Adults.

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Background. Insulin-like growth factor-1 (IGF-1) stimulates cell proliferation and inhibits cell apoptosis. Recent studies underline its important role as anabolic hormone and nutritional marker especially in older individuals. The regulation of IGF-1 is affected by several nutritional factors including selenium intake. However, whether circulating IGF-1 levels are influenced by plasma selenium, one of the most important human antioxidants, is still unknown.
Methods. To test this hypothesis we selected from the InCHIANTI study, a population-based cohort in Italy, 951 men and women ≥65 years with complete data on selenium and total IGF-1. Plasma selenium was measured by graphite furnace atomic absorption spectrometry using a Perkin Elmer Analyst 600 with Zeeman background correction. Within and between-run coefficients of variation (CV), were 3.1% and 7.1%. IGF-1 were measured by immunoradiometric assay, using commercial reagents (DSL, Webster). Inter- intra assay CVs for 3 concentrations were all less than 10%. Average daily intake of energy and alcohol were estimated using the European Prospective Investigation into Cancer and Nutrition food frequency questionnaire. Plasma selenium was analyzed as a continuous variable. Factors statistically correlated with total IGF-1 were identified using age-adjusted partial correlation coefficient and Spearman partial rank-order correlation coefficients, as appropriate. Parsimonious models obtained by backward selection from initial fully adjusted models were used to identify independent factors of total IGF-1. General linear models were used to test the relationship between selenium and total IGF-1 after adjusting for age and sex (Model 1) and further adjustment for Energy and Alcohol intake, ALT, and Congestive Heart Failure (Model 2).
Results. Overall, mean (SD) of plasma selenium and total IGF-1 were 0.95 (0.15) µmol/L and 113.4 (31.2) ng/mL, respectively. After adjustment for age and sex , selenium levels were positively associated with total IGF-1 (ß ± SE: 43.76±11.2, p=0.0001).After further adjustment for total energy intake, alcohol intake, ALT, congestive heart failure, plasma selenium remained significantly associated with total IGF-1 (β ± SE: 36.7 ± 12.2, p=0.003). **Conclusions.** Selenium and IGF-1 are positively associated, independently of confounders in older adults living in the community, suggesting a profound interaction between hormonal and nutritional pathways in this population.

Sources of Research Support: National Institute on Aging Contracts N01-AG-916413, N01-AG-821336, N01-AG-5-0002, and NIA Grant R01 AG027012. This research was supported in part by the Intramural Research Program, National Institute on Aging, NIH.

Nothing to Disclose: MM, FL, SB, JMG, GP, RDS, AN, LB, GC, FA, MB, LF, GC

P2-113

Alterations in the Chemokine Th1/Th2 Balance and Not the Mode of Dosing Hydrocortisone May Explain the Increased Fatigue in Addison's Disease.

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CONTEXT: The adrenalitis found in autoimmune primary adrenal insufficiency (PAI) is considered having a Th1-driven pathogenesis. The actions of cortisol on the immune system are probably more Th2-deviating than simply suppressive. Circulating Th1- and Th2-associated chemokines responsible for the trafficking of leukocytes to inflammatory sites can be used as markers for the Th1/Th2 balance. However, if Th1- and Th2-associated chemokines influences the subjective health or are modulated by the mode of daily cortisol replacement therapy in PAI is unknown.
OBJECTIVE: We investigated if the same daily hydrocortisone dose of 30 mg given in either 2 or 4 doses to patients with PAI could affect the Th1/Th2 balance of circulating chemokines and quality of life.
DESIGN: Fifteen patients (6 women and 9 men; mean age 44.6 ±15.7 yr) with PAI of autoimmune origin were included in this randomized, placebo controlled, double blind cross-over study (EuduraCT number 2005-001768-30). The patients started with either 20 mg hydrocortisone at 0700 h and 10 mg at 1600 h or 10 mg at 0700 h, 10 mg at 1200 h, 5 mg at 1600 h and 5 mg at 2200 h for 4 weeks, and then switched to the other treatment for another 4 weeks. Diurnal samples for chemokines, Th1-associated CXCL10 (IP-10), CXCL11 (I-TAC) and Th2-associated CCL17 (TARC), CCL22 (MDC) were drawn at the end of each treatment period and analyzed with Luminex. Questionnaires were used to evaluate quality of life. Seven control subjects did the same blood sampling once.
RESULTS: Subjects with PAI had higher levels of the Th1-associated chemokines than controls, CXCL10 (median 39.91 pg/ml vs. 20.18, $p<0.001$) and CXCL11 (median 34.00 pg/ml vs. 9.00, $p<0.001$), whereas no significant difference was found regarding the Th2-related chemokines. Similar chemokine levels were found when the same hydrocortisone dose of 30 mg was divided in 2 or 4 doses.
High Th1-related chemokine levels were correlated to a reduction in self-evaluated health according to the SF-36 questionnaire and increased fatigue according to the Fatigue Impact Scale (FIS).
CONCLUSIONS: High Th1-associated chemokine levels were associated with a reduced vitality and increased fatigue in PAI, but the Th1/Th2 balance was not affected by changing from 2 to 4 daily doses of cortisol. Thus, the decreased vitality and increased fatigue found in PAI may be caused by other mechanisms than inadequate cortisol replacement therapy.

Nothing to Disclose: JW, MB-L, TL, MCJ, BE

P2-114

Gene Therapy for Type 1 Diabetes: A Combinatorial Approach Using Neurogenin-3-Interleukin-10 Hepatic Gene Transfer Reverses Diabetes in Overtly Diabetic NOD Mice.

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A definitive therapy for stable reversal of autoimmune type 1 diabetes (T1D) must tackle all aspects of its pathogenesis, including autoimmunity and near complete β-cell deficiency. Thus, it is necessary to combine β-cell mass restoration with protection of the restored cells from autoimmune attack. We have previously shown that hepatic gene transfer of the islet transcription factor Neurogenin-3 (Ngn3) and the islet growth factor Betacellulin (Btc) induces islet neogenesis in the liver and reverses streptozotocin-induced diabetes in mice. This treatment failed to reverse diabetes in overtly diabetic NOD (non-obese diabetic) mice due to the autoimmune destruction of the newly formed hepatic β-cells. We investigated whether the addition of Interleukin-10 (IL-10) to the regimen would suppress the cell-mediated autoimmunity and protect the neo-β cells from immune destruction. IL-10 had been shown previously to prevent diabetes development in NOD mice, but was found to be ineffective in reversing diabetes once it has developed. Here, we present data on the effect of helper-dependent adenovirus-mediated hepatic delivery of a combined Ngn3/Btc and IL-10 regimen in overtly diabetic NOD mice. Our data show a long-term (>20 month follow-up) and complete reversal of diabetes in 50% of treated mice, consisting of a return to euglycemia, with the restoration of normal glucose tolerance as documented by a normal glucose and insulin response during a glucose tolerance test as well as normalization of fasting and postprandial glucose and insulin levels. Treated animals regained their body weight and developed insulin-positive islet-like cell clusters in the peri-portal region of the liver and insulin expression at the RNA and protein levels in the liver. The newly formed β-cells were able to appropriately turn off insulin production as efficiently as nondiabetic wild-type mice during a 72-hour fast. Characterization of the nature of the immune protection is under way: Experiments thus far demonstrate that splenocytes from treated mice which showed reversal of diabetes did not lose their diabetogenicity in an adoptive transfer experiment, suggesting a locally protective role for the combination regimen. In addition to gaining new mechanistic insights into islet neogenesis and immunopathogenesis of autoimmune diabetes, we are hopeful that our investigation will pave the way for an optimized gene therapy regimen for the treatment of patients with T1D in the future.

Sources of Research Support: NIH Grant R01DK068037-34 awarded to LC.

Nothing to Disclose: KO, VY, EB, VL, LC

P2-115

Diabetes-Associated Dry Eye Syndrome in a 'Humanized' Transgenic Model of Type 1 Diabetes.

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Patients with diabetes are at significant risk of developing corneal lesions due to lacrimal gland dysfunction (1). Even proliferative diabetic retinopathy appears to have a more severe course in patients with diabetes and lacrimal dysfunction (2). Whether or not this dysfunction is metabolic or autoimmune in origin is still controversial. However, it has been established (3) that the prevalence of dry eye syndrome (DES) is higher in children with type 1 diabetes (T1D), years before metabolic complications are even expected. Nonobese diabetic (NOD) mice usually suffer lacrimal gland dysfunction. Lymphocytic infiltration of various organs including salivary and lacrimal glands accompanies lymphocytic infiltration of pancreatic islets in NOD mice (4). A possible explanation for the association of pancreas and lacrimal gland lymphocytic attacks has been based on the existence of cross reactive antigens in both glands.
We have developed a new model of T1D using double-transgenic mice carrying HLA-DQ8 diabetes-susceptibility haplotype instead of mouse MHC-class II and expressing the human β cell autoantigen GAD65 in pancreatic β cells. Double-transgenic mice immunized with DNA encoding human GAD65, develop insulitis, glucose intolerance and diabetes while controls are insulitis free and glucose tolerant (5). We report here the development of eye manifestations classically associated with diabetes in NOD mice and proposed to be associated with human T1D. From corneal lesions to severe keratitis, our animals manifest obvious clinical signs of DES. Histological studies of peribulbar areas revealed lymphocytic infiltration of glandular structures. Indeed, severe infiltrative lesions were observed in lacrimal/Harderian glands within weeks following development of glucose intolerance. Lymphocytic infiltration ranged from focal lesions to complete acinar destruction. We did observe correlation between the severity of the pancreatic infiltration and severity of ocular disease.
Our results demonstrate development of DES in association with antigen-specific insulitis and diabetes following immunization with clinically-relevant human autoantigen, concomitantly expressed in β cells of mice carrying HLA-DQ8. As in the NOD mouse model and as in human T1D, our animals develop DES. This specific finding stresses the relevance of our model for studying the human disease. We believe our model will facilitate studies to prevent human diabetes and other associated syndromes.

(1)Cousen et. al., J Diabetes Complications 2007; 21:371
(2)Yu et al., Ophthalmologica 2008; 222:284
(3)Akinci et al., Eur J Ophthalmol 2007; 17:873
(4)Leiter and Serreze, Clin Immunol Immunopathol 1991; 59:323
(5)Elagin et al., J Autoimmun 2009; 33:50

Nothing to Disclose: RBE, JCJ

P2-116

The Murine Amniotic Fluid Macrophage: Upregulation of Classical Activation Markers Prior to Labor at Term Is Inhibited by Maternal Progesterone Treatment.

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Both term and preterm labor are associated with an inflammatory response, exemplified by increased levels of proinflammatory cytokines in amniotic fluid (AF), infiltration of myometrium, cervix, and fetal membranes by neutrophils and macrophages (Mφ), with activation of nuclear factor κB (NF-κB) and other proinflammatory transcription factors in myometrium. We previously obtained compelling evidence that the fetus provides an important signal for initiation of labor near term through developmental induction of surfactant protein-A (SP-A) expression by the fetal lung and its secretion into AF, where it activates Mφ, triggering their migration into the uterus. We propose that interactions of Mφ surface receptors with SP-A, at term, or bacterial lipopolysaccharide at preterm, initiate changes in Mφ phenotypic properties, resulting in the enhanced expression of genes involved in their polarization and chemotaxis to the uterus. The objectives of this study are: (1) to analyze the phenotypic changes of mouse AF Mφ associated with the developmental induction of SP-A synthesis and secretion by the fetal lung into AF, and (2) to determine whether a delay in labor caused by maternal progesterone (P_4) treatment can alter AF Mφ activation status. Using flow cytometric analysis we observed that the density of AF Mφ greatly increased between 15.5 and 18.5 dpc (19 dpc = term). Additionally, a significant increase in Toll-like receptors, TLR2 and TLR4/MD2, putative receptors for SP-A, was also noted. Interestingly, mice deficient in TLR2 or in both TLR2 and TLR4 exhibited a significant delay in the timing of parturition. Microarray and Superarray qRT-PCR-based analysis of WT AF Mφ from 15.5, 17.5 and 18.5 dpc mice demonstrated enhanced expression of markers associated with alternative (M2/antiinflammatory) and classical (M1/proinflammatory) activation toward term, suggested by increased expression of arginase 1, FIZZ1, YM1, YM2 (M2 markers), and IL-1β, IL-6 and TNFα (M1 markers). Intriguingly, AF Mφ isolated from P_4-injected mice demonstrated a marked decrease in expression of M1 markers, as compared to vehicle injected controls. Taken together, these findings demonstrate that AF Mφ express a mixture of M1/M2 associated genes and acquire proinflammatory, immunomodulatory, and suppressive effector functions as term approaches. Moreover, maternal P_4 treatment selectively suppresses classical/M1 activation, and in this manner, may compromise a fetal signal leading to labor.

Sources of Research Support: NIH P01-HD011149 and March of Dimes Prematurity Initiative Grant #21-FY07-601.

Nothing to Disclose: APM, CRM

P2-117

Modeling the Association between 25[OH]D and All-Cause Mortality in a Representative US Population Sample.

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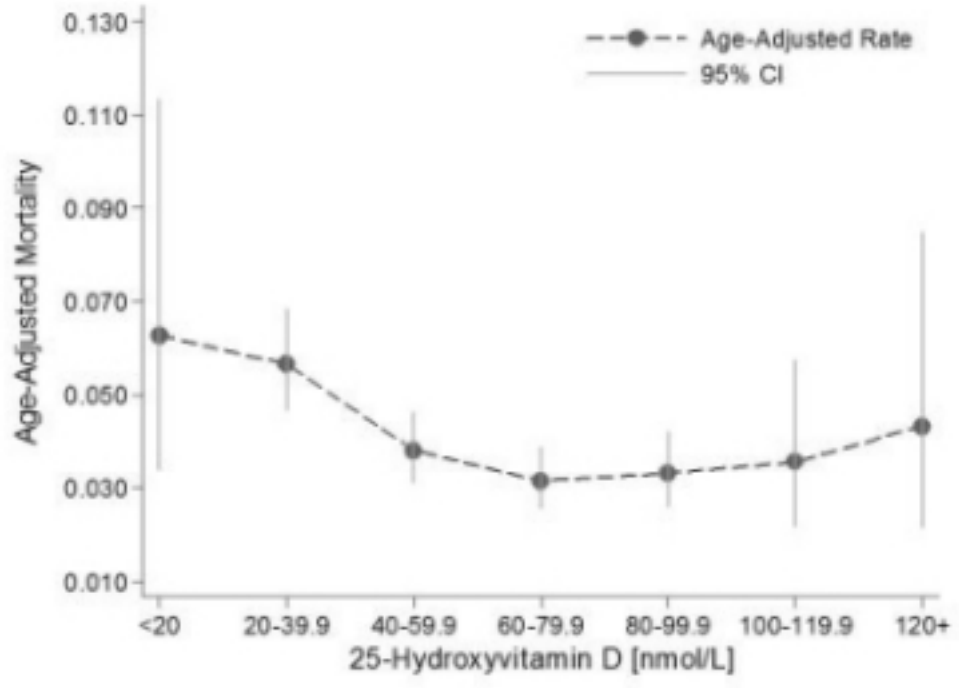
Vitamin D has been identified as a potential key risk factor for several chronic diseases and mortality. The association between all cause mortality and circulating levels of 25-hydroxyvitamin D (25[OH]D) has been described as non-monotonic with excess mortality at both low and high levels (1). However, the shape of this association appears asymmetric. Although the non-linear relationship has been modeled with up-to-date statistical methods, to our knowledge no attempt has been made to quantitatively estimate the 25[OH]D level at which minimum mortality occurs, accounting for the observed asymmetry. We modeled the non-monotonic, asymmetric association between 25[OH]D and all-cause mortality (Fig 1) in 15,106 participants aged 20 and older(6,355 Whites) in the NHANES III follow-up study using logistic regression, adjusting for age, education level, GFR, season, BMI, sex, race/ethnicity, medication use (anticonvulsants, estrogens, loop diuretics and/or thiazide diurectics) and blood pressure level. Two modeling approaches were used, namely 1) restricted cubic splines, and 2) transformation of 25[OH]D to normality. Maximum likelihood methods were used to estimate the parameters in the model and standard calculus techniques to obtain closed form solutions of the 25[OH]D of minimum mortality. The delta method was used to estimate standard errors of the parameters, along with normal-theory based confidence intervals(CI). Table 1 presents results for the entire population and for Whites. Estimates of 25(OH)D levels of minimum mortality based on the cubic splines (82 nmol/L) are lower than those obtained from the normal transformation approach (91 to 99 nmol/L). Similarly, the cubic splines approach yields narrower 95% CIs. In conclusion, the range of optimal 25[OH]D values appears rather wide, with excess mortality observed below 50 nmol/L and above 120 nmol/L. Interpretation for large levels of 25[OH]D deserves careful examination owing to the limited number of events.



Multivariable Adjusted Model-based estimates (95% CI) of the 25[OH]D of minimum mortality

	Whites	All
Transformation	91(57-124)	99(52-146)
Cubic Splines	82(48-116)	82(52-111)

(1) Melamed, et al, Arch Intern Med. 2008;168(15):1629-1637

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Nothing to Disclose: RAD-A, CS, AL, HK, EY, BD-H, GC, RLB, JTD, AJR, MFP

P2-118

Dexamethasone Synergistically Increases Secretion of the Anti-Inflammatory Cytokine Interleukin-10 in Murine Dendritic Cells upon Viral Infection: Potential Implication to Stress-Mediated Modification of Susceptibility to Viiral Infection.

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Glucocorticoid hormones are end-products of the hypothalamic-pituitary-adrenal axis, which play central roles in adaptive response to various stressors, including infection of pathogens, and are widely used as therapeutic agents for autoimmune, allergic and inflammatory diseases. The glucocorticoid receptor (GR), an intracellular receptor of the nuclear receptor superfamily, mediates diverse cellular actions of these hormones. It has long been known that individuals in a stressed condition show altered susceptibility to viral infection. Dendritic cells (DCs), on the other hand, are versatile and central regulators of host immunity. Upon pathogen infection, they sense pathogen components through their toll-like receptors (TLRs) and secrete cytokines to activate both innate and acquired immunity. Since it is not known how glucocorticoids influence the function of DCs upon viral infection, we infected murine bone marrow-derived DCs (BMDCs) with Newcastle disease virus (NDV) in the presence of dexamethasone (Dex), and examined mRNA expression of 84 genes, which play various roles in the TLR-mediated signaling cascade. We found that NDV infection potently induced mRNA expression of 24 genes. Among them, we observed 7 genes (interferon (IFN)-γ, interleukin (IL)-6, IL-10, lymphotoxin A, C-type lectin domain family 4, nuclear factor of κ light chain gene enhancer in B cell inhibitor β and prostaglandin-endoperoxidase synthase 2) were further up- or down-regulated significantly by pre-treatment with Dex. Particularly on the anti-inflammatory cytokine IL-10, Dex synergistically and potently up-regulated NDV-induced mRNA expression of this cytokine by 7-fold when compared to the NDV infection alone. This Dex effect was mediated by GR, as the GR inhibitor RU 486 completely abolished the effect. In contrast to IFN-γ and IL-6, which are Th1 cytokines and response at early time point (6 hours), kinetics of IL-10 mRNA expression showed the highest synergistic effect of NDV and Dex at a later time point (24 hours). ELISA results showed that NDV and Dex changed IL-10 secretion consistent with those observed at its mRNA levels. Taken together, our study indicates that glucocorticoids modulate host immunity against viral infection, altering TLR-related gene expression in DCs. Since IL-10 is an anti-inflammatory cytokine, our results may underlie increased susceptibility to viral infection in the subjects under stressed conditions.

Sources of Research Support: Funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD.

Nothing to Disclose: SSMN, AL, OK, TK

P2-119

In Vitro Glucocorticoid Sensitivity Partially Predicts Glucocorticoid-Therapy Outcome in Rheumatoid Arthrtitis.

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Introduction: Glucocorticoids (GC) are frequently prescribed drugs in rheumatoid arthritis (RA). However, the response to GC treatment is highly variable among patients, which may be explained by differences in GC-sensitivity. Therefore, assessment of a patient's individual GC-sensitivity would allow for tailor-made GC-therapy.
Aim of study: To study the correlation between *in vitro* cellular GC-sensitivity and *in vivo* GC-sensitivity in RA-patients treated with GC.
Patients: 36 consecutive patients with recent-onset RA (< 1 year) and 30 patients with established RA and active disease were included.
Methods: Disease Activity Scores (DAS44) were measured before and after standardized GC-treatment (either oral prednisolone or intramuscular depot of GC). The relative decrease in DAS44 was used as an index for *in vivo* GC-sensitivity. Peripheral blood mononuclear cells (PBMC) were obtained from healthy controls (n=20) and from all patients before start of treatment. Cellular GC-sensitivity was determined *in vitro* using our recently developed bioassay measuring GC-specific transactivation of the GC-induced leucine zipper (GILZ) gene and transrepression of the interleukin-2 (IL-2) gene using qRT-PCR. Half maximal effective concentration (EC_{50}) was used as a read-out for *in vitro* GC-sensitivity. In addition, in a subset of intramuscularly treated patients, the number and affinity of glucocorticoid receptors (GR) were measured.
Results: IL2-EC_{50} values were higher in RA patients compared to controls (p=0.003). In RA patients both IL2-EC_{50} and GILZ-EC_{50} values correlated with the relative decrease in DAS44-score (IL-2: R^2=0.13, p=0.03; GILZ: R^2=0.12, p=0.03). These correlations were only observed in the intramuscularly treated patients. Furthermore, GR number in PBMC correlated with the *in vivo* response (R^2=0.336, p=0.04). In contrast, the affinity of the receptor did not correlate with GC-therapy outcome.
Conclusions: *In vitro* GC sensitivity of PBMC is decreased in RA with respect to transrepressive effects of GC. In patients with early and established RA, effectiveness of GC-therapy could partially be predicted by *in vitro* assessment of GC-sensitivity. In addition, the number of GR, rather than the affinity of the receptor, is correlated with *in vivo* GC-sensitivity. Further studies are needed to establish whether assessment of *in vitro* GC sensitivity can support individualized therapeutic management of RA patients treated with GC.

Sources of Research Support: Dutch Arthritis Association.

Nothing to Disclose: RAMQ, FK, RvH, AEW, MAH, DvZ, FHdJ, SWJL, JMWH, RF

P2-120

Sexually Dimorphic Actions of Glucocorticoids: A Potential Link to Gender Prevalent Inflammatory Diseases.

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Males and females differ in physiology and in the incidence and progression of diseases. Females have a higher incidence of auto-immune diseases during reproductive years compared to males. Although sex hormones have been implicated in several sexually dimorphic diseases, alternatives have not been thoroughly considered. Glucocorticoids are the primary physiological anti-inflammatory hormone, and synthetic derivatives of these hormones are widely prescribed as anti-inflammatory agents. We previously demonstrated that glucocorticoids regulate common and distinct sets of genes in the liver of male and female rats. A comparison of the number of genes involved in inflammatory disorders between sexes revealed 84 additional glucocorticoid responsive genes in the male rat, suggesting that the anti-inflammatory actions of glucocorticoids are more effective in males than in females. To evaluate the proposed hypothesis, we tested the anti-inflammatory effect of glucocorticoids in a sepsis model. In agreement with the in silico predictions, glucocorticoids substantially improved survival rate and prevented the elevation of pro-inflammatory cytokines in the plasma and liver of endotoxemic males, suggesting that males are more responsive to the anti-inflammatory effect of glucocorticoids. We also evaluated the influence of sex hormones on the anti-inflammatory actions of glucocorticoids. The absence of endogenous androgens did not interfere with the survival rate of endotoxemic male rats treated with glucocorticoids. In contrast, ovariectomy increased the survival rate of female endotoxemic rats treated with glucocorticoids. Moreover, male specific glucocorticoid responsive genes involved in inflammatory disorders (for example, NFKBIB) were up-regulated by glucocorticoids in ovariectomized females, suggesting that removal of ovary potentiates the anti-inflammatory actions of glucocorticoids in females. Together, our data show that glucocorticoids regulate gene expression in a sex specific manner, the anti-inflammatory effect of glucocorticoids may be more dramatic in males, and in females the anti-inflammatory effects of glucocorticoids in females seem to also involve interplay with sex hormones. These findings suggest that glucocorticoids, through sexually dimorphic regulation of gene expression, modulate sex specific homeostatic functions in male and female livers, and provides a new explanation why females have a higher risk of developing auto-immune diseases.

Nothing to Disclose: DD, JAC

P2-121

IgG4-Positive Plasma Cells: A Marker of Fibrotic Variant of Hashimoto's Thyroiditis.

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Background: Hashimoto's thyroiditis (HT), which is characterized by the presence of goiter and serum thyroid autoantibodies, is the most common type of thyroiditis. Although the diagnostic criteria describe a well-defined disease entity, HT exhibits various clinicopathological presentations and outcomes. We herein describe a subsection of HT characterized histologically by dense lymphoplasmacytic infiltrate, stromal fibrosis, large numbers of IgG4-positive plasma cells, and serologically by elevated IgG4 titer. These cases seem to represent a distinct form of HT and have close relationship with IgG4-related sclerosing disease.
Methods: 83 cases of surgical samples from patients with HT and 2 cases of subacute thyroiditis were involved in this study. Immunostaining of IgG4 and IgG was performed on paraffin sections and the histopathological characterisitics of these cases were evaluated.
Results: On the basis of immunohistochemistry of IgG4 and IgG4/IgG ratio, the 83 patients with HT were divided into two groups: IgG4 thyroiditis (IgG4-positive plasma cell-rich group, 24 cases) and non-IgG4 thyroiditis (IgG4-positive plasma cell-poor group, 59 cases). Histopathologically, IgG4 thyroiditis showed higher grade of stromal fibrosis, lymphoplasmacytic infiltration and follicular cell degeneration than non-IgG4 thyroiditis. Histiocytic infiltration, foreign body type giant cells and colloid production within follicles were less frequently observed in IgG4 thyroidtis, but these features were common in subacute thyroiditis. In addition, serum IgG4 concentrations were significantly higher in IgG4 thyroiditis than non-IgG4 thyroiditis. Furthermore, patients with IgG4 thyroiditis are more frequently to suffer from subclinical hypothyroidism than patients with non-IgG4 thyroiditis. No other organs were found to be involved by IgG4-related sclerosing disease in this patients' series.
Conclusion: Hashimoto's thyroiditis can be subclassified into two groups, in regard of IgG4-positive plasma cell population: IgG4 thyroiditis and non-IgG4 thyroiditis. IgG4-positive plasma cell infiltrate is a marker of fibrotic variant of Hashimoto's thyroiditis, which shows atrophy of follicles and stromal fibrosis, resulting in subclinical hypothyroidism more frequent.

Nothing to Disclose: YL, KH, EN, ZL, TO, ET, IM, AM, KK

P2-122

Differential Inflammatory Gene Expression Profile in Graves' Disease.

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Introduction: Several studies identified an overlap of T helper(Th) 1 and Th2 responses in the autoimmune mechanism of Graves' disease. Considering it a systemic disorder, characterization of a pattern of mRNA expression of inflammatory genes could determine a specific signature of the disease and provide new information about its pathogenesis.
Objective: To characterize expression of genes of inflammatory response in Graves' disease and determine actual systemic inflammatory pathways involved.
Materials and methods: We evaluated 10 patients with Graves' disease diagnosed by laboratory hyperthyroidism associated with presence of anti-TSH receptor antibodies, treatment naïve, and 11 controls with normal thyroid function without any known autoimmune disorder. Total mRNA was extracted from peripheral blood mononuclear leukocytes and labeled cRNA was prepared. Gene expression studies were performed using commercial cRNA microarray Oligo GEArray SABioscience (SABiosciences, USA) that contained 440 pre-selected genes related to adaptative and inflammatory response.
Results: There were no significant difference between Graves' disease group and control group in sex(p=0.67) and age(p=0.39). Microarray analysis revealed 52 genes presenting 2 fold differential overexpression in Graves' disease patients(p<0.05).

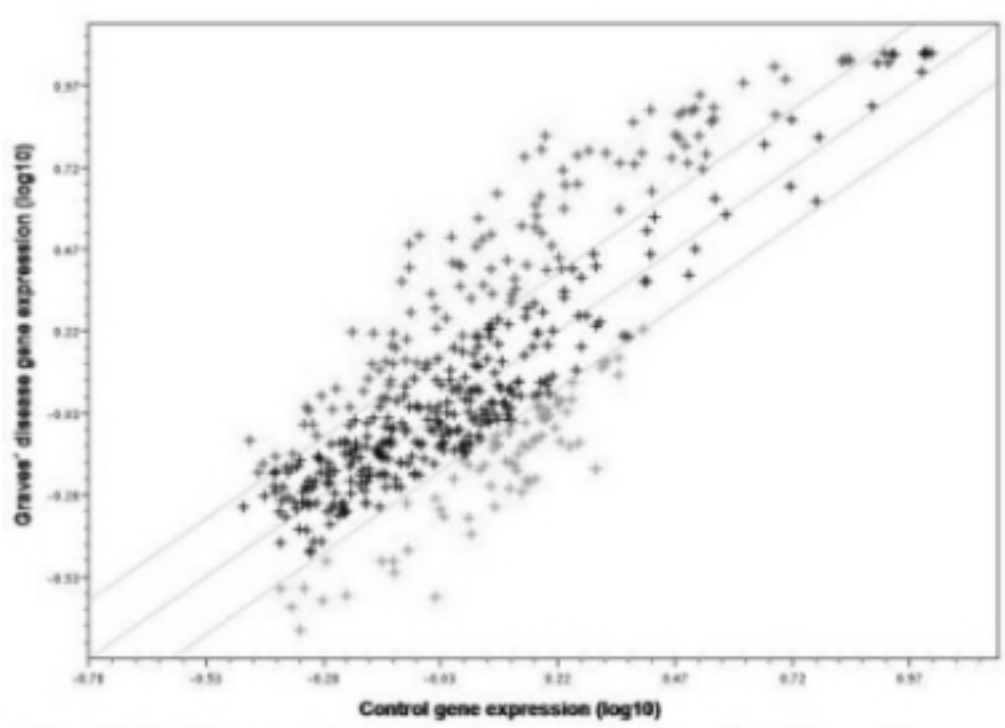

Figure. Scatterplot representation of different gene expression in control and Graves' disease subjects. Genes with increased expression in Graves' disease are identified above 2 fold limit lines.

We identified genes involved in antigen–presenting mechanism, such as CD74, CD86 and CD1C. We found overexpression of IFNGR1 and 2, FLT3LG and IL13 involved in Th1 response, CD22, IL4R and BCL6 in the Th2 response, and IL1B and Il10RA in both. Among chemokines and their receptors, we found CCR5 and CXCR3 overexpression, related to Th1 response, CCR2, IL8RA and CCR8 related to Th2. We also found intracellular genes overexpression, like NFKB, TRAP-1 and CEBPB, denoting that inflammatory responses are actually activated in peripheral leukocytes in Graves´ disease.
Conclusions: Graves' disease presents a differential inflammatory expression signature that can be detected in peripheral leukocytes. Our findings highlight the participation of both cellular and humoral responses and novel cytokines that could have a role in the autoimmune process

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Nothing to Disclose: DLSD, AML, CJL, MK, BBM, SM

P2-123

Compromised Ability of the Crh-/- Mice To Survive DSS Colitis: A Novel Role of CRF in the Resolution of Inflammation?.

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Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, is a chronic, relapsing disease . The pathogenesis of IBD involves complex interactions between genes and the environment and, although the exact mechanisms triggering the initial attack and the relapses are not well understood, increasing evidence supports the significance of innate immune responses in its pathogenesis. Several studies have supported the contribution of neuropeptides, including substance P, neurotensin and vasoactive intestinal peptide, in the development of IBD. In our previous studies, we have demonstrated the proinflammatory effects of Corticotropin Releasing Hormone, or Factor, (CRH or CRF), a neuropeptide acting as the major mediator of the stress response, in intestinal inflammation in rodents. Using an experimental model of innate immune responses-dependent ulcerative colitis, the DSS-induced colitis, we have demonstrated that CRH- null *(Crh-/-)* mice developed severe more severe local and systemic disease compared to similarly treated wild type *(Crh+/+)* mice.
DSS-colitis is a self-limited inflammatory condition, where, if no further challenges are given following the initial 7 days of DSS treatment, the injured tissue is repaired and regeneration of the epithelial layer occurs. We wanted to evaluate the ability of *Crh-/-* mice to recover the DSS-injury and repair the injured epithelium. Thus, we followed-up the recovery of both *Crh+/+* and *Crh-/-* mice for 5 days after completion of the 7-days DSS treatment. All DSS-treated *Crh-/-* became moribund by 4 days after the cessation of the treatment, whereas all *Crh+/+* mice recovered the injury and their initial wasting. Histological analysis of the injured colon, showed abnormal epithelial proliferation and fibrosis patterns in the *Crh-/-* tissue. The above together with increased angiogenesis and high levels of activated MAPK p38 indicated an on-going inflammatory process. Our findings (a) demonstrate a significant protective effect of CRF in innate immunity – dependent intestinal inflammation and (b) suggest a novel effect of this peptide in the resolution of the inflammatory process and the repair and regeneration of the mouse intestinal epithelial barrier.

Disclosures: ST: Employee, Merck & Co.
Nothing to Disclose: PG, ZC, SP, KPK

P2-124

Alpha (CXCL10) and Beta (CCL2) Chemokines Modulation by Peroxisome Proliferator-Activated Receptor-Alpha Agonists in Graves' Ophthalmopathy Fibroblasts.

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Context and Objective. Until now, to our knowledge, no data are present in literature about the effect of cytokines on the CCL2 chemokine in Graves' ophthalmopathy (GO), nor of peroxisome proliferator-activated receptor (PPAR)-alpha activation on chemokines secretion in fibroblasts or in GO.
Design. Here, we have tested the interferon (IFN)-gamma and tumor necrosis factor (TNF)-alpha effect on CCL2 and the possible modulatory role of PPAR-alpha activation on the prototype alpha (CXCL10) and beta (CCL2) chemokines secretion in normal and GO fibroblats in primary culture.
Results. The present study shows that IFN-gamma alone, or in combination with TNF-alpha, was able in stimulating the secretion of the CC chemokine CCL2 in primary orbital fibroblasts from patients with GO, at level similar to that observed in controls. IFN-gamma and TNF-alpha stimulated CXC chemokine secretion as expected. The presence of PPAR-alpha and -gamma in primary fibroblasts from patients with GO has been shown. PPAR-alpha activators were able in inhibiting the secretion of both CXC (CXCL10) and CC (CCL2) chemokines, while PPAR-gamma activators were confirmed to be able in inhibiting CXCL10 chemokine, but had no effect on CCL2. PPAR-alpha activators were stronger inhibitors of chemokines secretion than PPAR-gamma agonists.
Conclusions. The present study shows that CCL2 is modulated by IFN-gamma and TNF-alpha in GO. PPAR-alpha activators are able in inhibiting the secretion of the main prototype alpha (CXCL10) and beta (CCL2) chemokines in GO fibroblasts, suggesting that PPAR-alpha may be involved in the modulation of the immune response in GO.

Nothing to Disclose: SMF, PF, SF, ADD, CM, EF, AA

P2-125

The Effects of Resveratrol on T Cell Populations and the Immune Response to Vaccination in Rhesus Macaques on a Diet High in Fat and Sucrose.

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Resveratrol is a polyphenol that has been shown to produce similar beneficial effects to those of caloric restriction. These include anti-inflammatory, anti-platlet and anti-mutagenic effects that seem to provide protection from diabetes, atherosclerosis and cancer. It has also been reported to extend lifespan in every organism tested from nematodes to mice and protect mice against premature death from high calorie-high fat diet. In collaboration with the National Institutes on Aging as part of a larger study of the health benefits of Resveratrol on rhesus macaques on a diet high in fat and sucrose (HFS), we examined the effects of resveratrol on T cell populations and the immune response to vaccination in these animals. Earlier work done by our lab has shown accelerated immune senescence in young rhesus macaques chronically fed a diet high in calories, fat and fructose. We wanted to test whether resveratrol could protect animals on the HFS diet from premature immune senescence and improve the immune response to vaccination. Twenty four male animals matched for age and body mass were randomized to a HFS, HFS+Resveratrol and a control group with 10,10 and 4 individuals in each respective group. These animals were followed for 12-18 months before sacrifice and tissue evaluation. Six months after randomization each monkey received an Modified Vaccinia Virus Ankara (MVA) vaccine and a booster at Day 28 and a second booster 14 days prior to necropsy. Blood was collected at baseline and 7, 14 and 28 days after initial vaccine and 7,28, 63 and 90 days after booster. A last blood collection was done 14 days after the second booster prior to necropsy. At each time point, PBMCs and plasma was isolated to evaluate the kinectics and magnitude of the immune response by measuring: 1) plasma antibody titers by ELISA, T and B cell proliferation, and T cell cytokine production by intracellular cytokine staining using flow cytometry techniques. In response to MVA vaccination, all animal groups generated a comparable T and B cell proliferative response. Moreover, frequency of MVA-specific T cells and IgG titers were also similar in all three groups. These findings differ from earlier studies in rhesus macaques that indicated that high fat diet results in lower immune response to viral infection. The reason for the discrepancies is likely due to the relatively short duration of the HFS diet. Our results also suggest that resveratrol might not modulate immune function.

Nothing to Disclose: WCC, IM

P2-126
Triclocarban Modulates Cytokines *In Vitro*.

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Triclocarban (TCC), an anti-microbial agent, has recently been identified as a new type of endocrine disrupting substance (EDS). Increasing evidence now indicates that TCC is persistent in sludge and has the potential to be spread onto agriculture and into waterways. Widespread exposure to TCC therefore has the potential to alter physiology in both wildlife and humans. Unlike most classical EDSs, TCC acts as strong agonist by potentiating the transcriptional activity of estrogenic and androgenic steroid hormones. Earlier studies have shown that TCC alone has little or no biological activity in estrogen and androgen receptor signal transduction assays. Since glucocorticoids (GCs) also transduce signals through a nuclear receptor similar to those of estrogens and androgens, they may also be affected by TCC. Using MDA-kb2 cells which express a functional glucocorticoid receptor (GR), we demonstrate that TCC enhances the cortisol-induced transcriptional activity of the luciferase gene. To confirm this enhancement, RAW 264.7 cells, a mouse macrophage cell line, were treated with a synthetic GC and TCC concurrently with a LPS-induced inflammatory reaction. A combination treatment of TCC, GC and LPS did not induce consistent changes in IL-10, IL-6 and TNF-α mRNA expression. In contrast, treatment with TCC in the presence of LPS but in the absence of a GC induced consistent and significant concentration-dependent effects: a decrease in IL-10, GM-CSF, IL-1β and an increase in TNF-α mRNA expression. TCC also induced a concentration-dependent decrease in protein levels of IL-1β, IL-6, IL-10 and GM-CSF. TNF-α had no significant changes when compared to control.
These data demonstrate that unlike the effect of TCC on estrogenic and androgenic steroids, TCC does not require the presence of the natural ligand to induce glucocorticoid-like modulation of cytokine expression. The underlying mechanism(s) and the potential physiological importance of TCC actions on endocrine systems are currently unknown. Since TCC alone is able to alter cytokine expression, further investigations of its potential immunotoxic effects should be determined to understand possible human health risks.

Sources of Research Support: NIEHS Superfund Basic Research Program P42 ES04699.

Nothing to Disclose: NCO, JC, AJD, BLL

P2-127
Effect of Extracts from Porcine Testis on Decidualization.

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The physiological properties of a steroid hormone are depended on the physiological properties which are constructed by other steroid hormones. Interestingly, the boar has a number of unique features concerning the synthesis of uncommon steroid hormones including 19-nortestosterone. The extracts of porcine testis includes various steroid metabolites such as 19-nortestosterone, 4-androsten-17β-ol-3-one, and 17-β-estradiol, and the relative amount between the compounds can be defined by the extraction methods. These steroids have many physiological roles in female reproduction and can be applied in medicine. Therefore in this study, we examined the possible side effects of the extract during pregnancy especially decidualization. Artifical decidualization was induced in ovariectomized mice by following the well defined method and was analyzed the uterine responsibility through histological methods and analysis of decidua specific mRNA expression. Histologically the decidua was induced by trauma both in control and extract treatment groups. Alkaline phosphatase specific mRNA was increased by trauma both in control and extract treatment groups in a same patterns with histological changes. In addition the expression levels and patterns of CTLA-2β, Doc-1, cathepsin A and connecxin 43 were also similar between the control and extract treatment groups. In addition, the extract was not cause of abortion. Based on these results, it is suggested that the extracts of porcine testis did not inhibit the responsibility of uterus to embryo implanation and the compounds of the extract may work as a collaborator during pregnancy.

Nothing to Disclose: JHY, JHB, TC, KHL, IC, YPC

P2-128

The Tyrosine Phosphatase, SHP-1, Acts on Multiple Tyrosine Kinase Receptors to Negatively Regulate Human Cytotrophoblast Proliferation.

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Pregnancy complications such as fetal growth restriction are associated with abnormal placental cell (cytotrophoblast) proliferation and apoptosis. Regulation of these events is unclear but recently we have used a placental explant model to demonstrate that IGFs influence cytotrophoblast kinetics and demonstrated that the protein tyrosine phosphatase (PTP) SHP-2 is required for IGF actions in the placenta. However, SHP-2 accounts for only 20% of total PTP activity, which suggests that other PTPs may also be important. mRNA for a closely related PTP, SHP-1, has been reported in the placenta but its actions are unknown; in other systems it functions as a negative regulator of signalling events. We examined the hypothesis that SHP-1 negatively regulates IGF actions by measuring cytotrophoblast proliferation following siRNA-mediated knockdown of SHP-1.
SHP-1 siRNA or non-targeting siRNA (500nM) was delivered to BeWo cells or first trimester human villous tissue explants. Cells and tissue were maintained in culture for 72h, then treated with IGF-I or IGF-II (10nM) for a further 24h before western blot, immunohistochemical (IHC) and QPCR analysis. Following exposure to SHP-1 siRNA, SHP-1 expression was reduced in both BeWo cells (~85% knockdown) and in first trimester explants (~73%). IHC analysis for cell proliferation (Ki67) demonstrated that SHP-1 siRNA had no effect on IGF-induced proliferation but significantly enhanced levels of basal (serum-free) proliferation in both BeWo cells (from 19.7.4±2.6% to 52.3 ±2.9%; $P<0.05$, $n=4$) and placental explants (from 22.3±3.7% to 63.8 ±2.7%; $P<0.05$, $n=4$). The mechanism(s) by which SHP-1 regulates basal but not IGF-induced proliferation were investigated by examining the activation status of multiple receptor tyrosine kinases (RTKs), using antibody arrays and IHC, following depletion of SHP-1. This revealed that SHP-1 knockdown is associated with enhanced activation of EGFR and TrkB, suggesting that under basal conditions SHP-1 may interact with these molecules to inhibit proliferation.
This study demonstrates a role for SHP-1 in human trophoblast and establishes SHP-1 as negative regulator of multiple RTKs that regulate placental growth. Manipulation of RTK activation may have therapeutic potential in pregnancy complications associated with abnormal trophoblast kinetics.

Nothing to Disclose: KF, LS, JDA, MW

P2-129

Pharmacological Antagonism of Myostatin-ActRIIB Pathway Reverses Sarcopenia in Aging Mice.

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Aging-associated sarcopenia is believed to be a major cause of frailty and loss of independence for the elderly. In aging, sarcopenia often presents itself with reduced bone mass and increased adiposity, which further exacerbate frailty. Recent advance in understanding of myostatin pathway biology in regulating muscle homeostasis has opened an opportunity to develop new therapeutic approaches for age-related muscle loss. In the current investigation, we have examined the effects of myostatin pathway blockade on muscle mass and function as well as bone density and adiposity in old mice. Baseline characterization of muscle mass changes in mice at different ages revealed that hindlimb muscle mass (i.e., gastrocnemius and soleus) began to decline around 14 months of age. A significant decrease in muscle mass was found beginning from 16 months of age and by 24 months of age, the hindlimb muscle mass had lost approximately ~11% compared to its peak value at younger age. Concomitant with the muscle mass decline, ventral peritoneal fat mass was increased by 2-3 folds. Accordingly, we treated aging mice with a myostatin antagonist in the form of a soluble ActRIIB decoy receptor (sActRIIB) beginning at 24 months of age. 8-week treatment with sActRIIB dramatically increased LBM and muscle mass by 14% and 25 %, respectively, thereby completely reversing the age-related sarcopenia in the aging mice. sActRIIB treatment also resulted in a significantly increase in grip strength by 19 %. In addition, the treatment significantly increased BMD and BMC by 13%-18% and also markedly reduced the elevated adiposity by about 50% in the aging mice. These findings suggest 1) Myostatin-ActRIIB pathway activities may play an important role in the pathogenesis of aging-associated sarcopenia, bone loss and obesity; and 2) Pharmacologically antagonizing this pathway may have therapeutic potential for treating these age-associated disorders.
JWP and JKM contributed equally to this work.

Serum myostatin-immunoreactive protein is increased in 60-92 year old women and men with muscle wasting.
J Nutr Health Aging. 2002;6(5):343-8.
Antagonism of myostatin enhances muscle regeneration during sarcopenia
Molecular Therapy, Volume 15, Number 8, p1463-1470 (2007).
Myostatin inhibition enhances the effects of exercise on performance and metabolic outcomes in aged mice
The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 2009 64A(9):940-948

Nothing to Disclose: J-LW, XZ, KK, YS, EJ, JL, HQH

P2-130

Modulation of Wnt Signaling Pathway in the Absence of Endogenous FGF2.

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We previously reported that basic fibroblast growth factor (FGF2) is an important modulator of bone growth and differentiation. However, the mechanism by which FGF2 regulates bone formation or osteoblast differentiation is not well understood.
A single Fgf2 gene encodes several protein isoforms including low molecular weight 18kDa FGF2. We recently showed that mice specifically deficient in 18 kDa FGF2 display reduced bone mineral density together with increased expression of the Wnt antagonist secreted frizzled receptor -1 (sFRP-1) in bones and bone marrow stromal cells (BMSCs). sFRPs inhibit all Wnt-activated pathways. The Wnt signaling pathway is required in osteoblast differentiation. Similar to the phenotype of 18kDa-/- mice, Fgf2 all isoform null (Fgf2-/-) mice have markedly reduced bone formation as well as osteoblast differentiation. Here we further examine whether other components of the Wnt signaling pathway are altered in the absence of all endogenous FGF2 isoforms in osteoblasts. BMSCs from Fgf2+/+ and Fgf2-/- mice were cultured in osteogenesis medium. After 17 days, staining for alkaline phosphatase (ALP) and mineralized nodules were performed. Total RNA and protein were extracted from parallel cultures. Relative mRNA expression was measured by quantitative real time PCR and protein expression was analyzed by Western blots. We found decreased ALP-positive, mineralized nodules in BMSCs culture from Fgf2-/- mice. Consistent with our previous studies, we found significantly decreased mRNA expression of osteoblast differentiation markers Runx2 and osteocalcin. Interestingly, there was a dramatic decrease of β-catenin mRNA and protein expression as well as reduced canonical Wnt3a and Wnt10b mRNA expression in Fgf2-/- BMSCs. However, mRNA for non-canonical Wnt5a an inhibitor of canonical Wnt pathway was significantly elevated in Fgf2-/- BMSCs. These results suggest that impaired bone mass and bone formation in Fgf2 null mice may be due in part to modulation of the Wnt signaling pathway.

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Nothing to Disclose: YF, LX, TD, JDC, MMH

P2-131

Impacts of ERK and Akt Signaling Pathways on EGFR-Mediated Actions in Human DU145 Prostate Cancer Cells.

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Growth factor/receptor signaling pathways, especially epidermal growth factor receptor (EGFR) signaling, play important roles in the development and progression of a broad spectrum of human malignancies including prostate cancer. Impaired endocytic downregulation of signaling receptors including EGFR is frequently associated with cancer, as it can lead to uncontrolled signaling and oncogenic phenotypes such as metastasis. Thus, it is very important to understand the roles of divergent pathways in the regulation of EGFR trafficking and EGFR-mediated invasive migration as it will enable the development of more effective and selective therapies. We have previously reported differential roles of EGF-mediated ERK and Akt signaling pathways in prostate cancer cells: Akt plays a central role in EGFR-driven cell motility while ERK is more critical in regulating EGFR trafficking (Gan et al., 2009, Endocrine Society Annual Meeting). However, the underlying mechanisms remain to be explored. In this study, we first showed that in human DU145 prostate cancer cells, EGF induced threonine phosphorylation of EGFR in addition to tyrosine phosphorylation, and EGFR mediated cell migration in a ligand-dependent manner. Further, we found that EGF stimulation promoted epithelial-mesenchymal transition (EMT)-like cell morphological changes, accompanying by the disassembly of cell-cell adherens junctions, downregulation of E-cadherin (an epithelial marker), and upregulation of Snail (a transcriptional repressor of the E-cadherin gene). These effects were dependent on the activation/inactivation status of Akt and its downstream effector GSK3β. Finally, we demonstrated that pharmacological inhibition of ERK but not Akt eliminated the threonine phosphorylation of EGFR, which led to enhanced EGFR tyrosine phosphorylation (activation) and faster receptor turnover. Interestingly, the strength of EGF-induced Akt activation was also increased concomitantly, which may explain why blockade of ERK pathway rather promoted EGFR-driven cell motility. Based on these results, we conclude that 1) EGF-triggered ERK activation has profound feedback on EGFR signaling and trafficking via EGFR threonine phosphorylation; 2) Akt plays a pivotal role in EGFR-mediated cell migration, most likely via the route of Akt→GSK3β→Snail→E-cadherin→EMT; 3) targeting of ERK signaling may have undesirable outcomes under some circumstances (e.g. enhanced EGFR-driven motility).

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Nothing to Disclose: YG, MH, JB, YH

P2-132

Wnt10b Expression Stimulates NF-kappaB Activity in Osteoblasts.

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The Wnt family of secreted cytokines regulates numerous aspects of development including the formation and maintenance of bone. Activation of Wnt signaling by Wnt10b has been shown to enhance osteogenesis by increasing expression of key osteogenic transcription factors in cell culture models. Furthermore, genetic deletion of Wnt10b in mice is associated with a decrease in bone mineral density whereas transgenic overexpression is associated with a significantly higher bone mineral density, suggesting that Wnt10b is a positive endogenous regulator of bone formation. To identify the transcriptional targets of Wnt10b signaling in osteoblasts, we stably introduced Wnt10b in the U2OS osteosarcoma cell model and performed microarray analysis. A total of 370 genes were significantly regulated ($p<0.05$, fold change > than 3 SDs) including classical Wnt targets such as Axin2 and Lef1. Bone morphogenetic proteins (BMP)-2, -4, -6 and -7 were also upregulated confirming the role of Wnt10b as an activator of bone formation. Interestingly, Metacore pathway analysis revealed that the Wnt developmental pathway ($p=0.0016$) and the NF-kappaB pathway ($p=0.003$) were significantly upregulated in the U2OS-Wnt10b cell model. Increased expression of known NF-kappaB pathway activators, such as TNFα (7.57-fold, $p<0.001$) and IL1α (8.72-fold, $p<0.001$), were confirmed in subsequent QPCR experiments. To investigate this finding in a physiologically more relevant cell system, conditioned media generated from parental U2OS cells or U2OS-Wnt10b cells was used to treat primary mouse calvarial osteoblasts or mouse bone marrow stromal cells transduced with a NF-kappaB reporter vector. Wnt10b conditioned media stimulated reporter activity 10.5-fold ($p=0.0027$) and 2.5-fold ($p=0.015$) in these models respectively, demonstrating NF-kappaB pathway activation by Wnt10b in multiple systems. This data is seemingly inconsistent with a report that inhibition of NF-kappaB signaling in mature osteoblasts enhances bone formation in young mice. However, mouse bone marrow stromal cells maintained in osteoblastic differentiation media exhibited a steady decrease in Wnt10b expression throughout differentiation, suggesting that Wnt10b-mediated stimulation of the NF-kappaB pathway may be necessary at early stages of osteoblastic differentiation, however is downregulated for terminal differentiation to occur. In summary, this study identifies the NF-kappaB pathway as a novel Wnt10b target in osteoblasts.

Nothing to Disclose: UIM, DGM

P2-133

Essential Role of 2-O and N-Sulfation of Heparin on FGFR1 Regulation by Anosmin-1 in GnRH Neuron Migration.

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Anosmin-1 and FGFR1, known as KAL-1 and KAL-2 are associated with Kallmann syndrome (KS), a human developmental genetic disorder characterized by the loss of sense of smell caused by dysgenesis of olfactory bulb and hypogonadotropic hypogonadism (HH) owing to deficient hypothalamic GnRH neurons. We have found that in human embryonic GnRH olfactory FNC-B4 neuroblasts, anosmin-1 induced neurite outgrowth, cytoskeletal rearrangements and cell migration are through FGFR1-dependent mechanisms involving p42/44 and p38 mitogen-activated protein kinases and Cdc42/Rac1 activation. Anosmin-1 binding with heparin promotes FGF2/FGFR1/heparin signalling complex assembly and activity. Since sulfation of heparin is essential in the activation of FGFR1 signalling, we further investigated the specific heparin sulfation on FGF dependent FGFR1 regulation by anosmin-1. Single and double desulfated heparins deficient of 2-O, 6-O and N-sulfo groups were able to dose-dependently block anosmin-1 binding to immobilized heparin, while such competitive inhibition was not observed with fully desulfated heparin, demonstrating that sulfo groups in any potential sulfation sites are crucial for heparin binding to anosmin-1. Furthermore, binding of 6-O-desulfated heparin with anosmin-1 still led to complex formation with FGF2/FGFR1, whereas 2-O or N-desulfated heparin binding with anosmin-1 was unable to elicit complex formation. Consistently, in the transwell migration assay, only 6-O-desulfated heparin, but not 2-O, N- and completely desulfated heparin, was able to assist anosmin-1 to induce migration on the immortalized FNC-B4 cells. These results demonstrate that combined 2-O and N-sulfo groups are essential for the regulation of anosmin-1 on FGF2/FGFR1/heparin complex assembly and activation; therefore anosmin-1 might shift FGFR1 signalling activation from 6-O sulfation dependent to 2-O and N-sulfation associated mechanisms in GnRH neuron migration. These findings provide molecular basis for the complexity of FGFR1 signalling in GnRH ontogeny and implicate that the sulfotransferases specific for the 2-O and N-sulfation of heparan sulphate could be candidate molecules underlying pathogenesis of KS.

Nothing to Disclose: YH, SEG, S-HK, JET, PB

P2-134

Unique Properties of the Insulin Receptor Substrates (IRS)-1 and IRS-2 To Mediate IGF-1 Signaling.

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High complexity of signaling networks is required for the fine tuning of hormonal responses. Insulin/IGF-1 action is mediated in large part through the insulin receptor substrate (IRS) proteins family. Among them, IRS-1 and IRS-2 are the main mediators involved in the transmission of the signal and, although they share a similar overall structure, they play complementary and non-redundant roles in insulin/IGF-1 action (1). In the present study, we aim to define the specific roles of each of these two proteins in mediating IGF-1 signaling on gene expression. To this end, we performed microarray analysis using IRS-1 KO cells, IRS-2 KO cells and WT control cells stimulated or not with IGF-1 for 30 min or 6 h. 125 genes were significantly regulated after 30 min of IGF-1 treatment and 295 genes were significantly regulated after 6 h of IGF-1 treatment. Among those IGF-1 responsive genes, we found that 19 % and 3.7% of the genes were similarly dysregulated in the IRS-1 and IRS-2 KO cell-lines at 30 min and 6 h, suggesting that the two IRS proteins play complimentary roles in the regulation of expression of these genes. Interestingly, 7 % of the genes were specifically deregulated in IRS-1 KO at 30 min and 2.7 % at 6 h, while in IRS-2 KO cells none were deregulated at 30 min and 1.7% were deregulated at 6 h, indicating that some genes utilize one specific IRS protein to be regulated by IGF-1. Among the genes specifically dependent on IRS-1 were genes involved in the regulation of proliferation and differentiation, cell signaling and lipid metabolism. Genes depending on IRS-2 included genes involved in the regulation of RNA processing. The basis for these differences in function may relate to subcellular localization. Thus, while IRS-1 is mainly present in the cytosol and associated with intracellular membranes (high speed pellet or HSP) in both basal and insulin-stimulated conditions, IRS-2 is predominantly present in the cytosol. Thus, the relative abundance of IRS-1 was similar in the cytosol and HSP, whereas IRS-2 was 10-fold higher in the cytosol than in the HSP. Altogether, our results show that IRS-1 and IRS-2 play unique roles in mediating IGF-1 signaling and suggest that this might contribute to the action of IGF-1 on cell proliferation and differentiation. The different distribution of IRS-1 and IRS-2 in subcellular compartments may contribute to the different signaling properties of these proteins.

(1) Tseng YH et al., Mol Cell Biol 2004; 24(5):1918-29

Nothing to Disclose: BE, YHT, YM, CRK

P2-135

Human Serum Biomarkers for Detection of Erythropoietin Action.

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Erythropoietin (Epo) is mainly produced in the kidney upon low blood oxygen tension to stimulate erythropoiesis in the bone marrow. Recombinant human Epo (rHuEpo), a drug developed to increase arterial oxygen content in patients, is also illicitly used by athletes to improve endurance performance. Unfortunately, no sensitive test exists to detect this abuse. The aim of the present study was to investigate potential human serum biomarkers of Epo action using a proteomic approach.
Eight healthy male subjects (25 ± 4 yr, 183 ± 6 cm, 79 ± 7 kg) were injected s.c. with rHuEpo (5000 IU) every second day for a 16-day period. Serum was collected before, 8 and 16 days after treatment. At baseline, all measured hematological parameters of the individuals were within normal ranges. Several of the measured hematological values were significantly up-regulated at day 8 (reticulocytes) and day 16 (reticulocytes, erythrocytes, hematocrit, hemoglobin, and transferrin), showing that erythropoesis was increased. Furthermore, significant decreases in iron and ferritin levels were found at both day 8 and 16. For proteomic analysis, the samples were homogenized and proteins separated by two dimensional (2D)-gel electrophoresis. Protein spots that changed significantly ($p<0.05$) in response to rHuEpo treatment were identified by mass spectrometric (MS) analysis. Out of 97 serum protein spots, seven were found to decrease significantly at day 16 compared to pre-Epo administration. The spots were identified as four isoforms of haptoglobin, two isoforms of serotransferrin and a mixture of hemopexin and albumin. No protein spots were significantly altered eight days after the start of Epo injections.
In this study, proteins closely related to the transport of degradation products of erythrocytes were found to be significantly decreased. The decrease was consistent in all subjects, indicating that these proteins could be future markers of Epo action. Thus, a 2D-gel electrophoresis proteomic approach appears feasible for generating novel markers of Epo action.

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Nothing to Disclose: BC, LS-S, DC-T, JOLJ, NJ, CL, JJK

P2-136

Proteomics Profiling of Putative Growth Factors in Mouse Plasma and Pancreatic Islets by Mass Spectrometry.

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The discovery of novel circulatory growth factors has significant potential for advancing our understanding and therapeutic treatment of metabolic disorders such as obesity, insulin resistance, and diabetes. However, the relative low abundance of these putative factors within complex tissue samples presents a challenge for rapid identification and accurate 1quantification. In this work, we aim to establish methodologies for identifying putative growth factors in both mouse plasma and pancreatic islets by applying liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) based proteomics profiling. For mouse plasma, a SuperMix immunoaffinity depletion step was applied to remove nearly 100 proteins present in high and moderate abundance prior to LC-MS/MS analyses to enhance the detection of low-abundance proteins. Single dimension SDS-PAGE separation coupled with in-gel digestion and LC-MS/MS analysis was also applied to specifically identify low molecular weight (LMW) proteins. This initial profiling resulted in the identification of >30 known cytokines and growth factors and many other putative factors in mouse plasma, including insulin, leptin, adiponectin, IGF1, granulin, and prosaposin, among a total of ~980 plasma proteins identified. To identify pancreatic islet hormones, both global and LMW factions of the islet proteome were analyzed by LC-MS/MS. ~3000 islet proteins were identified, including all known islet secreting hormones and 19 other annotated growth factors and cytokines. A comparison of the identified proteins present in pancreatic islets and plasma revealed ~200 common proteins, suggesting that many of these proteins are potential secretory factors from pancreatic islets. Taken together, we have established a promising proteomic profiling methodology for effective identification of putative growth factors in plasma and tissue samples. The quantitative application of this methodology to different mouse models of insulin resistance, obesity, and diabetes, will provide a fundamental approach to discover novel regulatory growth factors critical for tissue function.

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Nothing to Disclose: WJQ, JYZ, CWL, VAP, RJM, DGC, RNK, RDS

P2-137

IGF-I Protects Human Subcutaneous but Not Omental Preadipocytes from FFA-Induced JNK Activation and TNFa Expression.

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Introduction: Subcutaneous and visceral adipose tissue depots differ in two significant ways: rates of adipogenesis and expression of inflammatory cytokines. Free fatty acids (FFA) are increased in visceral fat and increase insulin resistance through multiple mechanisms, including c-Jun N-terminal kinase (JNK) activation and expression of TNFa, MCP-1 and IL-6. Given that crosstalk between FFA and IGF-I signaling pathways has been demonstrated in 3T3-L1 cells, we hypothesized that IGF-I inhibits FFA-induced inflammation in human preadipocytes.
Methods: Preadipocytes isolated from abdominal subcutaneous and omental adipose tissue from obese subjects were treated with FFA (5mM of oleic, linoleic, arachadonic, lauric and myristic acids in 0.5%BSA) after pretreatment with 10nM IGF-I/1% BSA or 1% BSA alone for 60 minutes. RNA was isolated following 4 hr FFA treatment and analyzed by qRT-PCR. Total cell lysates were generated after 30 min FFA treatment followed by Western blot analysis.
Results: We observed greater JNK activation by FFA in omental preadipocytes, but we found attenuation of FFA-induced JNK activation by IGF-I in subcutaneous, not omental cells. As expected, FFA increased TNFa, MCP-1 and IL-6 expression in both subcutaneous and omental preadipocytes, but IGF-I pretreatment attenuated FFA-induced TNFa only in subcutaneous cells. There was a trend toward a protective effect of IGF-I in FFA-induced MCP-1 expression, and no effect at all on IL-6 expression. Our prior studies have found that IGF-I activation of AKT is decreased in omental cells, and pretreatment with a specific AKT1/2 kinase inhibitor (Sigma) blocked the IGF-I protective effect in FFA-treated subcutaneous cells.
Conclusions: These results suggest that IGF-I attenuates FFA-induced JNK and TNFa in human subcutaneous but not omental preadipocytes through crosstalk between the AKT and JNK pathways. These observations broaden the role of IGF-I in adipose biology and support emerging evidence that IGF-I regulates inflammation.

Nothing to Disclose: CMB, KC-D, TT, JLK

P2-138

Myofibers in 48 Hours: A Rapid Differentiation System for Normal Human Skeletal Myoblasts.

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We have developed a unique human skeletal myoblast system in which cryopreserved cells can be seeded directly into multiwell plate formats and differentiated into multinucleated myofibers with >70% of cells forming multinucleated scincitia in 48-72 hours. Skeletal muscle plays an essential role in maintaining physiological and metabolic health in humans. The loss of muscle mass which accompanies aging (sarcopenia), many types of chemotherapy (cachexia), injuries (burns) and genetic diseases (myopathies) is directly linked to poor patient outcomes. The mechanisms underlying these processes are under active investigation. Additionally, skeletal muscle plays a critical role in glucose metabolism. By mass, skeletal muscle represents the major tissue for insulin dependent glucose uptake, thus placing it centrally in normal glucose metabolism and in disease conditions such as diabetes. Tissue culture models of normal human skeletal muscle provide powerful experimental systems for the dissection of muscle biology and recent work from several laboratories has demonstrated cultured human myoblasts represent a much more physiologically relevant model than the rodent muscle cell lines commonly used. We have verified the expression of the insulin dependent glucose transporter, GLUT4, in these cells by immunoblotting and show insulin-stimulated uptake of ^{3}H-2-deoxy glucose. We have also demonstrated highly efficiency delivery and expression of exogenous gene products in these cells by utilizing BacMam technology (recombinant baculovirus encoding exogenous proteins of interest whose expression driven by a mammalian promoter). Furthermore we show that combining BacMam delivery with LanthaScreen® TR-FRET (time resolved fluorescent energy transfer) cellular assays enable robust and flexible interrogation of signaling pathways, including PI3K-Akt and TGFb-Smad, in both myoblasts and differentiated normal human skeletal muscle cells. For example, IGF-dependent activation of the PI3K-Akt pathway was interrogated by TR-FRET measuring phosphorylation of a GFP PRAS40 fusion protein that included an Akt consensus site (Thr246) following incubation with a terbium-labeled anti-phospho Thr246 antibody. Taken together our work demonstrates the feasibility of pairing easy to use primary human skeletal myoblasts with a highly efficient and sensitive assay platform to create sophisticated, physiologically relevant cell models.

Disclosures: DTK: Researcher, Life Technologies. PEK: Researcher, Life Technologies. CC: Researcher, Life Technologies. KB: Researcher, Life Technologies. GDS: Principal Investigator, Life Technologies.

P2-139

Osteoblasts Are the Primary *In Vivo* Targets of Inhibin A Skeletal Anabolism.

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Hormones in the hypothalamic-pituitary-gonadal axis are involved in regulating skeletal metabolism, including the non-steroidal hormone, Inhibin A (InhA). We have previously demonstrated that the gonadal peptide InhA is anabolic for the skeleton; systemic overexpression of InhA (serum levels 600-800 pg/ml) for 4wks in transgenic mice increases bone mass and strength (1). The InhA anabolism is defined by increases in trabecular architecture and cortical geometry in the tibia, spine and humerus. We hypothesized that InhA would be anabolic in normal wild-type mice and that the cellular basis of InhA anabolism would also involve increases in osteoblast number and/or activity. Therefore, male Swiss-Webster mice at peak adult bone mass (~3 months of age) were treated with physiological concentrations of recombinant human InhA continuously for 17d via osmotic minipump. Skeletal microCT analysis revealed that normal physiological levels of InhA (30-80pg/ml) significantly increased trabecular bone volume and architecture, as well as increased ortical thickness, cortical cross sectional area and total cortical diameter in these mice. Static and dynamic histomorphometric analysis was also performed on proximal tibial sections. InhA treatment stimulated significant increases in trabecular bone volume (BV/TV) in the secondary spongiosa. The most rapid and detectable cellular effect mediating this increased BV/TV is the stimulation of existing osteoblast activity, as indicated by increased osteoid thickness, mineral apposition rate and bone formation rates. These changes lead to the increased bone volume observed by microCT. In contrast, no demonstrable histomorphometric changes were observed in any of the measured osteoclast parameters *in vivo*. Surprisingly, while *ex vivo* marrow cultures from *in vivo* InhA treated mice demonstrated the expected significant increases in osteoblast recruitment and differentiation, the same treatment also significantly suppressed osteoclast differentiation *ex vivo*. Collectively these data demonstrate that physiologic concentrations of InhA are anabolic for the skeleton and reveal that although there are *ex vivo* InhA effects on both osteoblasts and osteoclasts, the primary *in vivo* effects driving bone anabolism are mediated by osteoblasts.

(1) Perrien et al., Endo 148:1654-65, 2007. [Epub 2006 Dec 28]

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Nothing to Disclose: KMN, DSP, NSA, TWF, LJS, DG

P2-140

Soluble Repulsive Guidance Molecule C/Hemojuvelin Inhibits BMP2- and BMP6-Mediated Signaling and Actions.

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Inactivating mutations in Repulsive Guidance Molecule c/Hemojuvelin (RGMc/HJV) cause juvenile hemochromatosis, an aggravated iron overload disorder in which expression of hepcidin, a key liver-derived iron-regulatory hormone, is severely diminished. Several growth factors within the bone morphogenetic protein (BMP) family, including BMP2 and BMP6, can stimulate production of hepcidin, and their actions may be modified by RGMc, potentially by interactions with BMPs. In the current study, we have investigated the ability of individual RGMc protein isoforms to broadly regulate BMP2- and BMP6-mediated signaling and gene expression. RGMc is produced by the liver and by striated muscle during development, and soluble single-chain RGMc species of 50 and 40 kDa (RGMc50 and RGMc40, respectively) have been detected in human and mouse serum. Treatment of the Hep3B liver cell line with either BMP2 or BMP6 and recombinant soluble RGMc50 or RGMc40 (as IgG Fc fusion proteins) but not IgG Fc alone led to a reduction in growth factor-stimulated Smad phosphorylation and to a decline in induction of BMP-responsive genes, including hepcidin and Smad7. Whole transcript microarray analysis revealed that BMP2 and BMP6 stimulated expression of a nearly identical cohort of ~40 mRNAs in Hep3B cells and further demonstrated that RGMc40 acted as a broad inhibitor of these growth factors, although its potency was less than that of the known BMP2-selective binding protein, Noggin. In addition, RGMc40 enhanced the accumulation of a small number of transcripts that were not induced by BMPs. We further find that selected disease-associated RGMc mutant proteins that retain their ability to bind BMPs also inhibited the actions of BMP2 and BMP6, but were less effective than their wild-type counterparts. Taken together, our observations show that soluble RGMc species can function as broad-spectrum BMP antagonists, and may regulate systemic iron metabolism by modulating the ability of BMPs to activate its receptors and stimulate hepcidin gene expression.

Sources of Research Support: NHLBI Grant F31HL095271 awarded to MN.

Nothing to Disclose: MN, PSR

P2-141

Two Distinct Regions of Latency Associated Peptide Coordinate Stability of the Latent TGF-β1 Complex.

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Transforming growth factor-β1 (TGF-β1) is a key regulator of cellular proliferation, differentiation, apoptosis, adhesion and extracellular matrix deposition, with well documented roles in organogenesis, wound healing, fibrosis and tumour progression. TGF-β1 is secreted as part of an inactive complex consisting of the mature dimer, the TGF-β1 propeptide (latency associated peptide; LAP) and latent TGF-β binding proteins (LTBPs). Using *in vitro* mutagenesis and functional analysis, we identified the regions of LAP that govern the cooperative assembly and stability of the latent TGF-β1 complex. Initially, hydrophobic LAP residues (Ile^{53}, Leu^{54}, Leu^{57} and Leu^{59}), which form a contiguous epitope on one surface of an amphipathic α-helix, interact with mature TGF-β1 to from the small latent complex. TGF-β1 binding is predicted to alter LAP conformation exposing ionic residues (Arg^{45}, Arg^{50}, Lys^{56} and Arg^{58}) on the opposite surface of the α-helix, which form the binding site for LTBPs. The stability of the resultant large latent complex is dependent upon covalent dimerisation of LAP, which is facilitated by key residues (Phe^{198}, Asp^{199}, Val^{200}, Leu^{208}, Phe^{217} and Leu^{219}) at the dimer interface. Significantly, genetic mutations in LAP (e.g. R218H) that cause the rare bone disorder, Camurati-Engelmann disease, disrupted dimerisation and reduced the stability of the latent TGF-β1 complex. Finally, our results indicate that the functionally significant regions of LAP are broadly conserved in the propeptides of other TGF-β superfamily members, and that variations within these regions determine the specificity and affinity of propeptides for their respective growth factors.

Sources of Research Support: NHMRC, Australia.

Nothing to Disclose: KLW, YM, JC, MCW, KLC, DMR, CAH

P2-142

Effect of Follistatin-Like 3 (FSTL3) Deletion on Testicular Development and Function.

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One in six couples is infertile and approximately half of the incidence is due to male infertility. In mammals microorchidism is linked to infertility, hence, identifying pathways to increase testicular size and germ cell proliferation is crucial. Activin and other TGFβ ligands are key regulators of testicular development and function. Follistatin-like 3 (FSTL3), a soluble glycoprotein that inhibits activin and related TGFβ factors, is strongly expressed in the testis. We found that FSTL3 gene deletion (FSTL3 KO) mice have larger testes that do not undergo age-related size regression. To address the cause of increased testicular size we undertook histomorphometric analyses. While seminiferous tubule structure appears normal Sertoli cell and germ cell numbers are significantly increased in FSTL3 KO mice compared to WT. Leydig cell numbers, however, are not altered. Flow cytometric analyses of propidium iodide stained testicular cell dispersates also demonstrate increased spermatogenesis in FSTL3 KO. To begin to elucidate the signaling mechanisms involved in increasing spermatogenesis in FSTL3 KO we first investigated whether SMAD factors are differentially activated in FSTL3 KO. Preliminary immunohistochemical analyses show that the SMAD2/3 pathway is activated more than the SMAD1/5/8 pathway in FSTL3 KO suggesting extended action of activin, a ligand inhibited by FSTL3. Importantly, as quantified by microdensitometry, the amounts of phosphorylated SMAD2/3 are most increased in late spermatogenic cells in FSTL3 KO testis suggesting a role for this pathway in the increased spermatogenesis in FSTL3 KO. Experiments to address the importance of SMAD2/3 signaling in germ-cell proliferation/survival are currently underway. To identify proteins and signaling pathways that are essential in preventing age-related testicular regression, quantitative mass spectrometric analyses were performed. Tryptic digests of WT and FSTL3 KO testis proteins were mixed after chemically modifying FSTL3 KO digests and analysed by LC-MS/MS. Of 45572 peptides analysed, 1285 proteins were differentially expressed between WT and FSTL3 KO testes. We are currently investigating the roles played by these proteins in maintaining testicular size. Taken together, these studies are a first step towards identifying proteins and signaling pathways that can contribute towards the stimulation of testicular size and function and thus might provide therapeutic targets to alleviate male infertility.

Nothing to Disclose: AM, JS, AP, JV, TG, PJO, SPG, ALS

P2-143

Modulation of Steroidogenic Enzyme Gene Expression and Androgen Production by Activins and BMPs in Ovarian Theca Cells.

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TGFB superfamily members are well established as playing vital roles in folliculogenesis. In this study, the effects of inhibin, activin and BMPs on ovarian theca cell function were investigated. Ligand receptors were mapped throughout folliculogenesis, and phosphorylated Smads 1/5 and 2/3 were detected in theca and granulosa cells showing active signaling pathways were present. Theca cells from sheep follicles (<3.5 mm diameter) were cultured under serum-free conditions[1] and androstenedione secretion measured at 48h to 144h after addition of ligands. Activins A/B, and BMPs 4/6 (1–100 ng/ml) have been previously shown to suppress androgen production[2]. Inhibin A alone caused an increase in androgen secretion, and this effect became more enhanced throughout the culture period. Since inhibin acts as an activin antagonist, rather than signaling in its own right, the inhibin-induced increase in androgen secretion suggests that primary ovine theca cells produce activin that appears to act in an autocrine manner to modulate androgen production. We have now shown that activin decreases the expression of steroidogenic enzymes, StAR, CYP17 and 3BHSD. In theca cultures the activin receptor antagonist SB-431542, and the intracellular Smad inhibitors, Smad6/7 increased androgen production by blocking BMP/activin signaling. Unexpectedly, Dorsomorphin, a BMP receptor antagonist[3], also inhibited androgen secretion and was not able to prevent BMP suppression, suggesting that BMPs may act through an alternative receptor signaling pathway. In summary, we have demonstrated that activins, inhibins and BMPs are involved in modulating the expression of steroidogenic enzymes and thecal androgen production.

1. Campbell et al., J Reprod Fertil. 1998, 112(1):69-77.
2. Young and McNeilly, SSR, 2009.
3. Yu et al., Nat Chem Biol. 2008, 4(1):33-41.

Sources of Research Support: Medical Research Council.

Nothing to Disclose: JMY, ASM

P2-144

Growth Differentiation Factor 9 Suppresses Follistatin and Follistatin-Like 3 Production in Human Granulosa-Lutein Cells.

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Background: We have demonstrated that growth differentiation factor 9 (GDF9) enhances activin A-induced inhibin β_B -subunit mRNA levels in human granulosa-lutein (hGL) cells by regulating receptors and key intracellular components of the activin signaling pathway (1, 2). However, we could not exclude its effects on follistatin (FST) and follistatin-like 3 (FSTL3), well recognized extracellular inhibitors of activin A, and undertook this study to further evaluate GDF9 in this regard.
Methods: hGL cells from women undergoing *in vitro* fertilization (IVF) treatment were cultured with and without siRNA transfection of FST, FSTL3 or GDF9 before treatment with GDF9, activin A, FST, FSTL3 or combinations. FST, FSTL3 and inhibin β_B-subunit mRNA, and FST and FSTL3 protein levels were assessed with real-time RT-PCR and ELISA, respectively. Data were analyzed by ANOVA followed by Tukey's test.
Results: GDF9 suppressed basal FST and FSTL3 mRNA and protein levels in a time- and dose-dependent manner and inhibited activin A-induced FST and FSTL3 mRNA and protein expression, effects attenuated by bone morphogenetic protein type 2 receptor extracellular domain (BMPR2 ECD), a GDF9 antagonist. After GDF9 siRNA transfection, basal and activin A-induced FST and FSTL3 mRNA and protein levels increased, but changes were reversed by adding GDF9. Reduced endogenous FST or FSTL3 expression, following corresponding siRNA transfection, augmented activin A-induced inhibin β_B-subunit mRNA levels by 12- and 5-fold respectively (P values all < 0.05). Furthermore, the enhancing effects of GDF9 in activin A-induced inhibin β_B-subunit mRNA were attenuated by adding FST.
Conclusion: GDF9 decreases basal and activin A-induced FST and FSTL3 protein levels, and this explains, in part, its enhancing effects on activin A-induced inhibin β_B-subunit mRNA expression in hGL cells.

(1) Shi FT et al., Endocrinology 2009; 150:3540
(2) Shi FT et al., The Journal of clinical endocrinology and metabolism 2009; 94:5108

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Nothing to Disclose: FTS, APC, HFH, PCKL

P2-145

Inhibitor of TGF-beta Receptor Reduces Spheroids of Leiomyomal Cells in Long Term Culture.

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Leiomyoma, a benign tumor derived from myometrium, is the most common gynecological problem to cause hysterectomy. Previous studies have shown that leiomyoma expresses higher levels of TGF-beta3 and extracellular matrix compared to myometrium. Most studies on leiomyomal cells used short-term culture. However, it was reported that leiomyoma cells formed ball-like cell aggregates after long-term culture in dish. In this study, we have investigated the role of TGF-beta in leiomyomal growth in long-term culture. Primary leiomyomal cells were isolated from leiomyoma tissue obtained from patients who underwent hysterectomy. Cells grew in 2% FCS-DMEM gradually formed different types of spheroids, which were classified into 5 types according to cell density and spheroid morphology by the phase-contrast photographs and Hoechst 33342 staining. Type 3-5 spheroids exhibited dark and compact mass. Time-course study showed that they were more advanced types of spheroid. The quantitative results showed that the growth rate of spheroids varied in leiomyomal cells from different tumors. The treatment with TGF-beta 3 (10 ng/ml) for 2-4 weeks promoted the formation of type 3-5 spheroid. To further investigate the role of endogenous TGF-beta, cells were cultured in the presence or absence of SB431542 (1 or 10 uM), an inhibitor of TGF-beta receptor kinase for 2 to 4 weeks. The analysis of 6 leiomyomal primary culture cells showed that SB431542 reduced the growth of type 3-5 spheroids, but not that of type 1-2. These results suggest that long-term culture system may provide a model to study the regulation of TGF-beta in leiomyoma growth.

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Nothing to Disclose: WCL, HMC, SYL, YMC, LYCW

P2-146

Dissecting the Role of the TGF-b Pathway in Genetically Mediated Vascular Disorders.

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The vascular form of Ehlers-Danlos Syndrome (VEDS), also known as the “acrogeric” EDS, is a rare hereditary connective tissue disorder caused by mutations in the *COL3A1* gene. Reduced or abnormal type III collagen leads to a weakened extracellular matrix (ECM). Tissue and arterial fragility are the core clinical features, causing early mortality from arterial or hollow organ rupture. Recently, involvement of the TGF-b signaling pathway has been implicated in Marfan Syndrome (MFS), and affected persons have been shown to have elevated circulating TGF-b levels in plasma. Since a similar pattern of elevated circulating TGF-b levels was seen in our cohort of 41 VEDS patients (p=0.0005), we hypothesized that derangement of the TGF-b pathway may play a role in the pathogenesis of VEDS. However, unlike MFS, western blot and PCR array analyses of endogenous levels of TGF-b markers (pERK1/2: p=0.323, phospho-p38 MAPK: p=0.212, TGF-b receptor II: p=0.406) or levels of stimulated markers (pSmad2: p=0.976, pSmad1/5/8: p=0.507) from 10 VEDS fibroblast cell lines as compared to matched controls did not indicate that the canonical TGF-b signaling pathway was involved. There was no significant correlation of the markers with plasma TGF-b (pERK1/2: r=0.097, phospho-p38 MAPK: r=-0.181, TGF-b receptor II: r=-0.055, pSmad2: r=-0.331, pSmad1/5/8: r=0.436), suggesting that elevated levels may be a response to ongoing damage to the arterial endothelium rather than a cellular consequence of the genetic defect. A clinically significant implication is that the inhibition of the TGF-b pathway with the angiotensin receptor blocker losartan, which was shown to be of benefit in MFS, may potentially exacerbate the disease process in VEDS. Studies are underway to explore the potential role of non-canonical TGF-b signaling and other candidate pathways in VEDS, as well as formulation and investigation of alternative hypotheses to explain the observed elevation of circulating TGF-b. While the goal is to develop an effective treatment for the disorder, a better understanding of VEDS at the cellular level has the potential to shed light on impaired ECM integrity and composition seen in aging.

Sources of Research Support: Intramural Research Program of the NIH, National Institute on Aging.

Nothing to Disclose: RM, FS, BG, JF, LS, OC, JVE, NM

P2-147

In Vitro and *In Vivo* Bioactivities of Glycosylated Isoforms of Inhibin A and Inhibin B.

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Inhibin A and inhibin B, important regulators of normal function in tissues of the reproductive axis, are either mono-(Asn^{268}) or di-glycosylated (Asn^{268} and Asn^{302}) on the common α-subunit to produce 31- and 34-kDa isoforms, respectively. In this study, we examined the ability of the 31- and 34-kDa inhibin A and B isoforms to suppress FSH release from pituitary gonadotrope cells, both *in vivo* and *in vitro*.
Glycosylated forms of recombinant human inhibin A and B were purified from conditioned medium using immunoaffinity chromatography and RP-HPLC. The masses of highly purified 31- and 34-kDa inhibin A and B were determined using amino acid analysis; these were utilised as reference preparations in the immuno- and bioassays. The immunoactivities of the purified inhibin preparations were determined by specific inhibin ELISAs and their *in vitro* and *in vivo* bioactivities were measured based on suppression of FSH release by rat pituitary cells in culture and suppression of plasma FSH in ovariectomised female rats, respectively. The binding affinities of the glycosylated inhibins were determined in 293T cells transiently transfected with betaglycan in the presence or absence of ActRIIA/B using either [^{125}I]-inhibin A or B as the tracer.
The 31-kDa isoforms of inhibin A and inhibin B were ~2.5-fold more potent than the corresponding 34-kDa isoforms in suppressing FSH release by rat pituitary cells in culture. The lower biological activities of 34-kDa inhibin A and inhibin B corresponded with a parallel reduction in affinity for betaglycan ± ActRIIA/B. Surprisingly, 31-kDa inhibin B was more bioactive than inhibin A, both *in vitro* (4.2-fold) and *in vivo* (1.45-fold). The greater potency of 31-kDa inhibin B could not be explained by affinity to known receptors as it bound more weakly to betaglycan, betaglycan+ActRIIA and betaglycan+ActRIIB (IC_{50} 2200, 400, and 750 pm, respectively) than did 31-kDa inhibin A (IC_{50} 190, 80, and 290 pm, respectively).

It is concluded that for the 34-kDa isoforms of inhibin A and B, the additional carbohydrate group at Asn^{302} of the α-subunit hinders the formation of the inhibin/betaglycan/ActRII complex, thereby reducing *in vitro* bioactivity. Conversely, the increased *in vitro* and *in vivo* bioactivities of monoglycosylated inhibin B compared to inhibin A cannot be explained by affinities to betaglycan or activin type II receptors. Therefore, inhibin B's potent action may be mediated by additional proteins.

Sources of Research Support: NHMRC, Australia.

Nothing to Disclose: YM, PDT-S, KLW, CAH, DMR

P2-148

A Drastic Shift from Positive to Negative Effects of Estrogen on BMP Signaling in Pulmonary Arterial Endothelial Cells in Response to Hypoxia.

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<BACKGROUND> Epidemiologic studies have revealed the female predominance in the morbidity of Idiopathic PAH (pulmonary arterial hypertension) in the world, suggesting a role of female sex hormones in the pathogenesis of PAH. Recent studies have identified a major role for the bone morphogenetic protein (BMP) signaling in the pathogenesis of PAH and we previously reported that the BMP signaling was reduced in pulmonary arterial endothelial cells (PAEC) *in vitro* and *in vivo*.
<PURPOSE> This study is to further investigate effects and mechanisms of β-estradiol (E2) on the BMP signaling in PAEC.
<MATERIALS and METHODS> Human and rat PAEC were cultured and the expression of BMP signaling including BMP receptor, Smad1/5/8, and Id1 was examined under 21%O_2 (normoxic) or 1%O_2 (hypoxic) condition, and regulation of HIF (hypoxia-inducible factor) -1α expression in the presence of E2 by western blotting and quantitative RT-PCR. We investigated the effect of estrogen receptor antagonist (ICI 182.780.) and cycloheximide, localization of Smad1/5/8 was examined by immunofluorescence staining and the binding of estrogen receptor (ER) and Smad or HIF-1α was examined by co-immunoprecipitation assay.

<RESULTS> In the presence of E2 (10^{-7}M), BMP signaling was augmented under 21%O_2 but suppressed under 1%O_2. In addition, these alterations of BMP signaling were not observed by ICI 182.780. These alterations were associated with the nuclear translocation of phosphorylated (p-) Smad1/5/8. Co-Immunoprecipitation experiment demonstrated a direct binding between ER, HIF-1α, and p-Smad1/5/8 proteins in a nucleus only under 1%O_2. In addition, HIF-1α accumulation by cobalt chloride under 21%O_2 led to suppression of BMP signaling, whereas HIF-1α inhibitor, YC-1, augmented the signal under1%O_2. By inhibiting de novo synthesis by administration of cycloheximide, p-Smad1/5/8 was suppressed under 21%O_2, but could not change under 1%O_2.
<DISCUSSION> We demonstrated that hypoxic stimulation and elevated level of HIF-1α lead to suppression of the BMP signaling in PAEC in the presence of E2. The direct interaction of p-Smad / HIF-1α / ER may contribute to the suppression of the signal. Our observations provide the new mechanism how sex hormone affects on BMP signaling, a key signal pathway for PAH, and novel therapeutic targets in the treatment of PAH.

Nothing to Disclose: HI, SK, HI, JN, KO

P2-149

Rapid Turnover of the Activin Type II Receptor ACVR2 Underlies Cycloheximide-Dependent Inhibition of FSH Production in Gonadotropes.

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Inhibins suppress follicle-stimulating hormone (FSH) synthesis and secretion by competitively antagonizing activin-stimulated FSHβ subunit (Fshb) transcription. The translational inhibitor cycloheximide (CHX) produces inhibin-like effects in rat primary pituitary cells, suppressing FSH without affecting luteinizing hormone (LH) secretion. We observed that CHX, like the activin bioneutralizing protein follistatin-288 or the activin type I receptor inhibitor, SB431542, reduced Fshb mRNA levels in murine primary pituitary cultures. These data therefore suggested that CHX might produce its effects by antagonizing endogenous activin signaling. Using the immortalized murine gonadotrope LβT2 cell line, we identified a potential mechanism underlying this antagonism. In these cells, activins stimulate Fshb subunit transcription via intracellular signaling proteins SMAD2, 3, and 4. CHX pre-treatment for 3-6 h inhibited both activin A-stimulated Fshb mRNA expression and C-terminal SMAD2/3 phosphorylation. This suggested an upstream effect of CHX, likely at the receptor level. Indeed, affinity labelling/cross-linking with ^{125}I-activin A demonstrated CHX-dependent suppression of ligand binding to both type I and II receptors within 3 h. Because activin binding to type I receptors depends on high affinity binding to type II receptors, we hypothesized that type II receptors were labile in these cells. Activins can bind to both ACVR2 and ACVR2B type II receptors, but RNA interference experiments in LβT2 cells suggested that ACVR2 was the preferred receptor. Attempts to measure endogenous ACVR2 expression by western blot were unsuccessful, likely due to its low abundance. We could detect transfected ACVR2, however, and observed an almost complete abrogation of its expression after 8 h CHX treatment. Inhibitor experiments indicated that degradation of the endogenous receptor was mediated via the proteasomal, rather than lysosomal pathway. Collectively, the data indicate that ACVR2 is rapidly degraded via the proteasome in gonadotrope cells. Blockade of de novo receptor synthesis with CHX thereby inhibits activin-stimulated Fshb transcription, leading to a decline in FSH levels. A review of the literature indicates that ACVR2 is also a low abundance protein in other cell types. Therefore, the results here may provide a more general understanding of post-translational processing of the receptor.

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Nothing to Disclose: CAR, YW, CCH, XZ, TEH, DJB

P2-150

Demonstrating the Expression and Origin of Mitochondrial-Derived Peptides.

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Mitochondria contain hundreds of proteins, most of which are encoded by the nuclear genome. The mitochondrial chromosome was traditionally believed to encode only 13 proteins that function primarily as components of the mitochondrial electron transport chain. However, we recently discovered that in addition to humanin (HN, a 24-aa peptide encoded from the 16S rRNA gene of the mitochondrial DNA) there are six ORFs within the 16S rRNA, challenging traditional beliefs about the mitochondrial genome. We synthesized the corresponding peptides, named SHLPs (small humanin-like peptides), and initial characterization of their biological activity indicates that they are potent bioactive molecules. SHLPs 1-5 act to induce cell survival like HN, whereas SHLP-6 has opposing actions, inhibiting tumor growth and angiogenesis in vivo. We generated antibodies against SHLPs, and revealed that they are a novel family of circulating hormones, and also have a highly-specific tissue expression pattern. SHLPs 2 and 3 are expressed in metabolically important tissues such as liver, pancreas and brain where they modulate insulin sensitivity; whilst SHLP6 is expressed in the breast, prostate and testis.
Nuclear mitochondrial DNA, NUMT, describes the transfer of mtDNA sequences to the nuclear genome; as such many chromosomes have copies of the 16s rRNA and SHLP ORFs. To determine whether endogenous SHLPs are of mitochondrial origin, we generated ρ° cells (which have mitochondria, but no mtDNA) by ethidium bromide treatment and demonstrated loss of expression of SHLPs in the absence of mitochondrial DNA, suggesting mitochondrial not nuclear transcription of the peptides. To confirm this, we purified mRNA from 22RV1 cells, PCR amplified the SHLP ORFs, and performed sequence analysis. The resulting sequences could only have come from the mitochondrial ORF due to sequence differences, confirming mitochondrial origin of SHLPs. To verify these findings, we repeated the PCR experiments on mRNA isolated from ρ° cells, and were unable to amplify SHLP mRNA, confirming mitochondrial origin. To determine whether the mRNA is translated in the mitochondrion or cytoplasm, we incubated cells with cyclohexamide to block cytoplasmic translation, and demonstrated that the peptides are translated in the cytoplasm. This unexpected finding suggests that there is an as yet undefined mRNA export system allowing peptides to be transcribed in the mitochondria and translated in the cytoplasm.

Nothing to Disclose: LJC, HN, HH, CA, PC

P2-151

Molecular Evidence for the Mitochondrial Origin of Humanin.

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Humanin (HN) is a recently discovered 24-amino-acid peptide with potent neuroprotective, anti-apoptotic, and insulin-sensitizing properties. Humanin was originally cloned from a cDNA library from the surviving region of a patient with Alzheimer's Disease. The cloned cDNA containing the open reading frame (ORF) of HN was identical to the mitochondrial 16S ribosomal RNA, suggesting that HN is a peptide derived from the mitochondrial genome. However, reports of several putative nuclear homologs of HN resulted in a controversy regarding the true origins of humanin. The object of this study was to confirm the sub-cellular site of translation of HN. Using highly purified mRNA from 22Rv1 prostate carcinoma cells, we carried out PCR amplification of the humanin gene with specific primers designed to amplify both mitochondrial and nuclear HN ORFs. Sequencing the PCR products revealed a sequence that was only compatible with the 16s rRNA HN locus, demonstrating HN is encoded from the mtDNA. To confirm these data, we also generated 22Rv1 rho-zero cells by exposing the cells to a long-term treatment with low dose ethidium bromide (EtBr) to selectively deplete mtDNA. Rho zero cells contain mitochondrial, but not mitochondrial DNA. We repeated the humanin PCR with cDNA obtained from EtBr-exposed cells lines, and saw no amplification of HN. In addition, we were unable to detect HN peptide expression in the rho zero cells by immunoblotting. Future studies will now focus on determining the site of translation of humanin mRNA using cyclohexamide to specifically block cytoplasmic protein translation. These studies therefore provide the first real evidence that HN is transcribed from mitochondrial DNA and not the nuclear chromosomes. This is a major finding, as it confirms that the mitochondria are capable of producing specific peptides which have potent metabolic effects.

Nothing to Disclose: HH, HN, HHM, PC, LJC

P2-152

Phosphate Activated Glutaminase (GLS2), a Novel p53-Inducible Regulator of Glutamine Metabolism and Reactive Oxygen Species.

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Tumor suppressor p53, which is mutated in over 50% of human cancers, coordinates multiple cellular functions including cell-cycle arrest, apoptosis, or senescence to preserve genomic stability. In addition to these functions, recent evidence has indicated the involvement of p53 in regulation of cell metabolism such as energy production, autophagy and reactive oxygen species (ROS) levels. In cancer cells, glucose uptake is much higher than in most normal tissues, and glycolysis persists even under aerobic conditions, a process known as the Warburg effect. P53 plays several roles in regulating this process. For exmple, p53 can inhibit the expression of the glucose transporters GLUT1 and GLUT4, while increasing the expression of TIGAR to slow glycolysis. P53 can also activate the expression of SCO2 to maintain mitochondria and drive oxidative phosphorylation. On the other hand, dysregulation of glutamine metabolism in mitochondria, notably glutaminolysis, has been also implicated in oncogenesis, but its role is not well understood. Here, we have identified phosphate activated glutaminase (GLS2) as a p53-inducible gene. GLS2 is a key enzyme to convert glutamine to glutamate, which further catabolized for energy supply or serves as precursor for glutathione synthesis. Real-time PCR and Chromatin immunoprecipitation analysis clarified that GLS2 was induced in response to DNA damage and/or oxidative stress in a p53-dependnent manner in both normal and stressed cells. Elevated GLS2 lowered intracellular ROS levels, resulting in overall decrease in DNA oxidation. Furthermore, downregulation of GLS2 compromised glutathione-dependent antioxidant system and increased intracellular ROS levels. High ROS levels following GLS2 knockdown coincided with stimulation of p53-induced cell death. Thus, p53 regulation of mitochondrial glutaminase links glutamine metabolism, energy and ROS homeostasis, providing a novel metabolic role for p53.

Nothing to Disclose: SS, TT, KS, KY, CP, IT

P2-153

Phosphorylation of ATF2 by GnRH Leads to Induction of c-Jun.

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The only transcription factors induced by GnRH known to directly regulate FSHβ gene expression are members of the AP-1 family. c-Jun is the prototypical isoform, which can bind DNA as a homodimer or a heterodimer with Fos or the ATF family of proteins. Thus, understanding the molecular mechanisms of c-Jun induction by GnRH will provide insight into GnRH regulation of FSHβ gene expression and will aid in elucidating the specificity of GnRH signaling pathways in the gonadotrope. The -1000/+200 sequence of the mouse c-Jun gene was linked to the luciferase reporter and determined to be sufficient for the expression in the gonadotrope cell and for induction by GnRH. With truncations of this region, we determined that GnRH induction of the c-Jun promoter maps between -100 and the start site of transcription. Internal deletions determined that the -90/-80 region from the start site of transcription contains an element critical for basal expression, while the region -70/-60 contains the element critical for GnRH induction. The -90/-80 region contains the CAATT element, and electrophoretic mobility shift assays (EMSA) determined that the NFY transcription factor binds this region. Point mutations determined that this site is critical for basal expression of c-Jun in the gonadotrope cells. The -70/-60 region, on the other hand, contains a motif that has only one base-pair difference from the canonical CRE site, which are bound by the CREB transcription factor, but also by an ATF subfamily of transcription factors that can dimerize with c-Jun. Mutation of this site abrogates GnRH induction of the c-Jun reporter. EMSA determined that five different complexes differ in their binding to DNA following incubation of the probe with nuclear extract from GnRH-treated gonadotrope cells compared to controls. Surprisingly, all five complexes for which binding is induced following GnRH treatment, require the CRE element for interaction with DNA, since mutation in the core sequences eliminate binding. This indicates that GnRH activates multiple proteins that can interact with the same sequence on the c-Jun promoter. Inclusion of the antibodies to identify the proteins by supershifts in EMSA, demonstrate that one of the complexes contains ATF2. Using antibodies specific for the phosphorylated form of ATF2, we determined that GnRH treatment leads to phosphorylation of ATF2 which then binds to the CRE site in the c-Jun proximal promoter.

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Nothing to Disclose: LLL, DC

P2-154

SV40 T-Antigen Transformation in Muscle, Pancreatic and Hypothalamic Cell Lines Results in Changes to Basal Cellular Gene Expression and the Phosphorylation Status of Key Signal Transduction Proteins.

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Mammalian cell lines have been used to understand biological processes in endocrine, muscle and reproductive physiology for decades. However, a potential issue of using cell lines is the prerequisite of neoplastic transformation of primary cultures to generate proliferative cells. This can lead to a transformed phenotype, which may not be wholly representative of the tissue of origin. Scientists have used T-antigen (T-Ag) of the Simian Virus 40 (SV40) to induce transformation. Previous studies suggest there are additional, undesired effects of T-Ag on cellular gene expression, but these are currently unclear. To evaluate the effect of cellular transformation, we 'knocked down' T-Ag mRNA using shRNA and assessed the expression of endogenous neuropeptides, receptors and activation of signaling proteins in smooth muscle (Movas-1), pancreatic (Min6) and hypothalamic (mHypoE-38 and GT1-7) cell lines. We characterized each cell line using RT-PCR and found that these cell lines retained several phenotypic characteristics of their original in vivo tissue counterparts. MTT studies demonstrated reduced T-Ag levels indeed impair cell viability and proliferation. Our results also revealed that some endogenously expressed genes were uniquely regulated by T-Ag. In the mHypoE-38, Min6 and Movas-1 cells, T-Ag knockdown resulted in a decrease in gonadotropin-releasing hormone (GnRH) transcript levels as demonstrated by real time RT-PCR. However, in the GT1-7 neurons, we found that the knockdown of T-Ag increased GnRH mRNA. Additionally, we found that T-Ag knockdown in the mHypoE-38 and GT1-7 neurons also resulted in a significant reduction in agouti-related peptide mRNA levels, however, no changes were observed in the two other cell lines. Conversely, T-Ag did not affect the expression of estrogen receptor-α or neuropeptide Y in these cell models. We also hypothesized that T-Ag may alter the basal activity of intracellular signal transduction pathways. Using western blot analysis, we found that T-Ag directly increased the phosphorylation status of several kinases and second messengers in the JAK/STAT, PI3K and AMPK signaling pathways in all cell lines. We conclude T-Ag can both enhance and repress transcriptional activity of endogenously expressed genes and alter the activity of signaling molecules. Understanding the effect of immortalization through transformation is crucial to ensure cell models accurately mimic the cellular characteristics of their in vivo counterparts.

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Nothing to Disclose: SSD, AHS, DDB

P2-155

Gonadotropin Regulated Testicular RNA Helicase (GRTH/DDX25), a Negative Regulator of LH/hCG Induced Steroidogenesis in Leydig Cells: A Central Role of StAR.

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GRTH/DDX25, is a testis-specific gonadotropin-regulated RNA helicase present in Leydig (LC)/germ cell that is essential for spermatid development and completion of spermatogenesis (1). The normal basal levels of Testosterone (T) in serum and Leydig cells (LC) observed in the GRTH$^{-/-}$ mice excluded lack of androgen as cause of the spermatogenic arrest. However, LCs of null mice have reduced lipid droplets and swollen mitochondria with a significant increased in T production over wild type (WT) by either *in vivo* or *in vitro* hCG stimulation (by 40-100%). Protein and mRNA level(s) of genes participating in early steps of steroidogenesis or linked to transport in the mitochondria were assessed to determine the impact by GRTH on LC steroidogenesis. Protein levels of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), sterol regulatory element-binding protein 2 (SREBP2), and steroidogenic acute regulatory protein (StAR), a transport protein of cholesterol to the inner mitochondria membrane - rate limiting for pregnenolone synthesis, were all markedly increased in the LC of GRTH$^{-/-}$ compared to WT. No differences from WT in cholesterol side chain cleavage or other steroidogenic enzymes were observed in the KO mice. hCG treatment of WT mice caused a significant increase in both protein and mRNA levels of StAR expression but no effect on HMGCR or SREBP2 in LC. In KO mice consistent with the magnification of T production over the WT mice induced by hCG *in vivo*, a robust increase in KO vs. WT in StAR mRNA levels and protein expression was observed, with no further increase on HMGCR and SREBP2. Immuno-precipitation analysis using a specific GRTH peptide antiserum revealed the association of StAR mRNA with GRTH protein, suggesting a negative role of GRTH in StAR message stability. The impact of GRTH as a negative regulator on the early step of steroidogenesis in LC appeared to be gene specific in response to gonadotropin stimulation. The presentation of swollen mitochondria without central cristae found in LC of GRTH$^{-/-}$ mice could reflect the exacerbation in LC of HMGCR, SREBP2 and StAR function in basal conditions, with the added magnification of StAR following the gonadotropin stimulus. The inhibitory action of GRTH associated with gonadotropin-mediated steroidogenesis provides insights into a new negative molecular control mechanism and on the role of this RNA helicase in the regulation of steroid production in the male.

1. Tsai-Morris CH et al., PNAS 2004; 101:6373

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Nothing to Disclose: MF, CHT-M, MLD

P2-156

Leptin-Induced Transcriptional Regulation of the Nescient Helix-Loop-Helix 2 (Nhlh2) Gene.

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The basic helix-loop-helix transcription factor Nescient helix-loop-helix 2 (Nhlh2) controls body weight and exercise energy expenditure through gene regulation in the hypothalamus. We have previously shown that food deprivation reduces Nhlh2 expression, while feeding or leptin injection causes increased in Nhlh2 expression. In an effort to identify promoter elements controlling Nhlh2 expression, we examined the proximal promoter region of human and mouse Nhlh2. The proximal promoter region for Nhlh2 (NHLH2 in humans) contains five conserved putative Stat3 binding motifs, two conserved NFκB sites and one conserved AP-1 site, each of which could play a role in modulating Nhlh2 expression during changes in energy availability. Transfection of the hypothalamic N29/2 cell line with an Nhlh2 promoter construct containing both NFκB sites, the AP-1 but only 3 of the Stat3 binding sites showed the expected 2.3-fold increase in expression compared to the longer construct. Moreover, leptin treatment of these cells for two hours after starvation increased expression of the shorter construct by 2.1-fold, as compared to the expression in starved cells. Transfection of N29/2 cell line with promoters containing site-directed mutations in either or both of the NFκB binding sites did not reduce the Nhlh2 expression. On the other hand, mutation of the NFκB1 binding site at -144 on the Nhlh2 promoter, caused increased expression of the promoter luciferase construct in the presence of leptin, suggesting this site may have a negative regulatory function. Of the three putative Stat3 sites on the Nhlh2 promoter, we found that mutation of the third site, at -91 on the Nhlh2 promoter, results in more than a 300-fold reduction in leptin-induced expression. Mutation of the fourth site, at -77 of Nhlh2 promoter, did not change the expression of the reporter construct. However, mutation of the fifth site, at -56 of the Nhlh2 promoter, increased the expression 5-fold when compared to the WT construct. These results indicate that the Stat3 transcription factor, or a related factor acting at the third putative Stat3 site on the Nhlh2 promoter, may serve as the positive transcription factor for basal Nhlh2 expression. These studies begin to define a signaling pathway, whereby normal and leptin-mediated expression of Nhlh2, through NFκB and Stat3 motifs in its proximal promoter, lead to downstream affects in Nhlh2 target genes and body weight regulation.

Nothing to Disclose: NA_R, DJG

P2-157

Administration of 3-Iodothyronamine (T1AM) Affects Gene Expression in the Rat Adipose Tissue Cells.

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3-Iodothyronamine (T1AM) is an endogenous derivative of thyroid hormone. Recent studies showed that administration of T1AM to rodents may reduce body fat mass and induce a switch in energy metabolism, favoring fatty acid oxidation over glucose oxidation. To investigate the molecular mechanisms that may explain the role of T1AM in the modulation of metabolism, we examined the effects of T1AM administration on gene expression in rat adipocytes.
Eight Wistar rats were treated with T1AM by intraperitoneal injection of 10 mg/Kg twice a day for five days. The rats were sacrificed by guillotine and the adipose tissue was immediately dissected from their inner thighs and nitrogen-frozen. Gene expression was investigated by two-colour microarray analysis, using the Whole Rat Genome G4131F microarrays (Agilent Technologies, Palo Alto, CA, USA).
T1AM administration did not produce any apparent behavioral effect nor any change in food intake. As compared to untreated rats, the treated rats showed 285 differentially expressed genes, 203 up-regulated and 82 down-regulated. Pathway analyses mapped 47 out of the 285 differentially expressed genes in 77 KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways, including 13 pathways from Metabolism, 7 pathways from Genetic Information Processing, 10 pathways from Environmental Information Processing, 25 pathways from Cellular Processes and 22 pathways from Human Diseases.
The leukocyte transendothelial migration and the cell adhesion molecules (CAMs) pathways scored the highest values of impact factor. Pathways involved in the phospholipid digestion and metabolism, including glycerolphospholipid metabolism and sphingolipid metabolism, were also retrieved. Finally, many differentially expressed genes were mapped in the adipocytokine signaling pathway, the insulin signaling pathway and the GnRH signaling pathway.
Our findings in adipose tissue cells indicate that T1AM affects the expression of many genes associated with known mechanisms of metabolic modulation and thus suggest that the phenotypical effects observed after T1AM administration may be due, at least in part, to a direct action at the gene expression level in the target cells.

Nothing to Disclose: SP, EM, VM, MDR, SF, GC, RZ

P2-158

Silencing Pax8 RNA Inhibits Sodium-Iodide Symporter Expression While Increasing hCG Expression in Placental BeWo Cells.

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Fetal thyroid hormones are important for fetal development especially that of brain. Fetal thyroid hormone synthesis is absolutely dependent on a supply of maternal iodide, which is transported by the sodium-iodide symporter (NIS) in trophoblasts. We have previously reported that placental NIS is up-regulated by hCG and down-regulated by intracellular iodide. In thyroid, the TSH modulated paired-box gene 8 (Pax8), a transcription factor, regulates expression of several essential thyroid genes including NIS during thyroid development. Human chorionic gonadotropin, which shares significant homology with TSH, regulates placental Pax8. Previous reports suggest however that PAX8 may not be involved in hCG regulation of placental NIS. To further understand the mechanism of NIS regulation in placenta, we transfected Pax8 small interfering RNA (siRNA) into BeWo cells and examined expression of NIS, beta hCG, and Pax8. In BeWo cells, transfection of Pax8 siRNA reduced Pax8 gene expression by 16%, 51% and 62% on day 1, 3 and 6 respectively. NIS mRNA was measured simultaneously and found to be reduced by 17%, 41% and 57% correspondingly. NIS protein was decreased by 48% after Pax8 siRNA transfection for 4 days. Interestingly, Pax8 gene silencing was associated with significant (more than 4 fold) increased beta hCG mRNA and protein. A similar inverse relationship between PAX8 and hCG mRNA expression and hCG protein was also seen over time in untransfected BeWo cells. Our findings strongly suggest that PAX8 is involved in placental NIS regulation. The paradoxical rise in HCG mRNA and protein after PAX8 silencing also suggests that hCG expression is under feedback inhibition through a PAX8 dependent process. The nature of this is at present unclear.

Nothing to Disclose: HL, RHM, KR

P2-159

Effect of Steroids Extract from Porcine Testis on Anabolism and T-Lymphocytes.

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The boar has a number of unique features concerning the synthesis of uncommon steroid hormones including 19-nortestosterone (nandrolone). Under the various physiological conditions, androgens including 19-nortestosterone are resulted in building up muscle, immune-suppression, and the survival of red blood cells. In this study, the steroids were extracted from the porcine testes and the effects of the extracts on bovine myogenic satellite cells (MSCs) proliferation and differentiation, and RBC and T-lmyphocytes proliferation were observed. MSCs proliferation and differentiation were induced by the extracts. Increase in the expression levels of myoD, desmin and myogenin also indicated the enhancement of MSCs differentiation by porcine testes extract. Similary, mouse RBC proliferation was increased by the extract. In contrast, the porcine testes extract inhibited mouse T-lymphocyte proliferation, indicating the possibility of the application of porcine testes extract as immune suppressant. Taken together, the anabolic steroids extract from the procine testes enhances the MSCs proliferation, differentiation and RBC proliferation, whereas decreases T-lymphocytes cell proliferation.

Nothing to Disclose: DML, PB, EJL, JEK, KHL, TC, YPC, KHL, IC

P2-160

Identification of the Glucose Responsive Element(s) That Mediates Transcription Regulation of Rat Pyruvate Carboxylase.

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Beta cell mitochondria play a key role in glucose-induced insulin secretion (GSIS) by not only providing energy in the form of ATP to support insulin secretion, but also by synthesizing metabolites (anaplerosis) that can act, both intra- and extramitochondrially, as factors that couple glucose sensing to insulin granule exocytosis (1). It appears that the anaplerotic enzyme pyruvate carboxylase (PC) participates directly or indirectly in several metabolic pathways which are important for glucose-induced insulin secretion. Suppression of PC expression by siRNA impairs GSIS in both insulinoma cells and in isolated rat islets, strongly suggesting the supportive role of PC in GSIS (2). The islet-specific promoter (P2-promoter) of the rat PC gene is regulated by interaction with transcription factors (Sp1/Sp3) and beta-cell specific transcription factor, Foxa2 (3)
To study whether PC expression is regulated by the level of exogenous glucose, INS 1 832/13 insulinoma cells were cultured in medium containing low (5.5 mM) or high (25 mM) glucose for 72 h. Quantitative real time PCR analysis showed that PC mRNA expression was increased 2-3-fold by 12 h, suggesting that PC can be induced by hyperglycemic condition. To examine whether this glucose responsiveness occurs at the transcriptional level, we transfected INS-1 832/13 cells with a 1.1 kbp P2-promoter-luciferase reporter construct and cultured them in medium containing low and high glucose. Luciferase reporter activity increased by 2-3-fold, suggesting the presence of a glucose responsive element (GRE) in the rat PC gene. To identify the GRE, we performed 5'-deletions and sited-directed mutagenesis of the 1.1 kb P2-promoter construct followed by transient transfection, and reporter gene assay. Deletion of nucleotides -496 to -421 or -408 to -403 resulted in the complete loss of the glucose-mediated increase in luciferase activity. Electrophoretic mobility shift demonstrated that USF1 and USF2 binding to –408/-403 mediated transcriptional regulation of the PC gene under hyperglycemic condition. We are currently characterizing the second GRE located at –496 and -421 region.

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Nothing to Disclose: AW, TB, JCW, SJ

P2-161

A Case of Severe IGF-1 Deficiency Secondary to a Novel Homozygous Deletion in the Growth Hormone Receptor (*GHR*) Gene.

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Severe IGF-1 deficiency (IGFD) is a rare cause of short stature, usually as a consequence of a localized deletion or substitution in the gene encoding for the GH receptor (GHR). The preponderance of reported mutations are in the GH-binding extracellular domain (ECD) of the GHR (encoded by exons 2 to 7), while mutations and deletions affecting the transmembrane (exon 8) and intracellular portions of the GHR (exons 9 and 10) are relatively rare. The ECD can be proteolytically cleaved to circulate as GH binding protein (GHBP). Lack of detectable GHBP is often indicative of a defect in the *GHR* gene.
Objective: To evaluate the cause of severe growth retardation in a patient who was severely IGFD.
Case Report: A 10 year old boy of South Asian descent presented with extreme short stature; height was 89.5 cm (-8.9 SDS), weight, 12.9 kg (-5.3 SDS), and bone age, 8 years. He was born at term in India, following an uncomplicated pregnancy, with a birth weight of 2.4 kg. His medical history was unremarkable, aside from poor growth and short stature beginning in the first year of life. Parents are non-consanguineous and of normal stature, as was his 3 year old sister. Testing for thyroid disease and screening for chronic illness was normal. Maximal GH level in response to Arginine was 80 µg/L and to Clonidine, 112 µg/L. Serum concentrations of the GH-dependent proteins IGF-I (17 ng/ml; normal, 123-275), IGFBP-3 (0.6 mg/l; normal, 2.0-4.6) and ALS (1.1 mg/l; normal, 5.6-16) were all markedly low. An IGF-1 generation test showed no increase in IGF-1 levels after 7 days of GH treatment. Serum GHBP was also remarkably low (<127 pMol/l; normal, 431-1892)
Results: The aberrantly low GHBP level concomitant with abnormally low IGF-I, IGFBP-3 and ALS levels strongly indicated a defective GHR. Molecular analysis of the *GHR* gene revealed a novel, homozygous, gross deletion encompassing exons 7 to 10. The deletion extended from intron 6 through the 3'UTR encoded by exon 10. Microarray analysis to detect additional deletions in neighboring genes was negative. Treatment of the patient with rhIGF-1 was commenced with good response (5.3 cm in first 5 months of treatment).
Conclusion: This is one of the largest deletions described to date in the GH receptor. The low serum GHBP concentrations suggest that the resulting protein is unstable and probably degraded. Further studies are needed to delineate the extent of the mutation.

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Nothing to Disclose: DK, MAD, VH, RGR, JH

P2-162

Characterization of IGF-II and IGF-II Precursors in Human Plasma.

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Insulin-like growth factor (IGF)-I and IGF-II exert their biological effects principally through the IGF-I receptor, although IGF-II also exhibits significant affinity for the A isoform of the insulin receptor (IR-A). IGF-II precursors that retain all (pro-IGF-II) or part of the C-terminal E-domain ("big" IGF-II) can be co-secreted with mature IGF-II, and increased levels have been reported in patients with non-islet cell tumor hypoglycemia. Additionally, circulating IGF-II levels have been reported to be associated with weight gain. One prospective study found that low baseline levels of IGF-II were associated with increase risk of weight gain later in life. However, conflicting results on the relationship between IGF-II levels and BMI have been reported in cross-sectional studies. We have previously demonstrated differences in signaling through both IR isoforms by mature, big, and pro-IGF-II, suggesting that the spectrum of IGF-II isoforms in the circulation could influence metabolic status. For identification of IGF-II precursors in human plasma, previous studies utilized column chromatography or immunoassays with antibodies directed against the first part of the E domain, which is present in both big and pro-IGF-II. As a result, it is not clear whether the precursors detected were big IGF-II, pro-IGF-II, or both. The purpose of this study was to determine which specific IGF-II precursors are present in human plasma and if there was any association between the levels of IGF-II isoforms and insulin sensitivity or percent body fat. We analyzed previously obtained, de-identified human plasma samples for mature, big, and pro-IGF-II by Western blot immunofluorescence analysis using mouse anti-rat IGF-II monoclonal primary antibody and fluorescent Cy5 anti-mouse secondary antibody. IGF-II levels were then compared to percent body fat assessed by underwater weighing and insulin sensitivity (Si) assessed by FSIVGTT. These studies demonstrated that mature, big, and pro-IGF-II are all routinely present in human plasma. The absolute and relative levels of each vary between patients, with significantly more variation evident in big and pro-IGF-II levels than in mature IGF-II levels. However, preliminary analysis suggested that none were strongly associated with percent body fat or Si.

Nothing to Disclose: AGM, JQP, CTR

P2-163

Effect of Pro-IGF-I E-Peptide on Scratch Wound Closure of CCD-1112SK Cells (A Human Foreskin Fibroblast Cell Line).

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E-peptide is the pro-peptide domain of pro-IGF-I. To date, multiple forms of pro-IGF-I have been identified in organisms from fish to mammals. Each of these isoforms contains an identical mature IGF-I but a different E-peptides. While four different isoforms of pro-IGF-I (i.e., pro-IGF-I Ea1, Ea2, Ea3 and Ea4) were identified in rainbow trout, three different isoforms of pro-IGF-I (i.e., pro-IGF-Ia, Ib and Ic) were identified in humans (1,2).
Previous studies conducted in our laboratory showed that the rainbow trout (rt) Ea4- or human (h) Eb-peptide of pro-IGF-I possessed activities against human cancer cells. These activities include: (i) inhibition of anchorage-independent growth of cancer cells, (ii) suppression of invasion and metastasis of cancer cells, (iii) inhibition of cancer cell-induced angiogenesis, and (iv) induction of cancer cell apoptosis (3-6). In this paper we report that rtEa4- or synthetic hEb-peptide (shEb) enhances the wound closure of CCD-1112SK cells (a human foreskin fibroblast cell line) in vitro. A dose dependent closure of the scratched wound of CCD-1112SK cells treated with rtEa4- or shEb-peptide was observed. Results of real-time RT-PCR analysis revealed that the mRNA levels of c-Jun in CCD-1112SK cells were induced by treatment with rtEa4-or shEb-peptide, but the levels of PTEN mRNA were repressed by treatment with rtEa4- or shEb-peptide.
To confirm that c-Jun transduction pathway is involved in rtEa4- or shEb-peptide induced scratch wound closure in CCD-1112SK cells, an in vitro scratch wound assay was conducted on monolayer cultures with the presence of rtEa4-peptide and various concentration of inhibitors such as PD098059, U016, SB203580 and SP600125. These inhibitors inhibit the closure of scratch wounds induced by rtEa4-or shEb-peptide. All together, results presented in this paper showed that rtEa4- or shEb-peptide exhibits an effect on wound closure via the c-Jun signal transduction pathway. Furthermore, it implies that rtEa4- or shEb-peptide may be developed into a therapeutic agent to enhance the closure of wounds.

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Nothing to Disclose: TTC, MJC, C-ML

P2-164
Impact of IGFBP-3 in Human Cardiovascular Diseases.

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Obesity is a health abnormality that increases the risk and incidence of developing atherosclerosis primarily in populations of developed countries including United States. Studies emphasize that obesity has a strong association with cardiovascular diseases (CVD). Visceral obesity is obesity due to increased deposition of fat in the abdominal viscera and is the initial stage for CVD. Chronic inflammation of the adipose tissue leads to release of cytokines and inflammatory factors that play a key role in promoting CVD.
Tumor necrosis factor α (TNF-α) is a cytokine produced by adipose tissue, endothelial cells and macrophages that play a major role in inflammatory response and monocyte recruitment to early lesions of atherosclerosis. TNF-α is known to be a potent activator of nuclear factor kappa B (NF-κB), a family of transcription factors involved in the atherosclerotic process. NF-κB regulates adhesion molecules like ICAM and VCAM and chemokines such as monocyte chemo-attractant protein (MCP-1) and IL-8 that contribute towards CVD. Another well characterized marker of cardiovascular risk is C-reactive protein (CRP). CRP activates NF-κB activity that further regulates the expression of MCP-1 and VCAM. Insulin resistance induced by high glucose and atherosclerosis are tightly intertwined disorders. Hyperglycemia induces damage to the endothelium through activation of NF-κB pathway amongst others.
Insulin-like Growth Factor Binding Protein-3 (IGFBP-3), a major binding protein of IGF modulates IGF by regulating IGF binding to its receptors. It also contributes to the pathophysiology of a variety of disease states in an IGF independent manner. With respect to IGF-I/IGF-I receptor independent actions of IGFBP-3, it is known to interfere and inhibit the NF-κB pathway. Since NF-κB plays a significant role in the progression of atherosclerosis, we explored the role of IGFBP-3 as a therapeutic target in cardiovascular diseases. In our experiments using human aortic endothelial cells (HAEC), we demonstrated that IGFBP-3 inhibits the elevated NF-κB activity induced by treatment with factors involved in CVD such as TNF-α, CRP and high glucose. This subsequently led to the inhibition of NF-κB regulated proteins such as ICAM, VCAM and MCP-1 at mRNA and protein levels. Taken together, our findings strongly suggest that IGFBP-3 may prevent/inhibit the initiation/progression of CVD and pose as an ideal therapeutic candidate for obesity induced CVD.

Nothing to Disclose: LM, YO

P2-165

Inhibitory Role of IGFBP-3 in the Pathogenesis of Asthma.

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Insulin-like growth factor-binding protein-3 (IGFBP-3) is a multi-functional protein known for modulating the actions of IGFs in somatic growth and a variety of human diseases such as cancer. Despite the critical role of the IGF system in the pathophysiology of many diseases, limited information is available for its role in bronchial asthma. IGFBP-3 fragments have been identified in asthmatic airway tissue extracts, however, whether there is any association between IGFBP-3 and asthma remains elusive.
We performed *in vitro* and *in vivo* studies to show that IGFBP-3 blocks specific physiological consequences of asthma in an IGF-independent manner. We used a mouse asthma model whereby normal as well as IGFBP-3 transgenic mice were challenged to ovalbumin (OVA). We found IGFBP-3 was suppressed in bronchial epithelial cells from normal mice after OVA challenge. Restoration of IGFBP-3 either by recombinant IGFBP-3 treatment or adenoviral IGFBP-3 gene transfer effectively reduced all physiological manifestations of asthma examined *in vivo* (airway hyperresponsiveness, cellular and pathological change in BAL fluid and lung tissue, and expression of numerous proinflammatory molecules). Furthermore, IGFBP-3 treatment restored airway functions as demonstrated by the reduction of OVA-induced AHR. These unique IGFBP-3 effects were IGF/IGF-IR-independent since IGFBP-3 mutant devoid of IGF binding affinity (IGFBP-3 ^{GGG}) had similar effects. The studies using IGFBP-3 transgenic mice further confirmed the effects of IGFBP-3 by demonstrating significant reduction of infiltration of inflammatory cells, cytokines production and OVA-induced AHR compared to that of normal mice after OVA inhalation.
Further *in vitro* studies using human bronchial epithelial cells demonstrated that IGFBP-3 blocks TNF-α-induced expression of proinflammatory molecules, attenuates the TNF-α-induced migratory response of eosinophils, and negatively regulates TNF-α-induced expression of the key NF-κB regulatory molecules, IκBα and p65-NF-κB, at the post-translational level. Taken together, our results strongly indicate that IGFBP-3 inhibits airway inflammation and airway hyperresponsiveness via an IGF-independent mechanism which involves cross-talk with NF-κB pathway. IGFBP-3 therefore plays a pivotal role in the pathogenesis of asthma, and thus can serve as a potential therapeutic for prevention/treatment of asthma.

Nothing to Disclose: YCL, SJ-B, AH, DYL, JH, LJM, YO

P2-166

Mesenchymal Stem Cells Expressing IGF-I Improve Fracture Repair: The Seed and the Soil for Tissue Regeneration.

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Introduction: Non-unions occur in 10-20% of the bone fractures. Limitations in mesenchymal stem cell (MSC) number and growth factor imbalance play critical roles in non-unions. Objectives: To determine whether MSC expressing IGF-I improve the fracture healing promoting new bone formation. Methods: MSC isolated from BM of FVB males were engineered to constitutively express human des(1-3)IGF-I (MSC^{IGF}) or infected with empty vector (MSC). A stabilized tibia fracture was produced in 8-10 week old syngenic females. Mice were transplanted with 10^6 MSC by IV tail injection. Fractured tibias were analyzed 14 days post-fracture by µCT analysis and distraction to failure biomechanical testing (BMT). Callus sections were subjected to in situ hybridization (ISH), histological and immunohistochemistry (IHC) studies to determine the tissue distribution and expression of bone and cartilage markers. For cell tracking, MSC-LacZ, MSC^{IGF}-LacZ were obtained from $ROSA26^{CMVCre}$ mice that constitutively express Lac-Z. Results: In MSC^{IGF} transplanted mice, BMT analyses showed significant improvements in several callus biomechanical properties mice including the ultimate force (4.13±1.76; n=3) when compared to MSC alone recipients (2.86±0.84; n=10; $p<0.05$) or untransplanted control (no cell, NC) (1.62±0.53; n=11; $p<0.01$). The increase in the ultimate force correlated with an increase of new bone content as determined by µCT analysis (7.82±2.34; n=7) compared to MSC alone (6.16±1.12; n=10; $p<0.05$) and NC (3.67±0.88; n=7; $p<0.01$). Furthermore, the fracture line of mice that received MSC^{IGF} showed a continuity net of new bone filling the fracture gap. While the fracture gap was filled with soft/cartilaginous tissue in mice that received MSC. ISH, IHC and specific staining studies, confirmed that calluses from MSC^{IGF} recipients had more new bone bridging the fracture line. By tracking transplanted MSC-$ROSA26^{CMVCre}$ and MSC^{IGF}-$ROSA26^{CMVCre}$ with X-Gal staining, we found that MSC^{IGF} integrated into the callus as bone cells, positive for osteoblast/osteocyte markers, in a greater number that MSC. Bone markers were also increased in recipient cells adjacent to MSC^{IGF}-LaZ. A greater number of positive p-IRS-1 cells were detected in MSC^{IGF}-LaZ calluses including MSC^{IGF}-LaZ as well adjacent recipient cells. Conclusions: MSC engineered with IGF-I improve the fracture repair by promoting new bone formation through autocrine (the seed) and paracrine (the soil) mechanisms.

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Nothing to Disclose: FG-M, TJM, JAW, YY, TL, LL, MIM, AS

P2-167

Insertion/Deletion (Indel) Polymorphisms in the IGFBP1 Promoter: Influence on Circulating IGFBP-1 and BMI in a Cohort of Type-2 Diabetes (T2DM) Patients.

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Dysregulation of the IGF system has been implicated in the aetiopathogenesis of obesity and T2DM. There are two clusters of two relatively obscure indel polymorphisms in the 5'-flanking region of the human IGFBP1 gene. Cluster A consists of two 4 bp indels: -724 -/TTTG and 721 -/GTTT, cluster B consists of one 5 bp indel: -712 -/TTGTT and one 4/5 bp indel: -706 /TTGTT/TGTT. Since multi-nucleotide insertions alter the PCR product size amplified using common priming sites outside the polymorphic region, they are resolvable by amplicon size using an ABI PRISM 3100 sequencer. This was done in 751 genomic DNA samples from subjects with T2DM from the City of Salford. Three fragment sizes were seen; 186 bp (59%), 190 bp (+4 bp, 16%) and 191 bp (+5 bp, 25%). It was inferred that there was only ever one 4 bp indel and/or one 5 bp indel present in a given subject (no +8 bp or +10 bp fragments were seen), although the identity of each indel was not confirmed. Treating the +4 bp and +5 bp indels as separate polymorphisms (named BP1P1 and BP1P2 respectively); the following genotype frequencies were as in the table.

Table 1: IGFBP1 Promoter Indel Genotype Frequencies

Indel	-/-	+/-	+/+	Minor Allele Frequency
$BP1P_1$	74.9% (526)	18.4% (129)	6.7% (47)	15.9%
$BP1P_2$	59.1% (415)	30.6% (215)	10.3% (72)	25.6%

Values are percentage (frequency) with numbers of subjects in parentheses

Minor allele homozygosity of BP1P1 but not of BP1P2 was associated with lower non-fasted circulating ln[IGFBP-1] (186/186: 2.78±1.2 ng.ml-1, 190/190: 2.6±1.3 ng.ml-1, regression coefficient: -0.51, CI: -1.0,-0.1, constant: 2.98, p = 0.040). In addition, BP1P1 but not BP1P2 was associated with lower rate of change of BMI over ten years compared with other genotypes, and rate of change of BMI was significantly lower if ln[IGFBP-1] fell within the highest quintile (regression coefficient: -0.10, CI: -0.2, -0.04, constant: 32.9 32. p = 0.001).
We conclude that the presence of one, or both, of the 4 bp indels in the 5' flank of IGFBP1 results in increased circulating protein levels and lower BMI increase over time. Direct sequencing will be needed in order to confirm the identities of the indels.

Sources of Research Support: Salford Royal Foundation Trust R&D Executive.

Nothing to Disclose: RHS, RO, WERO, JMG

P2-168

IGFBP-3 Is a Metastasis Suppression Gene for Prostate Cancer.

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In addition to its role as the major serum carrier of IGFs, Insulin-like growth factor binding protein (IGFBP)-3 is a potent pro-apoptotic and anti-angiogenic protein in prostate cancer (CaP) *in vitro* and *in vivo*. Epidemiological studies suggest that low IGFBP-3 is associated with more aggressive CaP. In collaboration with Lexicon, we generated IGFBP3KO mice to study the in vivo actions of IGFBP-3. Initial characterization of the BP3KO mice revealed that they have 60% reduced serum IGF-1, and no detectable circulating IGFBP-3 and are 20% larger than wild-type at 3 weeks of age. No spontaneous prostate tumor development was observed in BP3KO mice; however, we did observe splenic lymphomas in 30 of BP3KO mice, but not WT, at 80 weeks of age. To investigate the significance of endogenous IGFBP-3 in the development of prostate cancer *in vivo* we crossed BP3KO mice with the Myc model of prostate cancer (expressing human c-*Myc* driven by a prostate specific promoter). Myc mice develop slow growing, non-metastatic tumors. Surprisingly, at 17 weeks of age, the prostates of BP3KO/Myc mice did not develop cancer and only displayed Prostate Intraepithelial Neoplasia while control Myc mice had well differentiated CaP ($p<0.05$), which we attribute to the lower circulating IGF-I levels. By 24 weeks of age, well differentiated cancer was observed in all mice regardless of IGFBP3 status. However, when BP3KO/Myc mice were necropsied at 80 weeks of age, we identified lung metastases in 55% of them, while no WT/Myc mice exhibited any metastases. This is a remarkable finding, as metastases in the Myc model of CaP have never been described previously, and suggests that a major anti-tumorigenic role of endogenous IGFBP-3 as a metastasis suppressor. To identify a mechanism for these effects, we assessed the expression of GRP78 by immunohistochemistry in the prostates of WT/Myc and BP3KO/Myc mice. GRP78 is a molecular chaperone, previously reported to interact with IGFBP-3, whose increased expression has been implicated with cancer metastasis and poor prognosis. GRP78 was barely detectable in WT/Myc mice but was 3- to 5-fold up-regulated in mice lacking IGFBP-3, suggesting that the anti-metastatic actions of IGFBP-3 may occur, in part, by down-regulation of GRP78. These important findings reveal a novel role for IGFBP-3 (previously considered to be a tumor suppressor) as an anti-metastatic agent, opening new therapeutic possibilities for IGFBP-3 in cancer patients

Nothing to Disclose: HHM, LJC, JS, AL, DD, KWL, PC

P2-169

Novel Specific Kinase Receptor Activation Bioassays for the Human Insulin Receptor A (IR-A) and B (IR-B) To Study the Insulin-IGF System.

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CONTEXT Kinase Receptor Activation (KIRA) bioassays are novel methods to assess bioactivity on living cells. They quantify ligand bioactivity by measuring ligand-induced receptor tyrosine kinase activation in terms of receptor phosphorylation (1). Consequently, the measured signal is very specific in that only the response of the receptor for which it is designed is determined. Previously a specific IGF-I receptor (IGF-IR) KIRA assay was developed (2) and implemented in our laboratory (3). We hypothesized that KIRA bioassays specific for the insulin receptor isoforms A and B (IR-A and IR-B, respectively) will further help to unravel the overall functional activity of the circulating insulin-IGF system on each receptor.
AIM To develop KIRA bioassays specific for the human IR-A and IR-B.
RESULTS Human embryonic kidney cells were stably transfected with cDNA of the human IR-B or IR-A. Both IR bioassays were sensitive (detection limit 32pM) and accurate (intra- and inter assay CV: <6% and <12%, respectively). In both bioassays co-incubation of human insulin with IGF-I or IGF-II resulted in an additive increase in receptor phosphoryation, whereas co-incubation with IGF Binding Protein-3 (IGFBP3) could block this increase completely. In contrast, addition of IGFBP-3 to serum samples only reduced phosphorylation by 75%. Both bioassays were able to discriminate between bioactivity of healthy subjects and patients with insulin resistance or acromegaly.
CONCLUSION We developed highly sensitive and accurate IR-A and IR-B specific bioassays. They have a design that is similar to our in-house IGF-IR specific KIRA assay and therefore they can be run in parallel. By using these assays the overall function of the circulating insulin-IGF system can be assessed, providing more insights in this complex system.

(1) Sadick MD et al., J Pharm Biomed Anal. 1999 May;19(6):883-91.
(2) Chen JW et al., Am J Physiol Endocrinol Metab. 2003 Jun;284 (6):E1149-55.
(3) Brugts MP et al., J Clin Endocrinol Metab. 2008 Jul;93(7):2539-45.

Nothing to Disclose: AJV, MPB, JAG, AMW, SWJL, LJH, JAMJLJ

P2-170

A Unique Heparin Binding Domain of IGFBP-2 Is Active in Inhibiting Adipogenesis in an IGF-I Independent Manner.

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There are six insulin-like growth factor binding proteins (IGFBPs) which act to promote or inhibit the action of insulin-like growth factor-I and -II. Recent evidence has shown that the IGFBPs can also act independently of IGF-I. Insulin-like growth factor binding protein-2 (IGFBP-2) is the principal IGFBP secreted by white adipocytes and appears to play a role in inhibiting adipogenesis. We sought to investigate the activity of IGFBP-2 in fat cell development. We found that 3T3L1 cells treated with whole native IGFBP-2 at a concentration of 3 ug/ml, 1.5 ug/ml, and 0.75 ug/ml had a dose-dependent decrease in triglyceride accumulation as assessed by Oil Red O staining, compared to differentiation of cells exposed to an adipogenic cocktail containing 10 nmol insulin without IGFBP-2. We assessed expression of adiponectin and found that there was also a dose-dependent inhibition of adipogenesis. We repeated these studies in cultured primary preadipocytes and confirmed inhibition of adiponectin expression by IGFBP-2. While IGFBP-2 shares significant structural homology in the C-terminal and N-terminal regions with the other IGFBPs, the linker region of the protein is highly variable. The principal IGF-I binding site is located in the N-terminal, however the C-terminal facilitates IGF-I binding and is responsible for binding to extracellular matrix. We recognized that the linker region of IGFBP-2 contains a heparin binding domain (HBD) of 11 amino acids that is not present in any of the other IGFBPs and does not play a role in IGF-I binding. We synthesized this unique HBD peptide and treated cultured primary preadipocytes with 1.5 ug/ml of the synthetic peptide in the presence of the adipogenic cocktail containing insulin. The synthetic HBD significantly decreased adiponectin expression. These findings suggest that this unique region of IGFBP-2 harbors significant activity in inhibiting adipogenesis and that its mechanism of action occurs independent of IGF-I binding.

Nothing to Disclose: AMS, WHB, CJR, DRC

P2-171

Evaluation of Two Automated Methods for Measurement of Serum IGF-I: Comparison with a Manual Immunoassay.

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INTRODUCTION: Insulin-like Growth Factor I(IGF-I) serum(S) levels are useful for diagnosis and follow-up of Growth Hormone(GH) related disorders in children and adults. It reflects 24 hours secretion of GH without significant diurnal variation. Manual(IRMA,ELISA), and recently, automated chemiluminescence assays(QLIA) have been available for IGF-I S determinations in our country.**OBJECTIVE**: To compare the results of S IGF-I measured by 3 immunoassays: one IRMA and two QLIA and its relationship to clinical evaluation. **MATERIALS AND METHODS**: S samples from 135 patients(39 men,96 women,10-74 years old) were aliquoted. Samples were kept at -20ºC and used within 60 days from blood draw. IGF-I was measured in 135 samples using two QLIA: IMMULITE 2000, Siemens(IMM) and LIAISON, DiaSorin(LSN) and in 100 of them also by IRMA with extraction(DSL). Statistic Analysis: ANOVA test, Bland-Altman analysis and calculations of Pearson´s correlation coefficient(Analyse-it program) and concordance coefficient(Med-Calc program) were done. Differences were considered significant at $p<0.05$. **RESULTS**: Interassay variation coefficients(%) were: IRMA: 8.0/20.0 for 63.3/336.0 ng/mL; IMM: 2.9/7.0 for 78.7/529.3 ng/mL and LSN: 8.7/3.9 for 31.9/352.2 ng/mL, respectively. No statistically significant difference in IGF-I levels were found between both QLIA (ANOVA) but QLIA values were different from those by IRMA($p<0.0001$).Comparison results:

Comparison between methodologies

Comparison	n	Correlation coefficient (Pearson)	Bias % (Bland-Altman)	Concordance coefficient
IMM vs LSN	135	0.9949 *	2.1	0.974
IRMA vs IMM	100	0.9739 *	57.8	0.671
IRMA vs LSN	100	0.9810 *	59.8	0.580

* $p<0.0001$

A high correlation between methodologies was found. However, absolute IGF-I values by IRMA were different from both QLIA and linearly increasing with IGF-I concentrations (IRMA vs IMM: $y=1.7962x+10.282$; IRMA vs LSN:$y=2.1501x-39.488$). IGF-I S levels were in accordance to clinical data and reference range informed by each manufacturer in 134/135 IMM, 98/100 IRMA and 100/100 LSN results. **CONCLUSIONS**:1) A high correlation between results from the three immunoassays was found. However, the absolute values were not coincident. 2) Both QLIA were highly concordant and their absolute values were significantly different from those from IRMA, specially at higher IGF-I levels. 3) The three assays were suitable for diagnosis and follow-up of GH disorders.

Nothing to Disclose: PLG, AR, MFF, FV, ML, AF, MJ

P2-172

Association between Insulin-Like Growth Factor I and the Incidence of Metabolic Syndrome: Results from the Study of Health in Pomerania.

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Background A previous study showed an inverse association between insulin-like growth factor I (IGF-I) and the risk of impaired glucose tolerance or diabetes mellitus (DM). Moreover, myocardial infarction patients with high baseline IGF-I levels had a lower risk of DM. These data suggested a protective effect of IGF-I against the development of metabolic syndrome (MetS). However, there are no longitudinal data regarding IGF-I and MetS. The aim of the present study was to investigate the longitudinal association between IGF-I and MetS.
Methods Data from the population-based Study of Health in Pomerania, Germany were used for cross-sectional (N = 3,903) and longitudinal (N = 2,143) analyses. MetS was defined by three or more of these five components: abdominal obesity, elevated triglycerides, reduced high-density lipoprotein cholesterol, elevated blood pressure, and elevated non-fasting glucose. Serum IGF-I and IGFBP-3 were determined by chemiluminescence immunoassays. Logistic and Poisson regression analyses were performed to determine associations.
Findings Cross-sectional analyses revealed associations between high IGFBP-3 (men: OR 1.81, 95% CI 1.25-2.61; women: OR 1.71, 95% CI 1.14-2.57) or low IGF-I/IGFBP-3 ratio (men: OR 2.13, 95% CI 1.56-2.92; women: OR 1.64, 95% CI 1.08-2.47) and prevalent MetS. However, the direction of the relationship changed in longitudinal analyses: In men but not women, low levels of IGF-I (men: OR 0.66, 95% CI 1.43-0.99) were linked to a decreased risk of MetS.
Interpretation In concordance with previous studies, our cross-sectional analyses showed a relationship between a low IGF-I/IGFBP-3 ratio and prevalent MetS. In contrast, the longitudinal analyses indicated that a high IGF-I level was a risk marker for incident MetS.

Nothing to Disclose: NF, MN, SS, HV, GB, HW

P2-173

An Open Label Study of Mecasermin for HIV-Associated Metabolic Disease.

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Background:
Impairments in the growth hormone/insulin-like growth factor (IGF)- I axis are increased in HIV patients and may contribute to metabolic disease. Treatment with growth hormone reduces central adiposity but can exacerbate hyperglycemia. Growth hormone releasing hormone analogues reduce adiposity and improve lipids with a neutral effect on blood glucose.
Methods:
We conducted an open label study of the effects and tolerability of recombinant IGF-I (mecasermin) in adult patients with HIV infection and lipoatrophy. Treatment consisted of recombinant IGF-I dosed at 40 mcg/kg SQ BID for 24 weeks. Fasting glucose, insulin, lipids, IGF-I, IGF-binding protein 3 (IGFBP3) and body composition were examined before and after treatment. Data from 7 of 10 enrolled subjects were available for review.
Results:
IGF-I levels increased with treatment (134+/-25 ng/mL at Wk 0 vs. 436 +/- 250 ng/mL at Wk 24, P=0.03), while IGFBP3 levels declined (3751 +/- 1146 ug/L vs. 3370 +/- 1235 ug/L, P=0.02). Insulin at 12 weeks decreased significantly (12.2 +/ 6.7 uU/L vs. 3.9 +/- 2.8 uIU/mL, p=0.03). There was no difference in homeostasis model assessment (HOMA) of insulin resistance or insulin levels at 24 weeks. Fasting lipids (triglycerides, HDL cholesterol, LDL cholesterol, and total cholesterol) were unchanged following treatment. Total body weight was unchanged. On single slice abdominal computed tomography, total abdominal fat, as a percentage of cross sectional area, decreased (39.7%+/-8% vs. 35.2% +/- 8.9%, P=0.04). However, subcutaneous and intraabdominal fat did not significantly change suggesting that the decrease in abdominal fat was not specific to either compartment. There was no significant change in limb or trunk fat mass or lean mass as determined by dual energy x-ray absorptiometry. Hip circumference decreased 2.2% with treatment (97.8 +/- 9.6 cm vs. 95.6+/-9.0 cm, P=0.04), while waist circumference and waist to hip ratio were not significantly changed. There were no significant changes in biceps, triceps, thigh, or suprailiac skin fold measurements. Subscapular skin fold was slightly decreased. There was no change in HIV mRNA or in CD4 count. No patients had hypoglycemia, persistent headaches, arthralgia, or edema.
Conclusion:
These data suggest recombinant IGF-I may reduce insulin levels and decrease abdominal fat in patients with HIV lipodystrophy. Further investigation of this agent in a controlled trial is warranted.

Sources of Research Support: NIDDK-NIH K23 DK080644-01A1 (RK); National Center for Research Resources NIH UL1-RR-024134; Penn Center for AIDS Research NIH P30 AI 045008; mecasermin was provided by Tercica, Inc.

Nothing to Disclose: RJK, SV, LMZ, JHQ, PT, IF

P2-174

A GABA Agonist Does Not Increase IGF-1 Levels in Healthy Older Men and Women.

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Age-related decreases in growth hormone (GH) secretion and serum IGF-1, associated with increased body fat and reduced muscle mass, has been demonstrated in healthy elders. Similar changes observed in spinal cord injury patients (SCI) can be normalized by treatment with Baclofen (a GABA-ergic agent). We, therefore, explored the effects of Baclofen in 15 healthy older men and women (55-75 yrs old, BMI 24-32 kg/m^2) with serum IGF-1 levels less than 200 pg/L. Subjects took Baclofen 10 mg orally at bedtime for 2 weeks followed by 30 mg for 2 additional weeks. We measured serum IGF-1 and clinical and safety labs on days 0, 15 and 29. Serum IGF-1 levels were 85±34, 90±36 and 91±38 pg/L (mean±SD) at baseline, day 15 (10mg) and day 29 (30mg), respectively. These values were not significantly different when compared by paired t-test. All subjects completed the 10 mg treatment period and 12 subjects the 30 mg period. Two subjects developed adverse symptoms on 10 mg and 6 more on 30 mg. These were mild and included drowsiness (3), dizziness (3), vivid dreams (2), nausea (2), tinnitus (2), and skin rash (2). No hematological, electrolyte, renal or liver abnormalities were found. We conclude that, unlike observations in younger SCI patients, Baclofen administration does not restore IGF-1 levels and is poorly tolerated at higher doses in aged healthy men and women.

Sources of Research Support: Aurora Foundation.

Nothing to Disclose: PDT, FG, SMH

P2-175

Congenital IGF-I Deficiency Protects from Cancer Development – Additional Support.

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Backround: In a previous study (1) we have found that homozygous isolated congenital IGF-I deficiency (such as in Laron Syndrome) protects patients from future development of malignancies. This was confirmed by another group (2).
Aim: a) To find out whether congenital IGF-I deficiency combined with other pituitary hormone deficiencies also confers protection from cancer. b) To enlarge the population of patients with isolated congenital IGF-I deficiencies.
Methods: By survey among endocrinologists in Israel and other countries, using a pre-structured questionnaire for patients and first and second degree family relatives.
Results: The main data obtained until Jan 15, 2010 are summarized in the following table:

		Patients			First degree Family members	
Diagnosis	n	Age range (y)	Malignancies	n	Age range (y)	Malignancies
Laron Syndrome	207	1.5 - 75	0	189	1 - 78	17
Cong. IGHD	102	1.5 - 53	0	180	1 - 81	6
IGF-I R mutation	2	2.5 & 30	0	0		0
IGF-I gene deletion	2	6.5 & 11.5	0	2	40 & 45	0
GHRH-R defect	6	2.8 - 50	1M Hodgkins lymphoma diagnosed at 43 y	10	3 - 83	0
Cong. MPHD	97	0.25 - 86	1 F thyroid ca. diagnosed at 10.5 y	175	1 - 84	4

The data reinforce our previous findings (1) and those of Guevara-Aguirre in 75 LS pts. (2) that cong. IGF-1 deficiency protects from the development of malignancy. In the present study we found in addition that pts. with cong. IGHD, IGF-1 R mutation and IGF-1 gene deletion also fall into this category.
Out of 6 pts. with a GHRH-R defect and out of 97 pts. with cong. MPHD including GH def. we found 1 pt. with cancer in each.
Among the first degree family members (most heterozygotes) we found 27 cases of cancer. In addition, 30 out of 131 second degree relatives also reported malignancies.
Conclusions: a) The present survey underlines the important role cong. IGF-1 deficiency has on the development of malignancies. b) It is premature to define which pts. with GHRH-R defect or cong. MPHD are not protected from cancer.

(1) Shevah O., Laron Z., Growth Hormone & IGF Res. 2007;17: 51-57.
(2) Guevara-Aguirre et al., Hormone Research 2007;68 (Suppl 1):175.

Nothing to Disclose: RS, OS, ZL

P2-176

Polymorphism of IGF-1 Promoter Gene and Healthy Aging Condition in Spanish Population.

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Introduction: Genetic variations in genes belonging to the Insulin/IGF 1 pathway have been explored in relation to longevity, dementia, metabolic diseases and cancer.
Objectives: Our study investigated the 192bp allele of IGF-1 promoter gene and its relationship with metabolic syndrome components, mental and nutritional state, and functional capacity in old Spanish population. **Patients and methods:** 292 subjects (144 men and 148 women, mean age **77.0**±5.4) participating in the Mataro aging study (a population-based study) were included. Anthropometric variables, lipid profile, glucose and blood pressure were measured, as well as the presence of the 192bp allele of IGF-1 promoter gene. Genotypes were determined by polymerase chain reaction using the forward primer labelled with FAM. Metabolic syndrome according to IDF criteria was found in 57.9% (54.9 % in men and 61 % in women). **Results**: The 192 bp allele of IGF-1 promoter gene was distributed in the total population as: 41.9% homozygous, 44.3% heterozygous and 13.9% were non-carriers of this allele. No differences in anthropometric variables -including height-, blood pressure, lipid profile, glycaemia, were found in relation to this allele. Also, no differences were found in relation to total IGF-1 and IGFBP3 levels. Mental state measured by MMSE, nutritional state using MNA and Barthel scale were better in homozygous individuals compared to heterozygous and non-carriers (p=0.015, p=0.024 and 0.047, respectively). A lower prevalence of metabolic syndrome was observed in homozygous (41.9% vs 54.9% in heterozygous + non-carriers, p=0.031). When men were considered, same results were found in anthropometric variables, blood pressure, lipid profile and glycaemia, being MNA better in homozygous but no differences in MMSE and Barthel scales. In women, blood pressure was lower in homozygous individuals (p=0.009) with no significant differences in anthropometric variables, lipid profile and glycaemia. Barthel scale in women was also better in homozygous individuals (p=0,05) with no differences in MMSE and MNA. **Conclusion**: Homozygosis for the 192 bp allele of the IGF-1 gene polymorphism confers a healthier aging condition, with less prevalence of metabolic disturbances, and better mental, nutritional and functional state.

Nothing to Disclose: MM, MJP, MS-P, EP, XB, JO, MP-D

P2-177

Development of a Population Pharmacokinetic (POP PK) Model for rhIGF-1 Administration.

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Introduction: POP PK models can be used to describe serum study drug exposures when only sparse serum sampling is available. The PK parameters derived from these models can be used to determine differences in the drug disposition of subjects with varying characteristics (eg, age, weight, gender) as well as the relationship between these differences and the safety and efficacy of the study drug.
Objective: To describe the development of a POP PK model for rhIGF-1.
Methods: A total of 1873 serum samples were collected from 212 subjects (mean age 9, range 3-25 years) receiving rhIGF-1 during clinical trials. These serum samples were used to evaluate IGF-1 and IGFBP-3 levels. The POP PK models were built sequentially: first using data from frequent sampling after single and multiple dose administration and then sequentially using sparse multiple dose data from long-term trials. A total of 68 models were evaluated to determine the model structure and covariates that influenced the PK parameters.
Results: The time course of serum IGF-1 after subcutaneous rhIGF-1 doses (15-248 µg/kg) was best described by a 1-compartment open model with first-order absorption and elimination, and a zero-order rate of endogenous IGF-1 secretion. Five covariates were found to influence the PK profiles: endogenous secretion rate (KS) increases with age and baseline IGF-1 concentration; clearance (CL/F) decreases with increasing IGFBP-3 concentration; volume of distribution (Vd/F) increases with weight and rhIGF-1 dose and decreases with increasing IGFBP-3 concentration. A typical profile has an absorption rate of 0.789 hr^{-1}, CL/F of 0.753 L/hr, Vd/F of 5.68 L and KS of approximately 64 µg/hr. Inter-individual variability was low for CL/F and Vd/F (16% and 14%, respectively), and was moderate for absorption rate and residual variability (41% and 66%, respectively).The final model showed no dependency on dose frequency (once-daily or twice-daily) or treatment duration.
Conclusions: The POP PK model of IGF-1 allows prediction of rhIGF-1 exposure over a wide range of rhIGF-1 doses using baseline IGF-1 and IGFBP-3 levels, body weight, rhIGF-1 dose and age. The model can be used for different dosing regimens and varying treatment durations. Thus, this POP PK model may be used to optimize rhIGF-1 dosage, dosing intervals, titration schedules and exposure at steady-state IGF-1 concentration in children, adolescents and young adults.

Sources of Research Support: Ipsen, US.

Disclosures: JMC: Employee, Ipsen. AM: Employee, Ipsen. RO: Vice President, Ipsen. GB: Employee, Ipsen.

P2-178

Insulin-Like Growth Factor-I in Cord Blood Is Predictive for Catch-Up Growth in Monozygotic Twins with Discordant Growth.

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Objective: To investigate the growth of monozygotic twins with discordant birth-weight and the predictive value of birth-weight, -length, and cord blood concentration of growth factors on their catch-up growth until the age of four years.
Patients and Methods: 25 monozygotic twin-pairs (14 with inter-twin birth-weight SDS difference > 1) were studied at birth and at 4 years of age. In 20 pairs, several parameters including IGF-I were analysed in cord blood and at 4 years of age. Inter-twin difference Δ of birth-weight, -length and growth at 3, 6, 12, 24, and 48 months were correlated to Δ of the parameters analysed.
Results: We found a reduction of Δ height-SDS form birth until 4 yrs with the main catch-up occurring during the first year, but only a slight, statistically insignificant reduction of ΔBMI-SDS during the observational period. Correlation coefficients were used to identify factors predicting postnatal catch-up growth. Both, birth-weight difference (r=0.653; p=0.001) and Δ IGF-I in cord blood (r=0.613; p=0.007) were of similar predictive value. Analysis of variance was not able to differentiate between the individual impacts for these two parameters though both parameters were comparable strongly correlated with actual height.
Conclusion: We observed an approximation concerning height but not BMI until the age of 4 years between genetically identical twins with discordant birth-weight. Both birth-weight and cord blood IGF-I are predictive factors for subsequent catch-up growth. This might reflect an epigenetic mechanism acting during fetal development, through which environmental factors lead to programming of the GH-IGF-I axis, thereby influencing postnatal growth.

Sources of Research Support: Pfizer, Germany.

Nothing to Disclose: BCG, PB, JW

P2-179

Reference Values for Serum Levels of Insulin-Like Growth Factor-I and Insulin-Like Growth Factor Binding Protein-3 in Korean Children and Adolescents.

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Objectives: Measurements of serum insulin-like growth factor-I (IGF-I) and its major binding protein, IGF binding protein-3 (IGFBP-3), are utilized in the diagnostic work-up and clinical management of the children with growth disorder or other metabolic diseases. Establishment of the normal range of IGF-I and IGFBP-3 is essential to monitoring their serum concentration and estimating the growth state accurately. So we designed this study to help the physicians interpret these laboratory data properly and achieve the better results in treatment of children by establishing the reference value of IGF-I and IGFBP-3 according to age, sex and pubertal stage.
Methods: 1380 healthy Korean children and adolescents aged 0 to 19 years (724 boys, 656 girls) were randomly selected. 22 medical college hospitals participated in this study. Blood samples were collected and the stored sera were assayed for IGF- I and IGFBP-3 by IRMA (Immunotech). R 2.8.1 program (Bell Laboratories) was used to generate reference percentile curves for IGF- I and IGFBP-3 according to age, sex, and pubertal stage.
Results: Both serum IGF- I and IGFBP-3 concentrations tended to be higher in girls compared to boys before the age of 16. Serum levels of IGF-I and IGFBP-3 increased steadily with age in the prepubertal stage followed by a progressive decline thereafter. Peak IGF-I concentrations were observed earlier in girls than boys (14 years vs. 16 years). But we noticed the contrary results in IGFBP-3, declining after the peak at the age of 17 years in girls and 12 years in boys. Serum IGF- I and IGFBP-3 concentrations were highest at Tanner stage IV in both girls and boys.
Conclusion: Our referenece value model based on age, sex, and pubertal stage can improve the diagnostic utility in the evaluation and management for the Korean children and adolescents with growth disorders.

Nothing to Disclose: SEH, HSK, DHK

P2-180

Differences in Skeletal and Non-Skeletal Factors in a Diverse Sample of Men with and without Type 2 Diabetes Mellitus.

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Introduction: While fracture risk is higher in patients with type 2 diabetes mellitus (T2DM), patients with T2DM tend to have higher bone mineral density (BMD) than their non-diabetic counterparts. These observations motivate comparisons of both skeletal and non-skeletal factors in subjects with and without T2DM.
Methods: The Boston Area Community Health/Bone (BACH/Bone) Survey is a population-based cross-sectional survey of skeletal health in a random sample of 1,219 Boston men aged 30-79 y. Diabetes status, age and race/ethnicity were obtained via self-report. BMD and body composition were measured by DXA. Physical function was assessed via a composite physical function score derived from walk and chair stand tests. Grip strength was measured with a hydraulic hand dynamometer. Multivariate linear regression was used to examine the association of T2DM status with skeletal and non-skeletal factors.
Results: Of the 1137 men with complete data included in this analysis, the mean age was 48 y. Prevalence of T2DM was 12.5%, with an average duration of disease of 7.4 y. Of the skeletal factors considered, only lumbar spine BMD was significantly higher in men with T2DM. Of the non-skeletal factors considered, both the composite physical function score and maximum grip strength were significantly lower in men with T2DM. As shown in the table, skeletal factors were no longer significantly associated with T2DM status in multiple regression models. Non-skeletal factors including appendicular lean mass, arms lean mass, and maximum grip strength were negatively associated with T2DM after adjustment.
Conclusion: These results suggest that non-skeletal factors such as body composition and muscle strength, which may influence fall risk, could explain higher fracture rates among patients with T2DM.

Multiple regression models (adjusted for age, race/ethnicity, and body mass index) showing the association between T2DM and outcomes.

		T2DM		
Subgroup	Outcomes	β	SE	p-value
Skeletal Factors	Femoral neck BMD (g/cm^2)	-0.01	0.02	0.46
	Lumbar spine BMD (g/cm^2)	0.01	0.02	0.56
Non-Skeletal Factors	Lean mass (kg)	-0.54	0.82	0.51
	Appendicular lean mass (kg)	-1.04	0.50	0.04
	Arms lean mass (kg)	-0.42	0.15	0.006
	Legs lean mass (kg)	-0.62	0.37	0.097
	Composite score	-0.48	0.26	0.07
	Maximum grip strength (kg)	-3.02	1.25	0.02

Sources of Research Support: NIH Grant R01AG020727.

Nothing to Disclose: JMD, GRC, AVS, ABA

P2-181

The Interactions of Leptin, Osteocalcin, and Adiponectin with Indices of Insulin Resistance and β Cell Function in the Baltimore Longitudinal Study of Aging.

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Recent animal studies show that bone regulates glucose metabolism through osteocalcin, which stimulates insulin and adiponectin secretion as well as β cell proliferation. Furthermore, leptin modulates the bioactivity of osteocalcin. Based on these findings, Karsenty proposed that bone modulates energy metabolism through a pathway that includes adipocytes and islets(1). To test this hypothetical paradigm, we studied the relationship of leptin, adiponectin and osteocalcin with markers of insulin resistance (HOMA2-IR)(2) and β cell function (insulinogenic index [IGI]), using data from the Baltimore Longitudinal Study of Aging.
Five hundred and forty-one subjects had data on fasting glucose, insulin, leptin, osteocalcin, adiponectin and 20-min post-OGTT glucose and insulin (Table 1). Subjects on hypoglycemic medications were excluded. Variables with skewed distribution were log-transformed for analyses.

Table 1

Characteristic	Mean(25th - 75th percentile)
N(% women)	531(47.6)
Age, yrs	68(59-77)
Weight, kg	76.6(65.9-87.1)
BMI, kg/m²	25.9(23.6-29.2)
Fasting glucose, mmol/L	5.0(4.7-5.3)
20-min glucose, mmol/L	8.1(7.1-9.3)
Fasting insulin, pmol/L	51.4(33.7-74.9)
20-min insulin, pmol/L	264.8(171.5-405.2)
HOMA2-IR	1.0(0.6-1.4)
Insulinogenic index	68.4(35.9-126.9)
Leptin, ng/mL	13.3(6.6-26.1)
Osteocalcin, ng/mL	15.4(11.3-20.6)
Adiponectin, µg/mL	12.6(7.7-20.5)

Table 2. Linear regression

	HOMA2-IR
	β
Age	0.16†
BMI	0.26†
Adiponectin	-0.18†
Osteocalcin	-0.09*
Leptin	0.29†

β=standardized coefficient. * $P < 0.01$, †$P < 0.001$; Adjusted $R^2 = 0.301$.

In a linear model, age, BMI, adiponectin, osteocalcin and leptin were independently associated with HOMA2-IR (Table 2),but only leptin was associated with IGI (β=0.3, P<0.001). Structural equation modeling (SEqM) showed: leptin had a negative effect on osteocalcin and positive effects on HOMA2-IR and IGI; osteocalcin had a positive effect on adiponectin and a negative effect on HOMA2-IR; adiponectin had a negative effect on HOMA2-IR. The SEqM developed was highly supportive of the existence of an integrated biological mechanism connecting bone, adipocytes and islet in humans. This hypothesis should be further examined in longitudinal analyses and intervention studies.

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(2) Wallace TM et al., Diabetes Care 2004; 27:1487-1495

Sources of Research Support: Intramural Research Program of the NIH, National Institute on Aging.

Nothing to Disclose: KSG, JKN, RGS, ODC, EJM, LF, JME, CWC

P2-182

Ethnic Differences in the Association between Adiponectin and Bone Mineral Density in Nondiabetic, Postmenopausal African-American, Caucasian and Filipino-American Women.

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Adiponectin, an adipose-derived protein with insulin-sensitizing properties, may be a novel determinant of bone mineral density (BMD). In animal models and cell cultures, adiponectin induces osteoblast proliferation and differentiation, but may increase osteoclastogenesis by stimulating the RANKL pathway. Gender but not ethnic differences in the inverse association between adiponectin and BMD have been reported. We evaluated this association in non-diabetic, postmenopausal African-American (AA), Caucasian and Filipino-American women. Serum adiponectin was measured by radioimmunoassay in archived fasting blood samples obtained from 204 Caucasian women (mean age: 69.8 years), 306 AA (mean age: 60.7) and 208 Filipina women (mean age: 59.6 years). BMD was measured at the femoral neck, total hip, and lumbar spine by DXA. Mean adiponectin was significantly higher in Caucasians (13.0 µg/ml) compared to AA (8.1 µg/ml) and Filipinas (7.1 µg/ml, p=0.006). Age-adjusted BMD was significantly lower in Caucasians (femoral neck: 0.654, total hip: 0.799, spine: 0.878 gm/cm^2) and Filipinas (femoral neck: 0.714, hip: 0.806, spine: 0.890 gm/cm^2) compared to AA women (femoral neck: 0.793, hip: 0.885, spine: 1.015 gm/cm^2 p<0.001). Filipinas had higher femoral neck BMD, but similar hip and spine BMD and body mass index (BMI) as Caucasians. BMI and lean mass decreased with increasing adiponectin tertiles in all groups. Similarly, BMD decreased with increasing adiponectin tertiles at all sites in Caucasians, and at the femoral neck and total hip in AA women, but not among Filipinas. Multivariable analysis adjusted for age, lean mass, smoking, estrogen use and exercise showed adiponectin was inversely associated with BMD at the femoral neck (ß= -0.031, p=0.032), total hip (ß= -0.047, p=0.004), and lumbar spine (ß= -0.053, p=0.02) among Caucasians and African-Americans (femoral neck, ß= -0.030, p=0.03; total hip (ß=-0.036, p=0.008), lumbar spine (ß=-0.039, p=0.048)). However, among Filipinas, adiponectin was not associated with BMD at any site: (femoral neck (ß=0.008, p=0.62), total hip (ß=-0.008, p=0.65), lumbar spine (ß=0.004, p=0.86)) and persisted when limited to those with the highest adiponectin ($\geq$ 10µg/ml) concentration. Adiponectin is inversely associated with BMD in Caucasian and African-American women, but not among Filipino-American women. The reason for these differences is unclear.

Sources of Research Support: NIDDK R01 31801, R03 60575 and NIA AG 07181.

Nothing to Disclose: MRGA, DvM, EB-C

P2-183

Vitamin D Deficiency – Possible Link between Osteoporosis and Metabolic Syndrome.

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Osteoporosis and metabolic syndrome, two multifactorial disorders are medical problems with major epidemiological dimension. Several common pathophysiological factors for both entities have been suggested. These include many factors (lipid oxidation products, inflammatory factors, estrogens, parathormone, ect) and vitamin D defficiency could be one of the most important one. The aim of our study was to evaluate the relationship between bone mineral density (examined by DXA – dual energy x-ray absorptiometry), vitamin D3 levels and signs of metabolic syndrome. We examined 55 subjects (37 women, 18 men, age median 67,8 years) with no history of osteoporosis, suffering from metabolic syndrome (defined as abdominal obesity and more than 2 of other components – arterial hypertension, dyslipidemia and diabetes mellitus or impaired glucose tolerance, according to EASD and IDF, 2006). Osteoporosis (T-score less than - 2,5) was found in 32,7% (15 women and 3 men) and osteopenia (T-score between - 1,5 and - 2,5) in 29% (13 women and 3 men) of patients. We observed negative correlation between BMI and fat percentage (examined by DXA) and vitamin D3 levels. Low concentration of vitamin D3 was found in 90% of patients with median 19,36 ug/l (64 % measured in winter, 36% in summer, no relationship between vitamin D3 levels and season). We also observed negative correlation between low concentration of vitamin D3 and presence of diabetes mellitus as part of metabolic syndrome. A link between osteoporosis and metabolic syndrome could influence the therapeutic approach in both disorders and vitamin D supplementation may play an important role in prevention of these important conditions.

Nothing to Disclose: JP, KB, PJ, AD, TK, ZK

P2-184
Thiazolidinediones (TZDs) Inhibit Bone Turnover.

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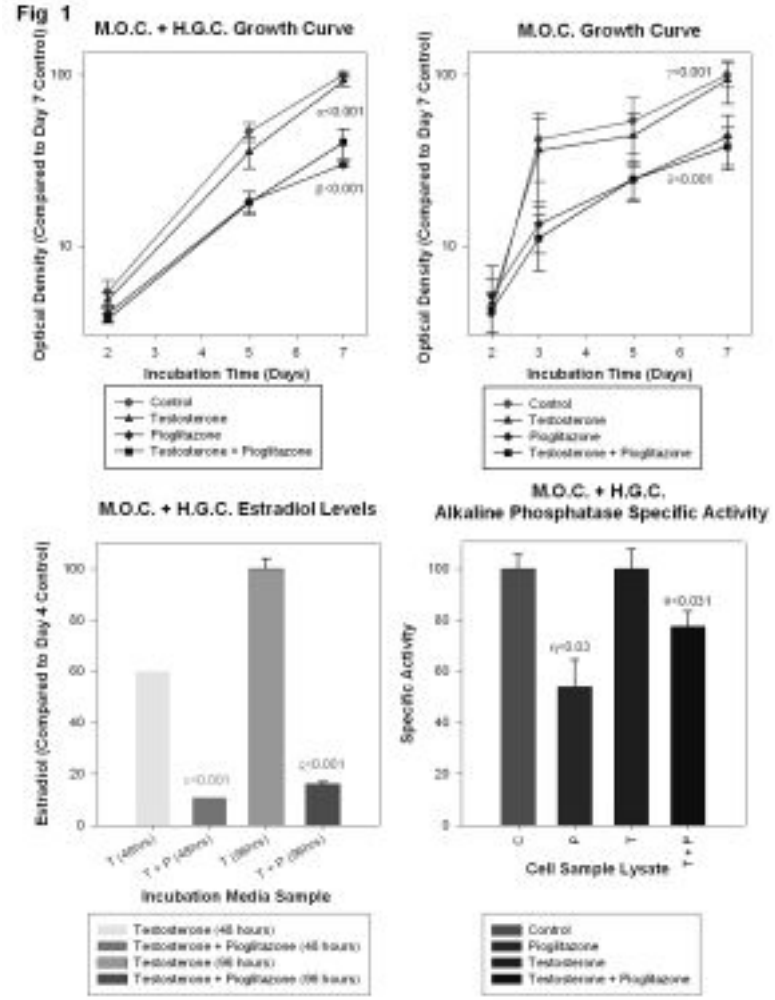
Introduction: TZDs (pioglitazone [Pio] & rosiglitazone [Rosi]), are insulin sensitizers used for diabetes treatment. Human & animal studies have suggested that TZDs may inhibit bone formation, decrease osteoblastogenesis, induce bone loss, & lead to a higher fracture risk.[(1-8)] We explored whether Pio affects bone turnover markers, estradiol (E_2) production, cell growth, & cell differentiation in mouse osteoblast cell (MOC) cultures and MOC/human granulosa cell (HGC) co-cultures. MOC/HGC co-cultures were used to determine whether TZD inhibition of aromatase (Arom) may play a role in bone metabolism. Rosi studies are in progress.
Materials & Methods: MOC were incubated in culture medium with and without HGC, testosterone (T), & Pio. E_2 levels, alkaline phosphatase (AP) activity, osteocalcin (OC), mouse pro-collagen expression, optical density for cell growth, & cell staining with hematoxylin, eosin, and oil red were analyzed.
Results: In the presence of Pio, cell growth in MOC, with or without HGC, was decreased by 60-70% regardless of T presence ($p < 0.0001$) (Fig.1). Oil red staining showed increased fat droplet accumulation (data not shown). When the MOC were co-cultured with HGC in the presence of T, E_2 production was significantly inhibited by Pio after 48 and 96 hours of incubation ($p< 0.0001$) (Fig.1). For MOC/HGC cultures, Pio inhibited AP specific activity by 45% ($p< 0.003$) in the absence of T and by 23% ($p< 0.031$) in the presence of T (Fig.1). Osteocalcin production and mouse procollagen expression analysis is in progress.



Conclusion: Pio inhibited MOC and MOC/HGC co-culture growth while increasing fat accumulation. Pio also inhibited AP specific activity regardless of T presence. Our previous studies have suggested that TZDs inhibit E_2 production in HGC through inhibition of Arom, which explains the inhibition of E_2 production in MOC/HGC co-cultures. Current results suggest that the TZD inhibition of MOC growth and AP activity is not related to Arom activity since both cultures exhibited decreased growth and AP activity regardless of whether the Arom substrate, T, was present.

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Sources of Research Support: Empire Clinical Research Investigator Program of the New York State Department of Health; Gerald J. and Dorothy Friedman Foundation; Chinese American Medical Society & Chinese Independent Practice Association.

Nothing to Disclose: AKS, DCG, PS, YF, SK, CR, RP, ZR, LP, DS-Y

P2-185

Defining the Effects of Thiazolidinediones on Osteoblast and Adipocyte Lineage Differentiation from Human Bone Marrow Stem Cells.

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Background and Objectives: Thiazolidinediones (TZDs) are effective and commonly prescribed oral agents for the treatment of diabetes. Recent studies have reported an association between TZD treatment and an increased risk of bone fractures. Despite the potential adverse effect of TZDs on fracture risk no previous human studies have determined the underlying mechanisms of the effects of TZDs on bone.
Methods and Results: We have therefore investigated the effects of TZDs on the differentiation of human bone marrow stem cells (hBMCs)to bone forming osteoblasts and adipocyte lineages. hBMCs were obtained from 4 non-diabetic and 4 diabetic patients via bone marrow aspiration. Cells were isolated, cultured and treated with adipocyte or osteoblast differentiation medium plus Rosiglitazone, Pioglitazone (0.3 to 10 μM) or vehicle for 14-21 days. Osteoblast differentiation and mineralization was assessed by Alizarin Red S staining, alkaline phosphatase enzyme activity, and gene expression. We observed that neither Pioglitazone nor Rosiglitazone directly alter osteoblastogenesis from hBMCs. Cell viability was also unaffected. However, both TZDs enhanced adipogenesis at a stage specific time as determined by quantitated lipid storage, an approximate 5 fold increase. Optimal enhancement of differentiation occurred when TZDs were added simultaneously with differentiation medium. TZDs in the absence of differentiation medium did not promote adipogenesis. A second mechanism by which TZDs might alter bone quality is to increase the differentiation and/or function of bone resorbing osteoclasts. These cells derive from human peripheral blood cells and are defined as TRAP (Tartrate Resistant Acid Phosphatase) positive multi-nucleated cells. The TZDs did not increase formation of multinucleated-TRAP positive cells which suggests, at least in vitro, that TZDs do not alter bone quality by increasing resorption. Significant differences between diabetics and non-diabetics have not been detected.
Conclusions: Our preliminary studies indicate that TZDs; 1) increase adipogenesis from hBMCs at a stage specific time, 2) do not directly alter osteoblastogenesis from hBMCs, 3) did not increase osteoclast formation from hPBCs. These results challenge the notion that TZDs directly alter osteoblastogenesis and/or increase osteoclastogenesis and raise the possibility that bone quality in humans is altered by factors secreted by TZD-enhanced adipogenesis.

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Nothing to Disclose: GRB, CEC, GB, LP, NBK, GEU

P2-186

Does Weight Gain Influence Bone Metabolism in Skeletally Mature Female Rats?.

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Body weight is correlated with bone mass during growth. However, it is unclear how bone mass is influenced by weight gain following skeletal maturity. The purpose of this study was to determine the effects of weight maintenance and two rates of weight gain on bone metabolism using skeletally mature female rats. Eight-month-old female rats were fed one of 3 diets for 13 weeks: Lieber-DeCarli liquid diet *ad libitum* (control diet), the same diet with caloric restriction to maintain initial body weight (calorie-restricted diet), and the same diet fed *ad lib* with the exception that appetite was enhanced (calorie-increased diet) by replacing a small quantity of maltose-dextran isolcalorically with ethanol (0.5% caloric intake). Compared to baseline, rats fed the calorie-restricted, control, and calorie-increased diets changed in weight by -1 ± 2% (mean ± SE), 10 ± 3%, and 21 ± 2%, respectively. Diet associated changes in serum leptin levels, suggest that fat mass increased in control and calorie-increased rats compared to baseline rats.
Compared to baseline, leptin levels were not significantly changed in the calorie-restricted group, and significantly increased in the control diet and calorie increased diet groups by 278 ± 49 and 689 ± 67%, respectively. Significant differences in tibial bone mineral content and density were not detected among treatments groups following dietary intervention or between treatment groups and the baseline group. Similarly, indices of cancellous bone architecture (area, trabecular number, thickness, and separation) and bone turnover (mineralizing perimeter, mineral apposition rate, and bone formation rate) did not differ among groups following dietary intervention. Our findings suggest that weight gain, with corresponding large increases in serum leptin levels, over the range evaluated, confers neither beneficial nor detrimental effects on bone metabolism in skeletally mature female rats. In other experiments, increasing the leptin levels in the hypothalamus of nine-month-old rats for 5 months using hypothalamic leptin gene therapy prevented age-related weight gain and lowered serum leptin levels without influencing bone mass or architecture. Taken together, these findings do not support weight per se, or serum or central leptin levels as important regulators of bone metabolism in skeletally mature female rats.

Sources of Research Support: NIH AA11140.

Nothing to Disclose: UTI, RTT

P2-187

Risperidone, a Second Generation Antipsychotic Alters Fat Distribuition and Has a Deleterious Effect on Bone Mass in Young C57BL6 Male Mice.

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Risperidone(R) is a second generation antipsychotic widely used not only in adults, but increasingly in children and adolescents. Adverse events such as weight gain, metabolic syndrome and hyperprolactinemia can be striking especially in younger individuals. In this study we hypothesized that R treatment in mice would result in a fat redistribution phenotype that might contribute to bone loss. Young(i.e. 4 week) male C57BL6 mice were kept in individual metabolic cages and orally treated with R at a dose of 0.84mg/kgxday^{-1} for 5 weeks. The drug was added to the food. Control mice(C) received a regular 10% fat calorie diet. During this period, body weight and food ingestion were measured daily. At the 5th week, body composition and bone mineral density were analyzed by DXA. In a smaller number of mice, body and marrow fat content were evaluated by MRI. At sacrifice, liver, epididymal and peri-renal adipose tissues were weighed. Bone micro architecture was evaluated by microCT. Liver histology and oil red O staining were performed. T test was used in statistical analysis, considering a 95% confidence interval. R treated mice(n=12) had greater weight gain than C mice (n=11) despite no differences in food intake. Surprisingly, total % body fat by DXA(R:14.6% C:18.6%,p<0.01), epididymal weight(R:14.5mg/gbw; C:20.8mg/gbw,p<0.01) and peri-renal weight(R:2.9mg/gbw, C:5.0mg/gbw,p<0.01) were significantly lower in R than C mice. R treated mice tended to have lower total bone mineral density by DXA and reduced femoral cortical thickness by uCT(p=0.08 R vs C) with only a 5% difference in femoral BV/TV (R vs C) that did not reach statistical significance. Overall, R treated mice had significantly less total body fat than C(p<0.005) but these mice had higher bone marrow fat content than C. Livers were also significantly heavier in R than C(p<0.008) and massive lipid droplet infiltration was noted on liver histology of the R mice. These results suggest that adipose distribution (visceral and marrow vs epididymal and subcutaneous) is significantly altered by R treatment of pubertal mice, even before excessive weight gain occurs. The detrimental effects of R on bone were principally in the cortical compartment although significant marrow adiposity was noted. In sum, R has a profound effect on fat metabolism. Prolonged use in younger animals and individuals may be associated not only with the metabolic syndrome but impaired peak bone acquisition and maintenance.

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Nothing to Disclose: ID-Dp, GW, SU, MK, AM, SB, CJR

P2-188

Locally Generated Glucocorticoids, Rather Than Pro-Inflammatory Cytokines, Directly Regulate Synovial Dkk-1 Expression in Inflammatory Arthritis.

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We have previously proposed a central role for locally generated glucocorticoids in the periarticular and systemic osteoporosis seen in rheumatoid arthritis (RA). Synovial fibroblasts (SFs) form a substantial component of inflamed synovium and increase endogenous glucocorticoid (GC) generation during inflammation through increased expression of the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). Recently, production of DKK-1 (a Wnt signalling inhibitor known to inhibit bone formation and support bone resorption) by SFs in response to inflammation has been proposed to be a master regulator of inflammatory bone loss. We tested the hypothesis that DKK-1 production in inflamed synovium was primarily mediated by GCs and indirectly by inflammatory cytokines as well as assessing the affects of GCs on expression of a range of Wnt agonists and antagonists.
Primary SFs were isolated from synovial biopsies from 4 patients with RA undergoing orthopaedic surgery. Fibroblasts were treated with either vehicle, Dexamethasone (Dex) (0.1-100nmol/l) or TNFα (0.1-10ng/ml) for 24hr. DKK-1 expression was measured by real-time RT-PCR and ELISA on cell culture supernatant. Gene expression of Wnt signalling components was determined by TaqMan gene expression plates.
High basal DKK-1 mRNA and protein expression were found in SFs with TNFα treatment resulting in a small increase in expression (2.3 fold mRNA; 1.4 fold protein). The active GCs cortisol and dexamethasone caused a substantially greater increase in mRNA and protein expression (3.1 and 3.2 fold increase in mRNA, $p<0.05$; 2.3 and 2.8 fold in protein respectively, $p<0.05$). The inactive GC cortisone also increased DKK-1 expression in SFs (2.7 fold in mRNA, $p<0.05$; and 1.7 fold in protein, $p<0.05$) to a much greater degree than TNFα, an effect blocked by an 11β-HSD1 inhibitor. In addition to DKK-1, treatment with Dex resulted in a significant increase in gene expression in the Wnt antagonist FRZB (10.7 fold, increase in mRNA; $p<0.05$) in combination with a decrease in expression of the Wnt agonist Wnt2, (7.3 fold, decrease in mRNA; $p<0.05$).
This study demonstrates that in SFs, GCs increase expression of the Wnt antagonist DKK-1 to a greater degree than the inflammatory cytokine TNFα. In addition, this study has identified several new factors involved in Wnt signalling that are regulated by GCs and may contribute to the imbalance in bone metabolism observed in inflammatory disease.

Nothing to Disclose: RSH, MA, PP, ER, AF, KR, CDB, PMS, MSC

P2-189

RANK Ligand Blockade by Denosumab Concurrently Prevented Bone Loss and Vascular Calcium Deposition in Mice.

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Receptor activator of NF-κB ligand (RANKL), its receptor RANK, and the decoy receptor osteoprotegerin (OPG) represent a potential mediator system for bone metabolism and vascular function. Here, we tested the hypothesis whether RANKL inhibition by denosumab (DMAb), a fully human RANKL antibody, could prevent glucocorticoid-induced bone loss and vascular calcium deposition in mice. Because DMAb does not recognize wild-type (WT) murine RANKL, we used human RANKL knock-in (huRANKL KI) mice (male, 8 months old) that exclusively express chimeric (human/murine) RANKL that is inhibited by DMAb. Vehicle or prednisolone (Pred) pellets were implanted sc to release 2.1 mg/kg of Pred/day over 4 weeks. Mice were concomitantly treated with DMAb (10 mg/kg, twice weekly) or vehicle (PBS). Bone mineral density (BMD) and vertebral strength were determined ex vivo. Bone resorption was determined by tartrate-resistant acid phosphatase (TRACP) staining of osteoclasts in histological sections, measurement of bone and serum TRACP-5b, and measurement of urinary deoxypyridinoline (DPD) excretion. Effects of DMAb on aortic morphology and mineralization were analysed by H & E and alizarin red S staining and calcium measurement. Pred treatment reduced vertebral and femoral BMD. Pred-induced bone loss was associated with suppressed bone formation and enhanced bone resorption based on increases in the number of osteoclasts, levels of TRACP-5b in bone and serum, and urinary excretion of DPD. DMAb treatment mitigated bone loss through a pronounced anti-resorptive effect. Compression tests of lumbar vertebrae revealed a detrimental effect of Pred on bone strength that did not occur with concurrent DMAb treatment. In the aortic wall, no morphological abnormalities were detected in huRANKL-KI mice, regardless of treatment. Pred treatment increased aortic calcium deposition compared to vehicle controls, which was lowered by concurrent Dmab treatment. Of note aortic calcium deposition in huRANKL-KI mice was negatively correlated with spinal BMD and positively with DPD excretion. In summary, RANKL inhibition by DMAb reduced glucocorticoid-induced bone loss and vascular calcium deposition in huRANKL-KI mice.

Sources of Research Support: Grant from Center of Regenerative Therapies Dresden (CRTD).

Disclosures: PJK: Employee, Amgen.
Nothing to Disclose: LCH, SH, CG, MS, RGE

P2-190

The Effect of a Dissociative Steroid Receptor Modulator on Molecular Markers of Bone Turnover.

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We previously reported on the characterization of a plant-derived and selective glucocorticoid receptor modulator (SEGRM), Compound A (CpdA), which efficiently inhibits the progression of collagen-induced arthritis, concomitantly in the absence of unwanted diabetogenic effects. In this abstract we want to compare the effect of CpdA on the expression of bone turnover markers with the gene-regulatory effects of the classical steroid dexamethasone (DEX).
Therefore, Q-PCR analysis was performed on cDNA from DEX- or CpdA-treated MG63b and Saos-2 osteosarcoma cells and on fibroblast-like synoviocytes (FLS), derived from patients with RA for the detection of osteocalcin, cathepsin K, monocyte colony-stimulating factor (M-CSF), osteoprotegerin (OPG) and receptor activator of NF-kB ligand (RANKL). Furthermore, we determined the effect of CpdA and DEX on TRAP5b and osteocalcin protein levels in vivo. Finally, we determined the anti-inflammatory effect of both compounds by means of Q-PCR analysis and ELISA.
Results: CpdA and DEX are equally potent in repressing the TNF-induced expression of pro-inflammatory mediators, such as IL-1b, TNFa, MMP-1, MMP-9, IL-6, IL-8 and MCP-1. However, in sharp contrast to DEX, CpdA does not upregulate the expression of cathepsin K, M-CSF and RANKL. Furthermore, CpdA does not downregulate the expression of OPG. As such, CpdA does not affect TRAP5b levels in vivo. Interestingly, the effect of CpdA on osteocalcin expression differs between mice and a human cell line; whereas CpdA downregulates osteocalcin in MG63b, CpdA did not affect osteocalcin levels in mice.
Conclusion: CpdA displays a more favourable profile of gene regulation compared to DEX regarding markers of osteoclastogenesis. Very interestingly and in contrast to previously described SEGRMs, CpdA does not stimulate the RANKL/OPG ratio.

Sources of Research Support: KDB, KVB, PJ & PVD are sponsored by the Fonds voor Wetenschappelijk Onderzoek (FWO)-Vlaanderen.

Nothing to Disclose: GH, VG, KDDB, PJ, KVB, BV, JT, DE

P2-191

Preventing Bone Loss and Weight Gain with Combinations of Vitamin D and Phytochemicals.

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Introduction: Vit. D and certain flavonoids have been shown to regulate both lipid metabolism and bone formation. Treatments that prevent or reverse age-related increase in bone marrow adipocytes could both increase new bone formation and inhibit bone destruction. In this study, we tested the hypothesis that dietary supplementation with a combination of flavonoids with Vit. D inhibits bone loss and decreases adiposity to a greater extent than control or Vit. D alone diets.
Methods: Ovariectomized female rats (12 mo old, N=10, initial BW=240g) were given control (AIN-93M diet alone), Vit. D (10 IU/kg BW/d) or Vit. D + resveratrol (R: 4, 20, or 100 mg/kg BW/d) + quercetin (Q: 20, 125 or 625 mg/kg BW/d) + genistein (G: 16, 64 or 256 mg/kg BW/d) in AIN-93M diet for 8 wk. Rats were weighed, sacrificed for terminal tissue collections. The right femora were collected to measure bone densitometry (DEXA). The right tibias were embedded with paraffin for H & E staining to visualize adipocytes and staining to demonstrate TRAP activity as a marker of active osteoclasts.
Results: The high dose combination treatment (Vit. D+R100+Q625+G256) significantly reduced BW gain compared to control (79±5 vs 62±3 g, $p<0.05$). Results from the DEXA showed that bone mineral density (BMD) and content (BMC) of femora were significantly increased by the high dose combination, although they weren't different from Vit. D alone. However, BMD corrected for BW was significantly greater in the high dose treated group compared to both control and Vit. D groups (4.7±0.05 vs 5.0±0.2 vs 5.6±0.2, $p<0.05$). Histomorphometric analysis of sections from the right tibia indicates that rats receiving the high dose combination have fewer marrow adipocytes (22.86±1.8 vs 17.25±2 vs 12.53±0.8, $p<0.001$) and significantly reduced osteoclast number in trabecular bone compared to both control and Vit. D groups (4.89±0.5 vs 5.51±0.7 vs 2.96±0.6 N.OC/B.Pm, $p<0.05$).
Conclusion: We conclude that in ovariectomized female rats supplemented with Vit. D when combined with R, Q and G improved bone density and reduced body weight gain and marrow adipocytes compared to control and Vit. D supplements alone. Thus, we propose that the synergistic effects of a combination of flavonoids with vitamin D would be effective in preventing osteoporosis and weight gain after menopause.

Sources of Research Support: The Georgia Research Alliance Eminent Scholar endowment (CAB).

Nothing to Disclose: C-YL, J-YY, MAD-F, MWH, SA, SR, AMW, DLH, RL, CAB

P2-192

Prevention of Glucocorticoid-Induced Osteoporosis by the Hexane Extract of Natural Plant *In Vitro* and *In Vivo*.

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Aim of the study: Effective therapeutic options against glucocorticoid-induced osteoporosis (GIO) are limited, which led to increasing demand for natural, effective alternatives. This study was performed to discover novel natural Korean botanical sources for effective GIO treatment *in vitro* and *in vivo*, and to further clarify their molecular mechanisms.
Materials and Methods: Ethanol extracts of 68 native edible Korean plants were screened. To investigate the activity of the hexane extract of 'plant A' against GIO *in vitro* and *in vivo*, cell viability analysis by MTT assay in two osteoblastic cell lines, C3H10T1/2 and MC3T3-E1, and bone mineral density (BMD) measurement in prednisolone (PD)-administered mice were performed. Screening of differentially expressed genes (DEGs) was performed using the annealing controlled primer (ACP) -based differential display RT-PCR.
Results: Ethanol extracts of 68 native edible Korean plants were screened to find effective natural plant sources for treatment of GIO, and 'Plant A' was selected as a final candidate because of its higher inhibitory activity and its novelty. The hexane extract of 'Plant A' inhibited apoptotic cell death of dexanmethasone (Dex)-induced osteoblastic cells in a dose-dependent manner and the most effective concentration of 'Plant A' was 50 μg/ml. The *in vivo* results indicated that 'Plant A' had not only a preventive effect on the bone loss caused by PD, but also promoted bone formation. Eight DEGs showing different mRNA expression levels in osteoblastic cells comparing Dex- with or without 'Plant A' treatment were screened and identified. The expression level of *AnxA6* was significantly decreased by 'Plant A' treatment in the PD-administered mice. Our results indicate that *AnxA6* may be one of the genetic regulators of bone mass and susceptibility to osteoporosis.
Conclusion: This study demonstrates that hexane extract of 'Plant A' possesses anti-osteoporotic activity both *in vitro* and *in vivo* of GIO model.

Sources of Research Support: “GRRC” project of the Gyeonggi Provincial Government, Republic of Korea.

Disclosures: Y-SC: Researcher, Hanlim Pharm. Co. Korea.
Nothing to Disclose: H-YY, S-IY, B-YK, E-RW, S-YJ

P2-193

Osteoprotective Effect of Alfacalcidol in Female Rats with Systemic Chronic Inflammation.

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Studies have shown that alfacalcidol (a hydroxylated form of vitamin D) mitigates glucocorticoid-induced bone loss. This study was undertaken to explore the mechanism and bone microarchitecture of alfacalcidol in rats with systemic chronic inflammation. Thirty female rats (3-month-old) assigned to 3 groups (n=10/group), placebo administration, lipopolysaccharide (LPS) administration, and LPS+alfacalcidol for 12 weeks. Efficacy was evaluated in femur and tibia by dual-energy X-ray absorptiometry and histomorphometric analysis, respectively. The femoral bone strength was assessed by 3-point bending test. Bone formation biomarker (osteocalcin) and resorption biomarker (tartrate resistant acid phosphatase, TRAP) were determined by respective kits. Expression of tumor necrosis factor-alpha (TNF-alpha) in proximal tibia was evaluated by immunohistochemistry. Data were analyzed by one-way ANOVA followed by post hoc test. LPS administration resulted in lower values for bone mineral density and strength of femur, and bone volume, trabecular number and thickness in proximal tibia, but higher values for TRAP, trabecular separation and osteoclast number in proximal tibia, bone formation rate at proximal tibia and periosteal bone, eroded surface at tibial shaft, and TNF-alpha expression in proximal tibia. In contrast to these negative impacts of LPS in bone, oral administration of alfacalcidol significantly increased bone mineral density and strength of femur, bone volume, trabecular number and thickness in proximal tibia, while suppressed TRAP, trabecular separation and osteoclast number in proximal tibia, bone formation rate at proximal tibia and periosteal bone, erosion surface at tibial shaft, and TNF-alpha expression in proximal tibia. Neither LPS nor alfacalcidol affected the femoral total area, osteocalcin, and endocortical mineral apposition rate and bone formation rate at tibia shaft. This study demonstrates that alfacalcidol administrated to female rats for 12 weeks protects bone microarchitecture due to chronic inflammation. Such an osteo-protective role of alfacalcidol may be attributed to a decrease in inflammatory mediator, TNF-alpha. This study suggests a potentially significant prophylactic role of alfacalcidol in bone health of women with systemic chronic inflammation-induced bone loss.

Sources of Research Support: Laura W. Bush Institute for Women's Health, Texas.

Nothing to Disclose: CLS, JJC, JKY

P2-194

Immune Alterations and Inflammatory Response after Iron Overload: A New Murine Model.

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Background: Osteoporosis is a frequent problem in diseases characterized by iron overload, such as thalassemia. The exact role of iron excess in the pathogenesis of low bone mass is still unclear. We have reported that iron overload in BL6 mice leads to bone loss characterized by trabecular and cortical thinning. Increased bone resorption, oxidative stress and reactive oxygen species (ROS) were also observed. Since ROS are associated with inflammatory response, we studied the iron-induced immune response to evaluate relationships to bone remodeling *in vivo*.
Methods: BL6 mice (n=12/group; 6M and 6F, age 2 mos) were treated *in vivo* with iron dextran (ID) (1g/kg IP weekly) or placebo for 2 mos. Circulating TNF-α, IL-6, and IL-1β levels were measured by ELISA. Splenic lymphocyte and myeloid cells were characterized by Immune phenotyping. Basal and activated intracellular cytokine (IC) levels of TNF-α and IL-6 were assessed *ex vivo* by flow cytometry and secreted basal TNF-α levels from parallel cultures by ELISA. Basal and activated ROS levels were tested *ex vivo* in the flow cytometric respiratory burst assay. In addition, macrophage RAW cells were treated *in vitro* with doses of ID and ferric ammonium citrate (FAC) for 24 & 48 hours. TNF-α and IL-1β secretion was tested by ELISA.
Results: Compared to placebo, iron-overloaded mice demonstrated: 1) elevated circulating levels of TNF-α, IL-6, and IL-1β ($p<0.01$) 2) increased basal IC production and secretion of TNF-α and IC production of IL-6 in splenic CD3+ T cells and Gr-1+ monocytes/granulocytes ($p<0.01$), 3) decreased numbers of both CD4+, and CD8+ splenic CD3+T cells with increased CD19+ B cells ($p<0.01$), 4) increased basal, ROS levels in Gr-1+ myeloid cells ($p<0.01$). In addition we found increased TNF-α and IL-1β secretion *in vitro* after RAW cells were treated with FAC, but not with ID, indicating that these effects were mediated solely by ferric iron.
Conclusions: We have previously shown that bone loss after iron overload is associated with increased ROS and resorption. Our present studies indicate that iron overload induces both ROS and inflammatory cytokine responses, including TNF-α and IL-6, and depletes CD3+ T cells. TNF-α is a known mediator of osteoclastogenesis. Overall, our data support the concept that iron overload leads to bone loss through an inflammatory process. Ongoing studies will delineate how iron induces the inflammatory cascade and mediates bone remodeling.

Nothing to Disclose: JT, ZY, HL, SC-R, FPR, RWG, PJG, MGV

P2-195

Diurnal Variation in Bioavailable Oestradiol and Sex Hormone Binding Globulin in Ageing Men with Osteoporosis.

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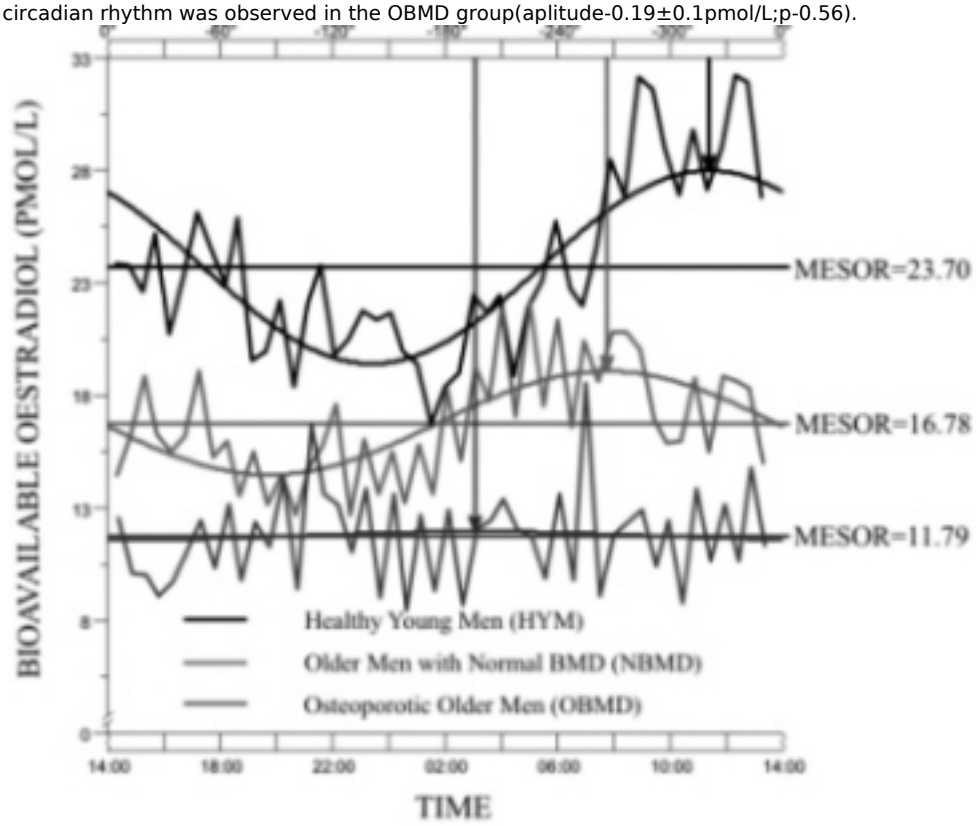
Introduction: The concentration of bioavailable estradiol (BE) is related to bone mineral density (BMD) in elderly men. Serum sex hormone binding globulin (SHBG) concentration is reported to be increased in elderly men and may be correlated with reduced BMD and increased fracture risk. However, there is little data on the exact relation of BMD with 24-hour mean concentration and circadian rhythm of BE and SHBG.
Methods: We measured 24-hour mean BE and SHBG concentration in 3 groups of volunteers matched for body mass indices: 6 healthy young men with normal BMD(HYM) (mean age-27.4±4.6 years),8 older men with normal BMD(NBMD) (mean age-70.5±2.09 years) and 10 older men with osteoporosis(OBMD)(mean age-69.8±3.2years).All individuals had normal plasma concentrations of insulin like growth factor 1 and testosterone. Circadian rhythm parameters were analysed using Chronolab 3.0. The differences between groups were determined using student's 't' test.
Results: The 24-hour mean concentration of BE in the OBMD group(11.79±0.4 pmol/L) was significantly lower than in the NBMD group(16.75±0.4pmol/L; P<0.001) which in turn was significantly lower than in the HYM group(23.7±1.8 pmol/L, p<0.001). BE in both HYM(amplitude-4.2±0.9pmol/L; p-0.02)and NBMD group(amplitude-2.3±0.6pmol/L; p-0.03) demonstrated a significant circadian rhythm with an early morning peak and nadir at night. However, no concerted circadian rhythm was observed in the OBMD group(aplitude-0.19±0.1pmol/L;p-0.56).



The 24-hour mean SHBG concentration in the HYM group (10.88±1.84pmol/L)was significantly lower than in the NBMD group (15.15±1.88pmol/L, p<0.001) which in turn was significantly lower than in the OBMD group (21.38±1.94pmol/L, p<0.001). There were significant SHBG circadian rhythms in all 3 groups with the peak at mid-day and the nadir at night. Circadian rhythm was more pronounced in the OBMD group. Amplitude of the OBMD group was significantly higher than that of the other two groups(p<0.01).
Conclusion: Changes in the 24-hour mean BE and SHBG concentration and alteration of their circadian rhythm are likely etiologic factors in development of osteoporosis in elderly men.

Nothing to Disclose: AM, FJ, AR, ST, AR, AJ, WDF, JPV

P2-196

Anabolic Steroids Slow Hindlimb Bone Loss in a Rat Model of Osteoporosis Due to Spinal Cord Injury: The Role of Wnt Signaling Pathway.

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Spinal cord injury (SCI) causes acute immobilization with immediate and irreversible unloading of skeletal regions. In patients with the most severe neurological compromise, approximately 50% of long bone mineral density (BMD) is lost below the level of lesion. The severe bone loss that occurs after SCI results in pathologic fractures, increased morbidity and cost (1, 2). Bisphosphonates prevent osteoporosis in the elderly, but, at best, modestly reduce SCI-related bone loss, and in some trials, have had no effect (3, 4). Because there are at least 250,000 individuals with SCI or spinal dysfunction in the United States, development of a safe, effective, and affordable therapy for bone boss after SCI remains a high priority. The present study addressed the potential use of nandrolone, a synthetic androgen, to reduce long bone loss after acute SCI. Male rats underwent a mid-thoracic spinal cord transaction; sham-transected rats served as controls. SCI reduced femoral and tibial BMD by about 20% at two months. Bone loss was significantly reduced by the administration of nandrolone along with physiological testosterone replacement during the second month of immobilization. Additionally, mRNA levels present in cultured osteoclasts and osteoblasts derived from bone marrow cells were assessed. SCI significantly increased TRAP and calcitonin receptor mRNA in osteoclasts; in osteoblasts, Runx2 and osteocalcin were dramatically reduced. In osteoclasts derived from marrow of nandrolone-treated SCI rats, TRAP and calcitonin receptor were remarkably similar to sham-SCI animals; in osteoblasts from the nandrolone-SCI group, Runx2 and osteocalcin were modestly, but significantly, increased relative to sham-SCI rats. Canonical Wnt signaling promotes mesenchymal progenitor cells to differentiate into osteoblasts, while blocking osteoclastogenesis by increasing the ratio of osteoprotegerin (OPG) and RANKL, both of which are produced by osteoblasts (5, 6). The OPG/RANKL ratio was reduced in osteoblasts from SCI-rats, but this unfavorable change was reversed in cells from nandrolone-SCI rats. In osteoblasts, SCI reduced LRP5 mRNA, a co-receptor of Wnts, and nandrolone attenuated this effect. Our results demonstrate a beneficial role of nandrolone on protection against bone loss after SCI and suggest a potential mechanism of such action, involving the Wnt signaling pathway in modulation of osteoclastogenesis and osteoblastogenesis.

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Nothing to Disclose: WQ, YP, LS, JP, XL, YW, MZ, WAB, CC

P2-197

Induction of Tibial Marrow Edema by Exercise and Low Energy Availability in Young Men.

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Stress fractures (SF) result from accumulation of microdamage in cortical bone 3 to 6 weeks after a sustained increase in repetitive mechanical loading. In response to various insults, including microdamage accumulation, tissue fluid invades bone marrow and appears in T2-weighted fast-STIR magnetic resonance (MR) images as regions of increased brightness. The incidence of SF in U.S. Army basic training is higher among the slower half of recruits in a 1-mile run test. To investigate the potential benefit of plyometric exercise for preventing SF in basic training, we imposed 100 min of high-impact plyometric exercise (400 drop landings/d at 15 s intervals from 30, 45, 60, and 75 cm) and 100 min of low-impact aerobic exercise (walking on a treadmill at 70% VO2max) on healthy, sedentary, young men (plyometric N=8, aerobic N=6) similar in age, size, body composition and 1-mile run time to the slower half of U.S. Army recruits. We administered these exercise regimens for 8 days in two trials at intervals of 3 months or more to permit recovery from the first trial. In each trial, we controlled diets to match vitamin D and calcium intakes at energy availabilities (EA = energy intake minus exercise energy expenditure) of 45 and 20 kcal/kgFFM/d in random order. Before and after each trial, we obtained fasting morning blood samples and MR images of the tibias in both legs. Low EA suppressed T3 by 14% and IGF-I by 32% (both $p<0.01$), confirming metabolic responses to energy deficiency. Quantifying the gray scale in MR images with image processing software (ImageJ, NIH) revealed that 10 ± 3% of the mid-coronal section of tibial marrow (mean ± SE, $p<0.001$) was brighter post-treatment than any marrow pre-treatment in 27 of 28 tibias. Separately, a blinded, board certified radiologist diagnosed marrow edema by visual inspection in MR images of one or the other tibia after 8 (29%) of 28 treatments, including 1 (17%) of 6 aerobic and 1 (13%) of 8 plyometric exercise treatments at 45 kcal/kgFFM/d and 2 (33%) of 6 aerobic and 4 (50%) of 8 plyometric exercise treatments at 20 kcal/kgFFM/d. 1-mile run time was 1 min slower in the diagnosed men (9:26 ± 0:22 vs 8:26 ± 0:14 min:s, $p=0.04$). We conclude that this experimental design and protocol are a useful human model of microdamage accumulation in the tibia. The incidence of diagnosed marrow edema was not greater in subjects doing plyometric exercise than aerobic exercise ($p=0.36$), but low EA may increase the incidence ($p=0.11$).

Sources of Research Support: US Army Bone Health and Military Medical Readiness Research Program Award Number W81XWH-07-1-0251 and Ohio University.

Nothing to Disclose: ABL, LB, MWC, DJD, JSB, LAD, RMH

P2-198
Effect of Diet Cola on Urine Calcium Excretion.

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Background: Earlier studies have shown that young, healthy women who drink colas, when all other factors are controlled, have lower bone mineral density and increased fracture rate compared to non-cola drinkers. The reason for this is unclear.
Our group has previously observed that within 45 minutes after diet cola consumption, there is a brief dip followed by a surge in PTH. This increase in circulating PTH was accompanied by a parallel increase in alkaline phosphatase in our prior study. We hypothesized based on that data that calcium would be mobilized from bone at this time, and since serum calcium levels did not change, it was possible that this mobilized calcium was excreted in the urine. In this study we tested the hypothesis that urinary calcium excretion would increase after diet cola consumption. We measured urine calcium and phosphate excretion in urine for 3 hours after ingestion of 24 ounces of diet cola or an equal volume of water.
Methods: We recruited 20 healthy women age 18-40 to participate in the study. Exclusion criteria were recent fracture within the previous 6 months, known bone disease or vitamin D deficiency, steroid or diuretic use, breast feeding and vitamin D supplementation above current US RDA. Subjects were randomized to drink 24oz of water or diet cola on 2 study days. Their urine was collected for 3 hours after ingestion of the designated beverage and assayed for calcium, phosphorous and creatinine by our clinical lab using standard assays. Data were analyzed for 16 subjects; 4 were excluded due to lab error or subject non adherence with the protocol. Paired data was unavailable for 2 subjects included in analysis. Calcium and phosphorous excretion per 3 hours after water consumption were compared to values after cola consumption using 2 tailed paired Students T test.
Results: Mean calcium excretion over 3 hours after cola ingestion was 6.85mg higher than after water ($p = 0.004$). Mean phosphorous excretion was 41mg higher in the cola group ($p = 0.003$)
Conclusion: Our study suggests that diet cola ingestion may result in a negative calcium balance acutely in young, otherwise healthy women. This may help explain the clinically observed decrease in BMD and increased fracture rate in women who consume these drinks regularly.

Nothing to Disclose: NSL, RA, CO, MAP

P2-199

Rapid Changes in Bone Mineral Balance in Response to Estrogen Depletion in Rhesus Monkeys Detected Using a Tracer-Less Calcium Stable Isotope Technique.

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Significance: Understanding of bone metabolism and therapeutic development are hampered by an inability to detect rapid changes in bone mineral balance (BMB) or bone mineral density. Conventional biochemical markers provide information on relatively short-term changes in bone formation and resorption separately, but they reflect bone remodeling rate not precise quantifiable changes in BMB. By contrast, the natural Ca isotopic composition of soft tissue and urine directly reflects BMB, not remodeling rate. High-precision measurements of changes in the natural Ca isotopic composition of urine reflect changes in BMB after four weeks of bed rest in humans(1). Here we test the ability of Ca isotopic analysis to detect changes in BMB within 2 weeks of estrogen-depletion bone loss using a well-developed nonhuman primate model, the female rhesus monkey.
Study design: Six adult (12-16 years) female rhesus monkeys were randomized to control (C, n=3) or treatment (MPA, n=3) groups. Animals in the MPA group received an intramuscular (i.m.) injection of 150 mg of medroxyprogesterone acetate (MPA, a known suppressor of ovarian estrogen lasting ~30 days/dose) on days 0, 28 and 56. At the same timepoints, C animals were given volume-matched saline i.m. Urine was collected for Ca isotopic analysis on days -7,-1,0,1,2,4,6,7,8 and 14. Ca analyses were done by inductively coupled plasma mass spectrometry (Neptune ICP-MS, Thermo Scientific, Waltham, MA). Conventional bone biochemical markers and circulating estradiol levels also were measured.
Results: Ca isotopes revealed a more negative BMB in MPA compared to C females from an increased negative slope ($p<0.05$) in Ca isotope ratios between baseline (day -7) and treatment period (days 1-14; C:-0.011±0.012, MPA:-0.046±0.018). All values were adjusted for age as baseline Ca isotope ratios were inversely correlated with age ($r2=0.87$, $p<0.01$), indicating naturally occurring increased bone mineral loss in older animals. Compared to baseline, estrogen was decreased ($p<0.05$) and osteocalcin and NTx levels were unchanged in MPA females.
Conclusion: Mass spectrographic measurements of urinary Ca reveal loss of bone Ca within a week of onset of estrogen depletion in adult female rhesus monkeys while traditional markers of bone turnover did not change. Such sensitive and rapid detection of bone mineral loss promises new insight into understanding short-term dynamics in bone metabolism.

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Nothing to Disclose: JLM, DHA, AA, GG, JLS, RJC

P2-200

Comparison of Skeletal Changes in Rat Models of Ovary-Intact vs. Surgical Menopause.

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The industrial chemical 4-vinylcyclohexene diepoxide (VCD) induces gradual ovarian failure in rodents by accelerating the natural process of ovarian follicle atresia. The residual ovarian tissue in follicle-deplete VCD-treated mice continues to secrete bone-protective androstenedione (ADIONE). Wright *et al.* have demonstrated that bone loss following VCD-induced ovarian failure is blunted and delayed vs. OVX mice. Experiments were undertaken to determine whether rats, commonly utilized in osteoporosis research, exhibit similar skeletal changes during the transition to and onset of VCD-induced ovarian senescence. Young (1 month) Sprague Dawley female rats were administered VCD (80 mg/kg or 160 mg/kg) or vehicle (DMSO, 1.25 µL/g) ip for 25 days to deplete primordial and primary ovarian follicles, then followed for 570 days to observe skeletal changes assessed by DXA analysis of proximal tibia bone mineral density (BMD). Prior to changes in ovarian function, assessed by circulating follicle-stimulating hormone (FSH), ADIONE, and 17b-estradiol (E2) levels and vaginal cytology, high dose VCD exerted an adverse effect on BMD that persisted throughout the experiment. Despite reduction in follicle counts by both VCD doses, only high dose VCD accelerated the onset of ovarian senescence, as defined by increased FSH and cessation of regular cyclicity. BMD in high dose VCD rats remained unchanged with the onset of irregular cyclicity and subsequent persistent estrus, an anovulatory phase unique to rodents. When persistent estrus ended and ovarian senescence ensued in high dose VCD rats (day 403), ovariectomy (OVX) was performed on a subset of age-matched, vehicle-treated controls. BMD fell by 15% in the OVX rats over the subsequent 5.5 months, while BMD in high-dose VCD and control animals remained unchanged. ADIONE levels, which were undetectable in OVX rats, remained at control levels in VCD rats, while E2 levels, which fell in OVX rats to 35% of age-matched controls (55% of E2 levels in young cycling), were also no different than controls. These studies suggest that depletion of ovarian follicles by VCD in rats, while accelerating the onset of persistent estrus and ovarian senescence, models neither the hormonal milieu nor skeletal changes characteristic of human menopause. Additionally, while bone loss in OVX animals does mimic that seen in menopausal women, the hormonal milieu does not, as depletion of androstendione, rather than E2, is its most striking feature.

Nothing to Disclose: ALL, JBF, LEW, SLM, PBH, JLF

P2-201

Diet Interactions in the Lipoxygenase 5 Enzyme System Is Associated with Significant Bone Loss.

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Lipoxygenase 5 (5-LO, *Alox5*) catalyzes the generation of LTB4 from arachidonic acid; LTB4 is a chemokine that enhances an inflammatory response and is implicated in plaque generation and development of atherosclerosis. Polymorphisms in the *Alox5* gene and its associated protein have been linked to cardiovascular disease; intriguingly, *Alox5* is also a candidate gene regulating peak bone mass in mice. We hypothesized that a high fat diet (HFD) in association with deletion of the *Alox5* gene could result in a major skeletal phenotype. We fed 3 week old male B6 controls and *Alox5* null mice either a regular chow diet (10% fat by kcal) or a HFD (45% fat by kcal) for 13 weeks post weaning. We then examined skeletal phenotype by DXA, uCT, serum bone turnover markers, histomorphometry, and cell culture. At 16 weeks on a regular diet, whole body and femoral areal BMD were significantly higher in *Alox5-/-* than in B6 ($p<0.01$). Similar phenotypic differences were noted by uCT in the femur. *Alox5-/-* mice had higher serum osteocalcin and PINP levels ($p<0.001$) than B6. In vitro, bone marrow stromal cell cultures from *Alox5-/-* mice demonstrated more ALP positive and von Kossa staining colonies compared to B6. The number of osteoclasts/bone perimeter was less by histomorphometry in *Alox5-/-* vs. B6 ($p=0.06$). On the HFD, there were marked strain differences such that B6 showed increased bone mass in the spine and femur. In contrast, *Alox5-/-* mice on a HFD had lower total body bone mass, femoral and vertebral trabecular BV/TV than *Alox5-/-* mice on a regular diet which is accompanied by reduced trabecular number and increased osteoclasts number/bone perimeter. Serum Trap5b in the *Alox5-/-* mice was greater on the HFD while P1NP decreased significantly ($p=0.04$). Serum leptin increased significantly in B6 and *Alox5-/-* mice on the HFD vs. regular diet ($p<0.04$) but was not significantly different between strains. In sum, mice lacking the gene for *Alox5* show significant bone loss with increased dietary fat. We postulate a gene associated with diet interactions in the lipoxygenase 5 enzyme system causes significant bone loss by suppressed bone formation and increased bone resorption. Polymorphisms in *Alox5* are common in the general population and are associated with atherogenesis. Our findings suggest an important interaction between dietary fat intake and genetic predisposition to osteoporosis.

Nothing to Disclose: PL, MK, SB, MH, CRR

P2-202
Assessment of FGF23 in Two Cases of Vitamin D Toxicity.

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Introduction: VD intoxication is characterized by hypercalcemia, but rarely hyperphosphatemia. The physiopathology of VD toxicity is not completely understood. The counterregulatory mechanisms involve PTH and 1a hydroxylase suppression and possibly an increase of FGF23 levels, explaining why phosphate (Pi) is not increased. To test this hypothesis, two different cases of VD intoxication were analyzed.
Case 1: 59 year-old woman presented with mental confusion, dehydration, nausea and vomiting. Laboratory tests disclosure: hypercalcemia (15 mg/dL, N: 8.6-10.2), normal Pi level (3.6 mg/dL, N: 2.3-4.6), low PTH (10 pg/mL, N: 10-62) and impaired renal function (Cr = 2.7 mg/dL, N: 0.5-0.9). She was initially hydrated and treated with bisphosphonate showing reduction of calcemia, renal recovery and improvement of symptoms. Four months later, she revealed normocalcemia, still had low PTH (16 pg/mL) and high levels of 25OHD (274-335 ng/mL, N 30-150) and $1,25(OH)_2D$ (77.3, N 15.9-55.6) that could not be studied before. 25OHD measurement by HPLC proved that VD toxicity was due to cholecalciferol but the source was not identified. At this moment, FGF23 value was increased (345 pg/mL, N: 10-50). During the next months, there was a progressive reduction of 25OHD and FGF23 levels until normal values.
Case 2: 45 year-old man who takes calcitriol due to auto-immune hypoparathyroidism revealed symptoms of VD intoxication after increase in calcitriol dosage. He presented hypercalcemia (11.2 mg/dL), normophosphatemia (2.9 mg/dL) and elevated FGF23 (133 pg/mL). Bone biochemistry was reestablished after treatment.
Discussion and conclusion: FGF23 is a hormone produced by bone which leads to renal Pi wasting and regulates the VD metabolism, however there is no report of FGF23 levels during VD intoxication. The main stimuli for FGF23 release are high Pi and increased $1,25(OH)_2D$ levels. In these two reported cases, the high FGF23 levels should help in the maintenance of normophosphatemia. Because during untreated hypoparathyroidism (low PTH and $1,25(OH)_2D$ levels), hyperphosphatemia is common and often decreases after therapy, calcitriol seems to be more important than hyperphosphatemia to stimulate FGF23 secretion. Therefore, we believe that in these two different cases of VD intoxication, the high levels of $1,25(OH)_2D$ may be an important inducer for FGF23 elevation and this rise perhaps represents another counterregulatory mechanism for the VD toxicity.

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Vieth R; J Bone Miner Res 2007; 22:64-68
Liu S et al.; J Am Soc Nephrol 2006; 17:1305-1315

Nothing to Disclose: APS, LZB, UZ, PHSC, RMM

P2-203

Vitamin D Intoxication Due to Manufacturing and Labeling Errors of a Dietary Supplement: A Case Report.

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Background: 45-55% of Americans use dietary supplements. However, the manufacturing of these supplements is not well supervised by the FDA and their intake by patients is not always specifically queried by physicians. Vitamin D intoxication is rare with only a few reported cases in the literature. We describe a patient who presented with Vitamin D intoxication from a dietary supplement with both labeling and manufacturing errors.
Case: A 58 year old male was admitted with obtundation and a serum calcium of 15.0 mg/dl. For the prior 3 weeks he had complained of fatigue, excessive thirst, polyuria, and poor mentation. Hypercalcemia was managed with IV saline hydration, lasix and calcitonin.
At first the patient denied taking any medication. Upon serial questioning he noted taking multiple dietary supplements for 2 months prescribed by a physician in London. Serum 25(OH)D 1220 ng/ml (30-80ng/ml) and 1,25(OH)2D 153 pg/ml (15-75 pg/ml). One of the supplements, Formula F, was purported to contain 40 mcg Vitamin D (1600IU), 99% as Vitamin D3. We analyzed the product by HPLC and found instead that a manufacturing error rendered each capsule to contain 4660mcg Vitamin D3. A labeling error had recommended taking 10 capsules instead of 1 per day. Thus he had been taking 46,600 mcg, (1,864,000 IU) Vitamin D3 daily for 2 months. He was also taking hormonal supplements prescribed a physician in California. Serum levels of these hormones were normal.
The patient was discharged on oral hydration, a low calcium diet and no dietary supplements. He was readmitted 1 week later with a calcium of 12.2. Pamidronate 60 mg IV and IV hydration were prescribed. He was discharged 1 week later on the former prescription, with the addition of 1-2 liters of IV hydration at a local hospital every 4 days as needed. This was required for 2 wks after which the patient stabilized.
Conclusions: 1. Vitamin D intoxication should be considered in the differential diagnosis of hypercalcemia. 2. It is important to include a history of dietary supplement use when inquiring about a patient's current medications. This may require repeated questioning as patients may not consider these supplements as potential health risks. 3. Patients may be taking more than 1 dietary supplement concurrently. 4. Errors in the manufacturing and labeling of dietary supplements may place patients at increased risks for side effects. There needs to be better monitoring for the vitamin D content in supplements.

Nothing to Disclose: TA, BA, MFH, LGN

P2-204

Vitamin A Toxicity: An Unusual Cause of Bone Pain in a Patient with Metastatic Breast Cancer.

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Introduction: Both patients and clinicians often overlook vitamins and supplements when discussing medication usage. We present a case of a breast cancer survivor with bone pain caused by vitamin A toxicity, rather than bone metastases.
Clinical Case: A 60 year old female was referred for evaluation of back and pelvic pain. She had been diagnosed with breast cancer at age 46, and treated with a right modified radical mastectomy and chemotherapy. After four years, she underwent total abdominal hysterectomy with bilateral salipingo-oophorectomy due to metastatic disease to the ovaries, and remained disease-free. The patient now reported back and pelvic pain of approximately four months' duration. Plain films of the lumbosacral spine showed no evidence of lytic or blastic lesions. Bone scan demonstrated increased tracer uptake in the right and left sacroiliac (SI) joints, consistent with metastatic disease. MRI was also consistent with metastases. CT scan revealed bilateral sclerosis of the sacral wings, as well as linear lucencies beneath and parallel to the SI joints bilaterally, consistent with stress fractures. The patient originally listed Halotestin as her only medication. On further questioning, she also reported taking numerous vitamins and supplements including > 60,000 IU of vitamin A and up to 7500 mg of calcium daily. Serum vitamin A level was 160 mcg (nl 30-95), calcium 9.3 mg/dL (nl 8.4-10.2), phosphorus 3.0 mg/dL (nl 2.5-4.5). Reluctantly, the patient eventually stopped taking vitamin A with rapid resolution of her bone pain. On repeat bone scan, she had persistent activity in the SI joints. Plain films demonstrated sclerosis near the SI joints consistent with healed fractures. Retinoic acid has been shown to increase bone resorption through multiple mechanisms including increased osteoclast formation, decreased osteoblast activity, and interference with the ability of vitamin D to regulate calcium homeostasis. Observational studies in humans indicate that excessive vitamin A intake can decrease bone mineral density and increase fracture risk. Given the clinical improvement in this patient and the evidence of healed fractures on radiographs, the patients bone pain was attributed to stress fractures due to vitamin A toxicity, and not to metastatic breast cancer.
Conclusion: This case highlights the importance of probing patients for vitamin and supplement intake when taking a history in order to better facilitate diagnosis.

Nothing to Disclose: YTH, SW

P2-205

Rhabdomyolysis Associated with Intravenous Zoledronic Acid Infusion for the Treatment of Postmenopausal Osteoporosis.

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Introduction: Bisphosphonates are widely prescribed for the treatment of osteoporosis and are generally well tolerated. While intravenous bisphosphonates are known to cause musculoskeletal symptoms including myalgias in up to one third of patients, there have been no published reports of rhabdomyolysis.
Clinical Case: A 74 year old female with a history of poor nutritional status was diagnosed with osteoporosis by routine screening. Her DXA showed a T score of -3.1 at the forearm and -2.4 at the left femoral neck (spine measurements were limited by artifact). Several months prior, her 25 hydroxy-vitamin D level was 21.7 ng/ml (n 30- 100) with a calcium of 9.1 mg/dl (n 8.5- 10.5) for which she received replacement vitamin D3. She was not able to tolerate calcium supplementation or multivitamin. Due to a history of peptic ulcers, oral bisphosphonates were avoided and she received an infusion of Zoledronic Acid 5mg. Approximately 12 hours after the infusion, she developed significant nausea and vomiting accompanied by generalized muscle weakness. After 24 hours of symptoms, evaluation revealed a marked elevation of serum CPK to 6793 IU/l (n 20- 165), with normal CK-MB. A diagnosis of rhabdomyolysis was suspected and no causative drugs, toxins or metabolic disturbances were identified. She was admitted and treated with intravenous hydration. Serum CPK peaked at 8171 IU/l and was associated with a corrected calcium of 7.8 mg/dl, phosphorus of 2.1 mg/dl (n 2.7-4.8), PTH of 107 pg/ml (n 10- 65) and 25 hydroxy-vitamin D of 39.8 ng/ml. Calcium and phosphorus replacement was attempted. The patient was discharged home 1 week later with a CPK of 486 IU/l and normal calcium and phosphorus levels.
Conclusion: This case demonstrates a temporal relationship between the infusion of a bisphosphonate and the development of rhabdomyolysis with subsequent hypocalcemia and hypophosphatemia. In-vitro studies have shown that bisphosphonates can induce apoptosis in L6 rat myoblasts by inhibition of farnesyl pyrophosphate synthase, an enzyme in the mevalonate pathway and potentiates muscular cell death (1). While not previously reported in humans, this case demonstrates rhabdomyolysis after IV bisphosphonate therapy. Bisphosphonates should be used with caution in patients with other risk factors for rhabdomyolysis (e.g. poor nutritional status such as hypophosphatemia, drugs that inhibit the mevalonate pathway such as statins).

(1) Nishiguchi T, et al. Synergistic action of statins and nitrogen containing bisphosphonates in the development of rhabdomyolysis in L6 rat skeletal myoblasts. J Pharm Pharmacol. 2009; 61: 781-8.

Nothing to Disclose: MWS, GG

P2-206

Lansoprazole Induced Hypocalcemic Seizure Fourteen Years after Thyroidectomy.

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Introduction: Hypoparathyroidism is a known complication of thyroidectomy. Calcium carbonate, a common form of calcium supplementation, requires an acidic gastric pH for absorption. Lansoprazole is a potent proton pump inhibitor (PPI) which impairs gastric acid secretion and thereby limits the absorption of calcium carbonate. We report a case of PPI induced hypocalcemic seizure and tetany.
Clinical Case: A 48 year old female with a history of Graves' disease, treated by total thyroidectomy 14 years prior, presented with hand cramps and a generalized seizure in the setting of hypocalcemia. After her thyroidectomy the patient had been asymptomatic on 1800 mg of elemental calcium, taken in the form of calcium carbonate, and 1200 IU of cholecalciferol (vitamin D_3) daily. Twelve days prior to her presentation, lansoprazole 30 mg twice daily was initiated as part of treatment for *H. pylori* infection.
Physical exam revealed a Chvostek's sign. Laboratory data was significant for low total serum calcium of 5.3 mg/dL (nl 8.5-10.5 mg/dL) with an ionized calcium of 2.51 mg/dL (nl 3.6-5.1 mg/dL), inappropriately low intact PTH of 5.8 pg/mL (nl 10-65 pg/mL) and elevated serum phosphorus of 4.8 mg/dL (nl 2.5-4.5 mg/dL). The serum magnesium, creatinine, 25- hydroxyvitamin D and 1,25- dihydroxyvitamin D levels were normal. EKG revealed a prolonged QT_c interval of 576 msec (nl ≤ 440- 460 msec).
The patient was diagnosed with hypoparathyroidism and treated with intravenous calcium gluconate, oral calcium carbonate and calcitriol. Her symptoms resolved after the initiation of intravenous calcium. Lansoprazole was discontinued on admission. As an outpatient she remained asymptomatic off lansoprazole, taking 1000 mg of calcium carbonate three times per day (equivalent to 1200 mg of elemental calcium daily) with meals and calcitriol 0.25 micrograms daily.
Clinical Lessons: Patients with a history of total thyroidectomy may have unrecognized hypoparathyroidism. Caution should be exercised in prescribing proton pump inhibitors to patients with a history of thyroidectomy or hypoparathyroidism when calcium carbonate supplementation is used.

Nothing to Disclose: SM, EJE

P2-207

Hepatitis C Associated Osteosclerosis (HCAO).The Effect of Interferon-alpha Therapy on Clinical and Biochemical Evolution.

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Background: Osteosclerosis is a rare complication of Hepatitis C virus (HCV) infection. There are no references as to whether specific HCV therapy with Interferon could modify or ameliorate the clinical picture. **Clinical Case:** A 48-year-old afro-caribbean man with a history of HCV infection was referred for evaluation of pain at pretibial areas, thighs and forearms with diffusely increased radioisotope uptake on bone scan (superscan pattern). Forearms and legs were painful on palpation. Increased temperature over the tibial surface, without deformities, was noticed. We found elevation of phosphorus 4.9mg/dl(2.3-4.6), alkaline phosphatase (ALP) 808UI/L(98-295), bone specific ALP(BALP) 90.9ng/ml(7.5-17), osteocalcine 86.69ng/ml(4-12), β-Crosslaps 2.5ng/ml(0.14-0.5) and $1,25(OH)_2vitaminD_3$ 150pg/ml(16-65). The rest of parameters were normal (25(OH) vitaminD, Intact PTH (iPTH), AST, ALT, serum and urinary calcium). Viral load was 2151.00 U/ml Log 3.33. Radiographic study showed generalized osteosclerosis with increased cortical thickness in long bones and lumbar spine. DXA showed an elevation of BMD and T-scores at lumbar spine (T-score: 5.1) and hip (T-score:5.2). At Tc99 bone scan, a generalized increased uptake, with the exception of cranium, was seen. The patient received vitamin D and analgesics with no improvement of limb pain during the following 6 months. By that moment the viral load raised to 2949 U/ml Log 3.33, detecting as well an increase in: AST (60UI/L)(5-45), ALT (72UI/L)(5-45), osteocalcine (156ng/ml), iPTH (83,2pg/ml) and $1,25(OH)_2vitaminD_3$ (129,9pg/ml). Interferon α and Ribavirine were initiated, observing that viral load was undetectable and AST and ALT were normalized in two months. Six months later the pain had disappeared and ALP and iPTH had fully normalized with a significant reduction in the rest of parameters: osteocalcine 65ng/mg, $1,25(OH)_2vitD_3$ 97 pg/ml and BALP 57ng/ml. BMD continued to rise on the hip (rate of change/yr 7.7%) but diminished on lumbar spine (change/yr:-3.1%). Bone scan showed a marked reduction in isotope uptake. **Conclusion:** Interferon therapy can improve the clinical evolution of HCAO by reducing the accelerated bone turnover, suggesting that the persistence of HCV infection or the linked disturbed immunity play a role in the unbalanced bone remodeling and derangement of vitamin D metabolism.

Nothing to Disclose: CB, EG, RS, RM, FH

P2-208

High Serum 25-Hydroxy Vitamin D in an Albino African-American.

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Background
Skin pigment has been postulated to be a cause of variability in serum 25-hydroxy vitamin D (25OHD) levels between African American (AA) and Caucasian individuals. Although differences in the parameters of vitamin D endocrine system between Caucasians and persons of African ethnicity have been studied, data on how decrease of skin pigmentation in albinism effects vitamin D regulation is lacking. A very high level of serum 25OHD and other parameters of the calcium-vitamin D endocrine system in an albino AA are reported.

Clinical case
A 39-year-old AA woman with hypertension and systemic lupus erythematosus was referred to the Endocrine clinic after she was found to have a serum 25OHD level of 95 ng/ml (ref 30-80) on routine testing. She had history of osteoarthritis and fibromyalgia. She never had a fracture or renal calculus. She was obese (BMI 61 kg/m^2), had hypopigmented skin, white eyebrows and eyelashes, light brown scalp hair and pale irises, consistent with albinism. She had horizontal nystagmus. Her sister and second cousin also had features of albinism. Her biochemical parameters were: serum calcium 9.4 mg/dl (ref 8.5-10.1), phosphorus 3.4 mg/dl (ref 2.5- 4.9), serum albumin 3.9 g/dl, magnesium 1.86 mg/dl (ref 1.8-2.4), parathyroid hormone (PTH) 59 pg/ml (ref 7-53), 25OHD 106 ng/ml, 1,25 dihydroxy vitamin D 45 pg/ml (ref 15-75), alkaline phosphatase 81 U/l (ref 33-110). Thyroid function tests, renal and liver function tests were normal. 24 hr urinary volume was 2 liters, calcium 80 mg/24 hours and phosphorus 931 mg/24 hours. Bone mineral density (BMD) at left forearm was 1.154 gm/cm2 (T score 3.0, Z score 2.3). BMD at spine and hips could not be measured because of the high BMI.

Conclusions
Genetic lack of skin pigmentation leads to high levels of serum 25OHD, without any acute toxic effects. The vitamin D endocrine system regulates itself to keep the levels of serum 1,25 dihydroxy vitamin D, serum calcium, and phosphorus in the normal range. As has been reported in African Americans without albinism, the serum PTH still continues to be slightly high, urine calcium low, and BMD high, although higher than normal serum PTH value is not completely explainable. Studies on calcium vitamin D endocrine system in albino African population may lead to insight into the complex regulation of this system.

Nothing to Disclose: AS

P2-209

Bisphosphonates for the Treatment of Premenopausal Osteoporosis: Perils, Pitfalls, and Pragmatism.

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Traditionally, osteoporosis is considered a disease of elderly females. However, a new generation of younger, premenopausal women with osteoporosis is emerging. While the evaluation of premenopausal osteoporosis is similar to postmenopausal women, treatment options differ. We outline two cases of premenopausal osteoporosis and the treatment courses.
Case 1: HS is 23 year old Caucasian female referred for an incidentally found T 11 compression fracture. DEXA showed a Z score of -2.4 at L2-L4 spine and -1.7 at the femoral neck. Risk factors for osteoporosis included: prednisone bursts for asthma and a 10 year smoking history. Lab evaluation was normal. Her osteoporosis was due to extensive smoking and steroid use. She was educated on lifestyle modifications, including weight bearing exercise and smoking cessation, and started on calcium and vitamin D. Given the patient's age, desire for pregnancy, and reversible risk factors, she was not treated with bisphosphonate therapy.
Case 2: SS is a 35 year old Caucasian female referred after a DEXA, done 9 months after the birth of her first child, revealed a Z score of -2.6 at L1-L4 spine and -1.8 at the femoral neck. She was now 4 months post delivery of her second child and nursing. SS also had a history of DVT treated with low molecular weight heparin (LMWH) and family history of osteoporosis. She was diagnosed with Pregnancy and Lactation-Associated Osteoporosis (PLO) in the setting of LMWH. Given that PLO is typically reversible, we recommended cessation of breast-feeding and daily calcium/vitamin D. Repeat DEXA six months later showed no improvement. As she had no further plans for pregnancy, bisphosphonate therapy was considered.
These cases illustrate the issues in treating premenopausal osteoporosis. If a clear etiology is identified, treatment consists of correcting the underlying cause, lifestyle modifications, and calcium and vitamin D. Bisphosphonates are the only pharmacological agents that play a role and are presently FDA approved for premenopausal women on glucocorticoid therapy. These agents are safety Category C and contraindicated in pregnancy. Preconception and first trimester exposure has been associated with neonatal hypocalcemia, lower birth weight, and increased rate of spontaneous abortion. While bisphosphonates are frequently used for postmenopausal osteoporosis, they should be judiciously used in women of reproductive age given the lack of efficacy and safety data.

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Nothing to Disclose: SKD, ESN, KLB, MJT, SGK

P2-210

Severe Asymptomatic Hypocalcemia and Elevated Serum Intact PTH Levels Induced by Intravenous Magnesium Sulfate Administration.

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Background: Hypermagnesemia resulting from intravenous infusion (IV) of magnesium sulfate ($MgSO_4$) to treat patients with eclampsia can lead to rapid suppression of parathyroid hormone (PTH) secretion and a subsequent decrease in serum calcium (Ca) concentration. We report a case of severe asymptomatic hypocalcemia and elevated serum PTH levels induced by IV $MgSO_4$ administration.
Clinical case: A 37 year-old woman, 2-week-postpartum, was admitted with a diagnosis of postpartum preeclampsia. Pregnancy was complicated by gestational hypertension and hyperemesis gravidarum. During the evaluation of gestational hypertension, 24-hr urine protein was normal. After an uneventful delivery, her BP remained normal. Two weeks after discharge, patient presented to hospital with headaches, blurred vision and BP of 195/125 mm Hg. A diagnosis of delayed postpartum pre-eclampsia was made and IV $MgSO_4$ (2-4gm/hr) was started.
On hospital day 2, severe hypocalcemia was noted. EKG revealed QT prolongation 545 ms. At this time, IV $MgSO_4$ was stopped. Examination revealed negative Chvosteks' and Trousseau's signs. Laboratory values are shown below.

Correlations of serum Mg, Ca, and PTH levels.

Time points	Mg (mg/dL)	Ca (mg/dL)	Phosphorus (mg/dL)	Intact PTH (pg/mL)
Before Mg infusion	2.2	9.7	3.7	35
During Mg infusion	7.2	6.5	3.5	not obtained
12hr after infusion discontinuation	3.4	7.4	3.1	192
36 hrs after infusion discontinuation	2.2	8.8	3.7	132
At discharge	2.0	9.4	4.1	49
2 weeks follow-up	2.0	9.7	4.8	52

Serum albumin was 4.0-4.3 g/dL. Normal range: serum Ca 8.7-10.4 mg/dL, Mg 1.7-2.4 mg/dL, PTH 15-65 pg/mL, Phosphorus 2.5-4.5 mg/dL.

Other labs: ionized Ca 4.5 mg/dL (4.8-5.6), calcitriol 80 pg/mL (18-72), calcidiol 40 ng/mL (30-80). Within 48 hours of discontinuation of $MgSO_4$ infusion, serum Mg, Ca and PTH levels normalized as did the QT interval. Labs remained normal 2 weeks later.
Conclusions: The etiology for the asymptomatic nature of the hypocalcemia may be due to the suppressive effect of hypermagnesemia on neuromuscular transmission. The discrepancy in serum PTH levels noted in our patient versus previous studies may be due to (1) the use of first generation PTH assays and /or (2) the timing of PTH measurements which missed the initial suppression (described in prior reports) but captured the exuberant recovery of PTH as serum Mg level decreased. These findings indicate that the degree of hypocalcemic stimulus on PTH is related to the severity of hypermagnesemia.

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Nothing to Disclose: TDH, VQM, HDN, PWC, BCG, KMMS

P2-211

Hypercalcemia Due to Parathyroid Carcinoma Presenting in the Third Trimester of Pregnancy.

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[1]Univ of Auckland Hamilton, New Zealand ; [2]Royal North Shore Hosp Sydney, Australia ; [3]Waikato Hosp Hamilton, New Zealand and [4]Kolling Inst of Med Res Sydney, Australia.

Background: Primary hyperparathyroidism in pregnancy is uncommon and management in the third trimester of pregnancy is controversial. There is a known association between parathyroid adenomas and pre-eclampsia however only rare case reports of parathyroid carcinoma in pregnancy exist.
Case: A 33 year old woman presented with pre-eclampsia at 32 weeks gestation. She had an incidental finding of hypercalcemia (corrected Ca^{2+} 3.27mM [RR 2.1-2.55]) with an elevated PTH (19.1pM [RR 1.6-6.2]). She had recent onset fatigue, anorexia and nausea. No previous Ca^{2+} levels were available. She had no relevant family history and no history of renal calculi, bone pain or fractures. No neck masses were palpable. Renal function was normal. Ultrasound confirmed bilateral nephrolithiasis and in the neck a 3cm mass consistent with a solitary parathyroid tumor.

Despite medical management serum Ca^{2+} remained 3.25-3.45mM. As fetal ultrasound showed reduced growth, she was induced at 34 weeks after a course of glucocorticoids. A 1.88kg female infant was delivered by emergency Cesarean due to fetal distress. No neonatal hypocalcemia occurred. The mother was discharged 4 days postop with a serum Ca^{2+} level of 3.17mM. Elective parathyroidectomy was planned for 4 weeks postpartum.

Ten days postpartum she represented with symptomatic hypercalcemia (serum Ca^{2+} 3.81mM) and PTH 25pM. A Tc^{99m} sestamibi scan of the neck and mediastinum was negative. At surgery a 3cm hard mass was adherent to the right thyroid lobe, and resected *en bloc* with the right hemithyroid due to suspicion of parathyroid carcinoma. Histology revealed an 8g mass consistent with parathyroid carcinoma. There was no extracapsular invasion. Parafibromin staining was negative and PGP9.5 staining was positive supporting the diagnosis of carcinoma.(1) Postoperatively her Ca^{2+} and PTH normalized to 2.28mM and 1.6pM, respectively. Gene testing for HRPT2 mutation is awaited.
Clinical Lessons: Parathyroid carcinoma is rare but can occur in pregnancy and, is likely associated with pre-eclampsia. Whilst the outcome in this case was satisfactory, parathyroidectomy in pregnancy has a low complication rate(2,3) and early surgical intervention for primary hyperparathyroidism in pregnancy should be considered particularly if there is concomitant pre-eclampsia or a poor response to medical treatment, to allow time for fetal maturation. All patients with parathyroid carcinoma should be offered genetic testing for HRPT2 mutation.

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Nothing to Disclose: RGP, MSE, AJG, IDB, DJM, LW, GYM-R, JVC

P2-212

A Case of Hepatitis C-Associated Osteosclerosis with Chronic Cholecystitis.

Y Komatsu MD[1], H Chusho MD[1] and Y Hataya MD[1].

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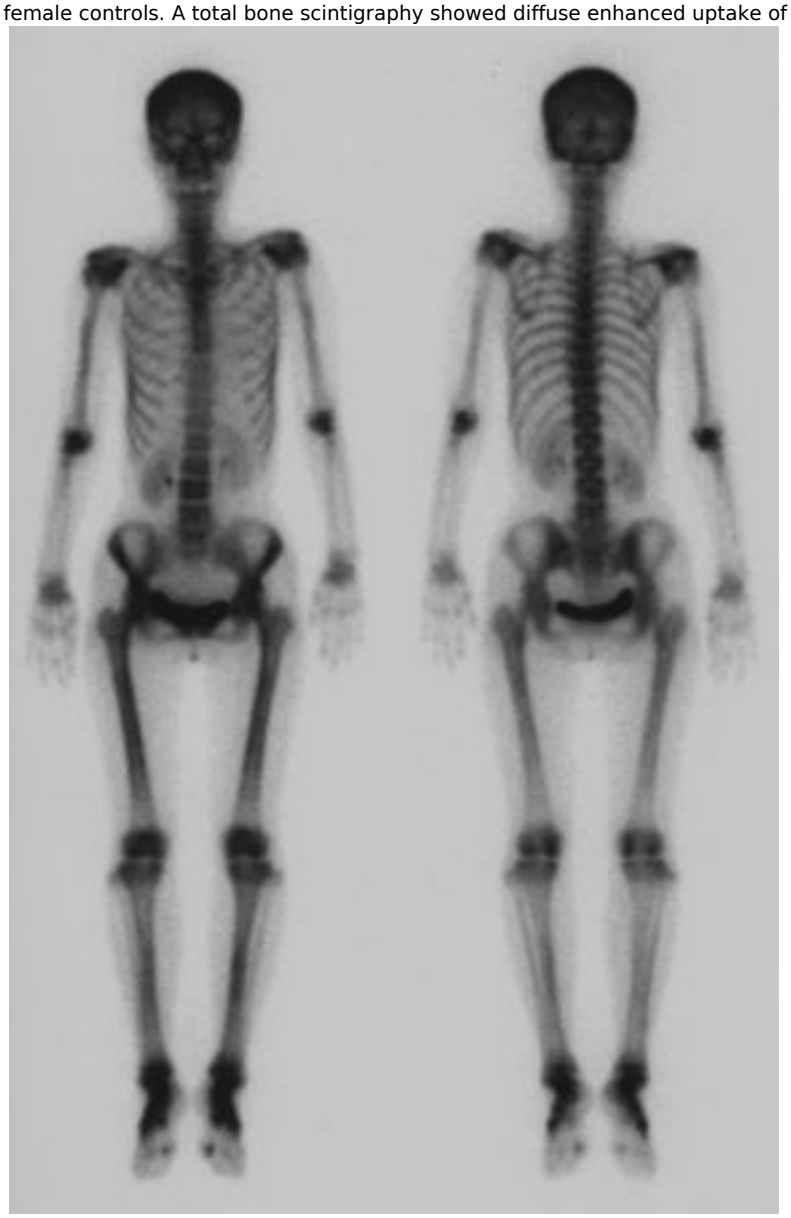
A 62-year-old woman presented the markedly increased serum alkaline phosphatase (ALP) during the regular follow-up of chronic hepatitis C. She had no subjective symptoms. Her ALP level was 4259IU/L (110-350), and ALP isoenzyme fractionation demonstrated predominantly ALP of skeletal origin. Serum calcium, phosphorus, and intact-PTH levels were normal, while 1,25-dihydroxyvitamin D level was slightly elevated to 81pg/ml (20-60). NTX/Cr was elevated to 1020nmBCE/mMCr (9.3-54.3) and osteocalcin was 34.1ng/ml (3.1-12.7). Bone mineral densities (BMD) of the lumber spine and the femoral neck were above the mean peak bone mass of young women (BMD 1.162g/cm^2 and T score 129%, and BMD 1.284g/cm^2 and T score 115%, respectively), and 139% and 173% of mean values for age-matched female controls. A total bone scintigraphy showed diffuse enhanced uptake of all major bones.



These findings were consistent with hepatitis C-associated osteosclerosis (HCAO), a rare syndrome characterized by a marked increase of bone mass in adults infected with the hepatitis C virus (1). As FDG-PET demonstrated strong accumulation on the gallbladder, the gallbladder cancer was suspected. Therefore, she was underwent cholecystectomy. However, histopathological findings demonstrated chronic cholecystitis. Six months after the operation, the serum ALP level gradually decreased to 1419IU/L and NTX/Cr to 140 nmBCE/mMCr, while BMD of lumbar spine and femoral neck rose to 1.49g/cm^2 (+32.0%) and 1.193g/cm^2 (+2.6%), respectively. The finding that the serum ALP level decreased after cholecystectomy in this patient may indicate the existence of the novel osteogenic factor in the gallbladder in HCAO, considering with the resent findings that Klotho/FGF19/FGF21 pathways play important roles in

both mineral metabolism and bile acid synthesis (2).

(1) Kaji H et al., Exp Clin Endocrinol Diabetes 2006; 114:599
(2) Tomiyama KI et al., Proc Natl Acad Sci USA 2010; [Epub ahead of print]

Nothing to Disclose: YK, HC, YH

P2-213

Guar Gum as a Putative Cause of Hypercalcemia in a Hospitalized Patient.

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Background: Animal studies have shown that ingestion of guar gum hydrolysate increases intestinal calcium absorption in rats with suppressed gastric acid secretion.
Clinical Case: A 62 year old woman with multiple medical problems, including type 2 DM and failed renal transplantation, now on regular dialysis, developed persistent hypercalcemia during prolonged hospitalization for a subdural hematoma. She required placement of PEG tube for nutritional support. Review of medication history indicated that patient had been started on an intravenous proton-pump inhibitor upon admission to the intensive care unit, and that guar gum had been recently added as a thickening and bulk-forming agent to relieve the irregularly loose stools associated with her PEG tube feeds. Serum calcium concentration was 9.2 mg/dL (normal 8.9-10.7) prior to initiation of guar gum and showed a persistent steady increase over the next three weeks to a peak of 11.3mg/dL at the time that endocrine consultation was requested. Patient was hemodynamically stable and euvolemic. Initial laboratory evaluation revealed PTH 55.3pg/mL (normal 12-65), albumin 3.2g/dL (normal 3.7-5.2), TSH 5.44mIU (normal 0.35-5.5), Vitamin D-25, OH of 12.4ng/mL (normal 32-100), and Vitamin D 1, 25 OH of <5pg/mL. SPEP interpretation indicated hypoalbuminemia and without evidence of monoclonal gammopathy. After discontinuation of guar gum, the patient's serum calcium levels returned to 9.2mg/dL over the following two weeks in the absence of other sustained changes to the patient's medications, tube feeds, or degree of physical activity and mobilization.
Conclusion: This case demonstrates a possible association between guar gum and hypercalcemia which occurs due to enhanced intestinal calcium absorption. Guar gum is a galactomannan derived from the guar bean, which is commonly used as an emulsifier and binder in foods and pharmaceuticals. It increases calcium absorption in large intestine with the aid of short-chain fatty acids which are cecal fermentation products of guar gum. It is important to look at nutritional additives, howsoever rare, as a cause of metabolic derangements, especially hypercalcemia as exemplified by this index case.

Hara et al. Ingestion of guar-gum hydrolysate partially restores calcium absorption in the large intestine lowered by suppression of gastric acid secretion in rats. Br J Nutr (1999) vol. 81 (4) pp. 315-21

Nothing to Disclose: RSG, TL, KS

P2-214

Systemic Mastocytosis Presenting as Osteosclerosis and Osteoporosis.

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Introduction
Systemic mastocytosis (SM) consists of a group of disorders characterized by excessive mast cell proliferation and accumulation involving the bone marrow and other extracutaneous tissues. The skeletal features of mastocytosis include diffuse osteosclerosis and rarely osteoporosis. We describe the case of a 61 years old Caucasian female who was referred to our bone clinic for osteosclerosis and was diagnosed with systemic mastocytosis on further workup.
Case report
A 61 years old Caucasian female was referred to our clinic for the evaluation of sclerosis of bones. Patient was allergic to multiple medications and had a history of allergic rhinitis. She had no history of urticaria or other skin rash. She had an elevated alkaline phosphatase level of 188 U/L (Normal 30-122) and a CT scan of abdomen and pelvis had shown diffuse sclerosis involving the pelvic bones and lower spine. DEXA scan showed osteopenia in lumbar spine (T score -2.0), normal bone density in the hip (T score 0.4) and osteoporosis in the distal forearm (T score -3.8). PET scan and bone scan did not show any abnormality. Bone specific alkaline phosphatase was elevated at 36.1 ug/l (Normal 0.0-21.3). Skeletal survey showed diffuse osteosclerosis of pelvis, lower spine and long bones with sparing of skull and upper spine. Work up for secondary causes of osteoporosis was negative. Patient underwent a transiliac bone and bone marrow biopsy. The biopsy showed the morphological and staining pattern consistent with the diagnosis of systemic mastocytosis.
Discussion
Mastocytosis is a rare disease of mast-cell proliferation characterized by a combination of symptoms due to the release of vasoactive substances, such as histamine. A well recognized radiological feature seen in 70-75% of patients with mastocytosis is diffuse osteosclerosis, affecting primarily the axial skeleton and the ends of the long bones. A subset of patients appears to be at increased risk of developing osteopenia and osteoporosis due to effects of mast cell mediators on bone turnover. Bisphosphonates may be used in these patients to treat osteoporosis.
Conclusion
Systemic mastocytosis is a rare cause of osteoporosis but should be considered in the differential diagnosis in the presence of other suggestive clinical or radiological features. Bone marrow biopsy remains the gold standard in the diagnosis of systemic mastocytosis.

Nothing to Disclose: MMH, AY, OB

P2-215

Massive Metastatic Calcification in an ESRD Patient with Uncontrolled Secondary Hyperparathyroidism.

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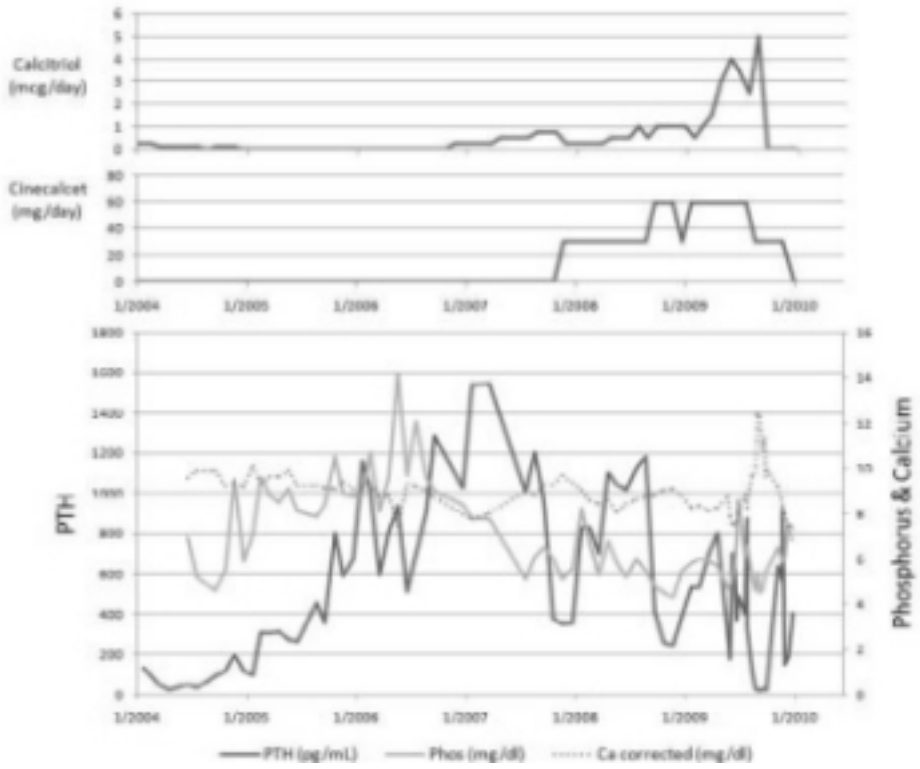
Background: Extra-osseous calcification is well described in patients with end stage renal disease (ESRD) and secondary hyperparathyroidism (sHPT). However, intra-abdominal extra-osseous calcification is rare and only described in patients receiving peritoneal dialysis (PD). We describe a patient on PD with uncontrolled sHPT and a large calcific mass arising from the iliopsoas muscle.
Clinical Case: A 17-year-old female with idiopathic ESRD, on PD for 6 years, developed right leg swelling and pain after 4 years on PD (10/2007). CT revealed a 10.6 x 22.2 x 7.0 cm diffuse calcific mass arising from the right iliopsoas, causing partial occlusion of the common femoral vein and mass effect on the common femoral artery. There were no radiographic features of tumor. Smaller subcutaneous calcific masses were present adjacent to both hips. For 5 years, she had severe uncontrolled sHPT. With aggressive increases in cinecalcet and calcitriol doses, PTH and phosphorus (P) normalized, at the expense of hypercalcemia (figure 1), and rebounded after stopping calcitriol. Repeat CT scan showed a significant decrease in the size of the calcified mass, 9.4 x 18.3 x 6.7 cm.



Discussion: This case illustrates two points (1) the huge size possible for extra-osseous calcifications in ESRD on PD complicated by severe sHPT and elevated P; as far as we are aware, this mass is the largest reported, (2) using 1,25-D analogs to lower PTH in patients with severe sHPT and elevated P does not necessarily cause serum P to increase further. We speculate that lowering PTH reduces P mobilization from bone, preventing a rise in serum P with 1,25-D use. Dialysis protocols hold 1,25-D analogs when P is elevated. This case shows that use of 1,25-D analogs can be associated with lowering of P, making a trial of 1,25-D analog prudent in dialysis patients, in spite of elevated P.

Nothing to Disclose: ABD, SRM, EAS

P2-216

The Management of Post-Thyroidectomy Hypocalcemia in Post-Gastric Bypass Patients.

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Background: Most calcium active transport sites in the gastrointestinal tract are located in the duodenum and proximal jejunum and are bypassed during Roux-en- Y gastric bypass (RYGB). Furthermore, 25-OH-Vitamin D (25OHD) levels are commonly low in this patient population. Thusly, the efficacy of oral calcium to treat transient or permanent hypoparathyroidism after total thyroidectomy is reduced.
Case: A 37 year old woman with a history of RYGB underwent total thyroidectomy. One parathyroid gland was autotransplanted. Preoperative calcium was 9.0 mg/dL (8.5-10.5) and 25OHD was 31.6 ng/mL (10-60). The final pathology showed a 2.0 cm papillary thyroid carcinoma arising in a background of Hashimoto's thyroiditis. Two lymph nodes were negative for malignancy.
On post-operative day (POD) #2 she developed extremity and perioral paresthesias. Serum calcium on POD#3 was 6.2 mg/dL and ionized calcium was 0.64 mmol/L (1.12-1.32). She was initially treated with IV calcium gluconate, calcium citrate 2g po q6hours, calcitriol 0.5 mcg po BID, and Mg oxide 400 mg po BID. Over the subsequent several days, her calcium citrate dose was increased to 5 g po Q6hrs and her calcitriol dose was increased to 1 mcg po BID. On this regimen, serum calcium stabilized at 7.5 mg/dL (ionized calcium 0.95 mmol/L), she remained asymptomatic, and was discharged home on POD#8, not having required any IV calcium for 48 hours. On POD#15, serum calcium was 9.2 mg/dL, ionized calcium 1.17 mmol/L, and PTH 26 pg/mL (10-69). Her medication doses were decreased, and over the subsequent 2 months, she was weaned back to her baseline calcium citrate dose of 1 g daily.
Conclusion: This case illustrates the difficulty in managing a patient with a history of RYGB, despite normal pre-operative 25OHD levels, on an oral calcium and calcitriol regimen during a period of transient hypoparathyroidism after total thyroidectomy. Administration of calcitriol is of particular importance in the perioperative setting because it increases active transport and passive diffusion of calcium in the colon and ileum. While the great majority of patients who develop post-thyroidectomy hypocalcemia can be managed as outpatients, treating physicians should be aware that patients who have undergone RYGB will probably need to be managed as inpatients, and will require higher doses of oral calcium and calcitriol than usual.

Nothing to Disclose: CS, DME

P2-217
Unusual Presentation of Intrathyroidal Parathyroid Carcinoma.

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[1]Hosp Britanico de Buenos Aires Ciudad Autonoma de Buenos Aires, Argentina.

Introduction
Parathyroid carcinoma (PC) is a rare disease, accounting for less than 1% of Caucasian patients with primary hyperparathyroidism[i]. Intrathyroidal localization is even more unfrequent, described only in a few cases.

Clinical Case
A 48-year-old woman, who had undergone a nodular resection for colloid nodule 15 years ago, concurred for a recurrence of thyroid nodule.
There was no history of psychiatric complaints, hypertension, nephrolithiasis, nor recent bony fractures. She referred no neck pain neither compressive symptoms.
Clinical examination was unremarkable, except for the presence of a right painless thyroid nodule.
Ultrasonography showed an enlarged right thyroid lobe with a nodule of 28x18x4mm in the anterior-inferior area.
Thyroid function tests were normal under 100mcg of thyroxin.
X-ray showed osteopenia and Dual energy X ray absorptiometry showed a T score of –2.0 and –2.1 at lumbar spine (LS) and right femoral neck (FN) respectively.
An elevated calcium level of 11.3 mg/dL (normal 8.4 to 10.5 mg/dL), confirmed by a second determination, with a concomitant increase in intact PTH level 150.1 pg/mL (normal 10 to 65 pg/mL) was detected in laboratory testing for osteopenia.
Fine needle aspiration of the thyroid nodule was consistent with colloid nodule.
Parathyroid scan using 99m Tc-SestaMIBI-99c-Pertechnetate was consistent with scintigraphic features of a parathyroid adenoma in right lower thyroid lobe.
Total thyroidectomy found a large intrathyroid parathyroid gland in the right thyroid lobe with local invasion of surrounding tissues. A PC inside benign thyroid tissue was revealed on pathology.
Postoperatively, calcium level dropped to 9.7mg/dL, and PTH level returned to normal values (19 pg/mL).
Patient had not shown evidence of recurrence of the carcinoma after 4 years of follow up. Serum calcium levels, as well as PTH levels, persist within normal ranges.
Dual energy X ray absorptiometry has improved in both areas, showing a T score of –1.3 at LS, and –1.0 at right FN.

Discussion

To the best of our knowledge, intrathyroidal PC has been reported only in five cases[i].
Clinical manifestations of hyperparathyroidism in patients with PC are usually more severe than in parathyroid adenoma, with markedly elevated serum calcium levels and PTH levels[ii].
This patient was asymptomatic and with mild hypercalcemia, leading to the conclusion that this is a rare case of asymptomatic intrathyroidal PC.

i. Temmim, L; Sinowatz, F; Hussein, WI; Al-Sanea, O; El-Khodary, H. Intrathyroidal parathyroid carcinoma: a case report with clinical and histological findings. Diagn Pathol. 2008. 3:46.
ii. Schmidt, JL; Perry, RC; Philippsen, LP; Wu, HH. Intrathyroidal parathyroid carcinoma presenting with only hypercalcemia. Otoloryngol Head Neck Surg. 2002. 127:352-3.

Nothing to Disclose: MGL, NE, AI, AC, MH, MVM, ALM

P2-218
Vanishing Bone Syndrome.

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[1]Cleveland Clin Cleveland, OH.

Background
Gorham-Stout syndrome is a rare clinical disease characterized by progressive osteolysis and invasion by benign vascular tissue of the surrounding soft tissue structures. It was first described by Gorham and colleagues in 1954.We report a case of a 49 yr old lady we saw in our clinic with this pathology.
Case presentation
A 49 year old lady was referred to the metabolic bone centre because of chronic back pain for the last 5 years. The pain started after a sledge riding accident. X rays at the time ruled out. but the pain persisted. Examination was significant for limitation of movement of the lumbar spine as well as weakness and atrophy of the right calf. A CT scan of the pelvis showed an extensive, cribriform, lytic mass 8.7 X 8.5 cm involving the right hemisacrum. An internal bony matrix was present within the lesion with a sunburst appearance favoring a benign etiology most likely a hemangioma. Pathology of an open biopsy was positive for benign vascular proliferation consistent with a hemangioma. Labs at the time of presentation at our institution showed normal electrolytes, bone turn over markers as well as alkaline phosphotase .On clinical, radiological and histological grounds, a diagnosis of Gorham's disease was made.
Discussion
Gorham's disease is a rare bone disorder characterized by replacement of normal trabeculae by intensively developing benign vascular tissue which resembles hemangiomatosis or lymphangiomatosis and results in complete or partial osteolysis. Patient usually present with persistent pain after trivial trauma and a high index of clinical suspicion is required to make a diagnosis. The pathogenesis is incompletely understood. Gorham and Stout hypothesized that hyperemia and changes in local pH triggered by trauma might be responsible while Devlin and colleagues maintain that enhanced osteoclastic activity and increased IL-6 leads to increased bone resorbtion. The imaging finding depend on the stage of the disease and vary from multiple intramedulary and subcorticol luscencies in the early intraosseus stage to subsequent complete resorbtion of bone and replacement with fibrous tissue in the late stages. There is a distinct lack of new bone formation at all stages which is characteristic of the disease process. There is no standard treatment and available modalities include surgical resection and radiation therapy. Bisphosphonates have been reported to be effective in stabilizing the disease process.

Nothing to Disclose: VG, AL

P2-219

Acute Renal Failure and Hypercalcemia Secondary to Mediastinal Parathyroid Adenoma in Elderly Female.

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[1]Banner Good Samaritan Hosp Phoenix, AZ and [2]Alsham Endocrinology Chandler, AZ.

An 86 yo female with past medical history of HTN, DM2 and remote h/o breast cancer was referred by her PCP to come to the hospital after her routine blood work revelaed BUN 50, Creatinine of 2.63 calcium 11.3, K 6.6 .Patient was admitted for ARF, hypercalcemia, hyperkalemia.
Further inpatient work up was significant for intact PTH of 117, albumin of 3.0 and corrected Calcium of 11.6 consistent with primary hyperparathyroidism. SPEP done to r/o Multiple myeloma did not reveal any evidence of hyper globulinemia.
A thyroid sonogram was done and revealed bilateral thyroid nodules. FNA revealed benign thyroid nodule. A SPECT parathyroid scan revealed no scintigraphic evidence of parathyroid adenoma in the anatomical area of parathyroid but revealed a focus of intense tracer labeling noted in the superior mediastinum suspicious for ectopic parathyroid adenoma. CT chest failed to reveal any masses in the superior mediastinum. Full body PET/ CT scan failed to reveal any metabolically active lesion.
Surgical exploration was performed after injecting Tc sestamibi , by inserting a gamma probe from the sternal notch and advancing it into the superior mediastinum. The Gamma probe was advanced to follow the hot area in the posterior tracheoesophageal groove and the ectopic mass was dissected out, noted to be large and hypercellular mass measuring 665 mg. A parathyroid level done intraoperatively prior to resection of ectopic parathyroid gland was 120 pg/ml, a repeat level 30 minutes after resection was 27 pg/ml indicating that mass identified and resected was the source of the parathyroid hormone. Pathology report later confirmed dx of Parathyroid adenoma with mixed adenoid hyperplasia and oxyphil cell. Patient Calcium level and PTH normalized post operatively and patient was taken off cinacelcet.
Conclusion : In established cases of PHPT with equivocal preoperative localization studies or negative neck explorations, an ectopically placed parathyroid adenoma should be considered. Gamma probe is a very useful device to differentiate the ectopic parathyroid adenoma from surrounding tissues for complete surgical excision.Such localization shortens the duration of the operation and reduces the possibility of complications Intraoperative rPTH is another useful tool which can correctly guide removal of hyperfunctioning glands.

Nothing to Disclose: WA, MA, MHH, AWN

P2-220

Potential Treatment Options for Pregnancy and Lactation-Associated Osteoporosis: A Case Report.

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[1]Fed Univ of Sao Paulo Sao Paulo, Brazil.

Background
Pregnancy and lactation associated osteoporosis (PLO) is an uncommon condition characterized by the occurrence of fragility fractures, most commonly vertebral, in third trimester or early post partum period. The prevalence, etiology and pathogenesis of this osteoporosis remain unknown. Despite its relative rarity, PLO can be a limiting condition that causes severe, often prolonged pain and height loss in affected women. To date, approximately 100 cases have been reported[1,2,3].
Case report
A 44-years-old female presented to a tertiary endocrine clinic for evaluation of osteoporosis. She had a long history of back pain that began immediately after the delivery of her unique child, twelve years ago. At that time rib and vertebral fractures were diagnosed. Dual energy X-ray absorptiometry (DXA) of lumber spine revealed marked osteoporosis (L1-L4 Z score -4.03). She had no medical history of any systemic disorder. There were no abnormalities in laboratory findings. After lactation, alendronate (70mg/weakly) and calcitonin nasal spray (twice monthly) were started. After 5 years, this therapy was replaced by teriparatide 20 mcg daily. It was used for 18 months and then discontinued. Alendronate was restarted and used for two years, when it was replaced for strontium ranelate 2 g/day. She used strontium for 18 months.
When she arrived in our clinic she was taking strontium ranelate 2g/d, with no history of recent fractures and no abnormalities in laboratory findings. The DXA revealed Z score L1-L4 -3.1. The thoracolumbar magnetic resonance imaging revealed six vertebral fractures (T11-L5).
Discussion
Longitudinal studies show that women experience a transient loss of 3-7% of their bone density during pregnancy and lactation, which is regained after weaning[1]. For some unknown reasons some women lose it more and do not recover. We don't know if osteoporotic fractures during pregnancy reflect a previous fragility or if pregnancy itself is responsible for the bone loss.
Currently, there is no consensus on PLO treatment. In most cases, women receive calcium supplements and are advised to wean. Biphosphonates use increased spine [3] and femur [4] bone density. Stump et al related the successful use of teriparatide in a PLO case with substantially increased in BMD [5]. Strontium ranelate was also used with improvement in lumbar and femoral BMD [6]. Despite many options, the therapeutic management of PLO remains undefined[3,4,5,6].

(1) Kalkwarf HJ et al Endocrine (2002) 17 49-53
(2) Athimulan S et al Arch Gynecol Obstet (2007)276:175-177
(3) O'Sullivan SM Osteoporos Int (2006) 17: 1008–1012
(4)Ofluoglu O et al Rheumatol Int (2008)29:197-201
(5)Stumpf et al Adv Med Scien (2007) 52: 94-97
(6)Tanriover MD et al The Spin Jour 9 (2009) e20-e24

Nothing to Disclose: TV, TPO, ML-C

P2-221

Hypercalcemia Associated with Vertebral Osteomyelitis and Immobilization.

MN Iyer MD[1], M Kaur DO[1], NB Watts MD[1] and DA D'Alessio MD[1].

[1]Univ of Cincinnati Cincinnati, OH.

Hypercalcemia is most frequently associated with primary hyperparathyroidism and malignancy as well as a number of less common conditions. We report a case of hypercalcemia associated with vertebral osteomyelitis exacerbated by immobilization, which, to our knowledge, has not been reported before.
A 23-year-old man presented with neck pain of 2 days duration and was found to have a serum calcium (Ca) corrected for albumin of 12.9 mg/dL (8.6-10.2), phosphorus 5.6 mg/dl (2.5-4.5), alkaline phosphatase 254 U/L (40-115) and creatinine 0.30 mg/dL (0.5-1.3). He was injured in an auto accident 6 months previously, sustaining a cervical spine fracture causing quadriplegia. At that time he underwent fusion of C5-C6 and subsequently developed soft tissue abscesses of the neck secondary to infected hardware, treated with incision and drainage (I&D) and antibiotics. Exam revealed a cachectic man with normal vital signs and left neck tenderness. He had flaccid paralysis and sensory loss below his neck. Serum calcium was normal at the time of his accident but gradually increased over the month before admission.
Intact PTH was <2.5 pg/dL (14-72), 25-OH vitamin D 34.6ng/dL (32-100), 1-25 dihydroxy vitamin D 7.1 pg/mL (15.9-55.6), and PTHrp <0.3 pmol/L (0.0-1.5). An 8 am cortisol was 19.8 mcg/dl, TSH 5.45 mIU/L (0.45-4.50), and 24-hr urine Ca 344.9 mg/ 24hr (100-300). Treatment with IV fluids lowered the serum calcium to 11.3 mg/dL.
Review of CT scan of the neck and spine two months prior to admission was consistent with focal regions of osteomyelitis within the C7 and T1 vertebral bodies. An MRI at the time of admission showed multivertebral osteomyelitis. CRP was 124.4 mg/dL (<5). He underwent I&D and was started on a three-month course of antibiotics. He was also given zoledronic acid. After these therapies his CRP was 36.80 mg/dL and his corrected Ca 9.9mg/dL.
In persons with normal renal function immobilization is associated with hypercalciuria and normocalcemia. Hypercalcemia associated with immobilization has been described in patients with renal impairment. In this man with normal renal function we hypothesize that severe local bone lysis, in addition to immobilization, led to high rates of calcium mobilization. The resolution of hypercalcemia with treatment of the osteomyelitis supports this hypothesis. We believe that this is the reported first case of vertebral osteomyelitis exacerbating hypercalcemia.

Nothing to Disclose: MNI, MK, NBW, DAD

P2-222

Bili in the Kid: A Case of Osteoporosis Due to Cholestasis in a Young Adult.

Robert F Ennis MD[1], Kathleen C Belbruno MD[1] and John H Turco MD[1].

[1]Dartmouth Hitchcock Med Ctr Lebanon, NH.

BACKGROUND
Osteoporosis with an associated high fracture rate is a well-known complication among patients with chronic liver disease, particularly those with cholestatic rather than hepatocellular liver disease. We present a case of osteoporosis presenting as a hip fracture in a young male with a rare syndrome characterized by chronic cholestasis.
CLINICAL CASE
A 22 year old male with a past medical history significant for Alagille's syndrome was admitted to the Orthopedic Surgery service at our institution for evaluation and management of a pathologic left hip fracture. Alagille's syndrome is an autosomal dominant disorder characterized by atresia or hypoplasia of the intrahepatic biliary tree and is often associated with 5 features: chronic cholestasis, cardiac anomalies, butterfly vertebrae, ocular anomalies, and dysmorphic facies.
The patient was in his usual state of relatively good health until 2 months prior to this admission when he developed left hip pain without antecedent injury. Evaluation in an urgent care setting was initially unrevealing, but the pain persisted. A CT obtained prior to admission revealed a left intertrochanteric fracture as well as diffuse osteopenia. Prior to this fracture, the patient had participated regularly in low contact sports and had 2 previous fractures, each of which occurred in the setting of significant trauma. The patient was noted to have a Z-score of -3.4 at the lumbar spine on DXA scan. With the exception of an elevated bilirubin (total bilirubin 7.6 mg/ dL) and a history of chronic cholestasis, an evaluation for secondary causes of osteoporosis was unrevealing. Therapy with teriparatide is planned, but follow-up has been challenging.
CONCLUSION
We bring this case to the attention of medical practitioners to highlight the association between cholestatic liver disease and osteoporosis, even among young adults who may have some form of congenital cholestasis. Available research on the association between chronic liver disease and osteoporosis has generally focused on older adults with acquired cholestatic liver disease. The pathogenesis of osteoporosis in the setting of cholestatic liver disease is poorly understood and is likely multifactorial, but it seems that decreased osteoblast activity perhaps due to a toxic effect of bilirubin may play an integral role. Additional research is needed to elucidate the mechanism of osteoporosis associated with cholestasis and to guide therapeutic decisions.

Nothing to Disclose: RFE, KCB, JHT

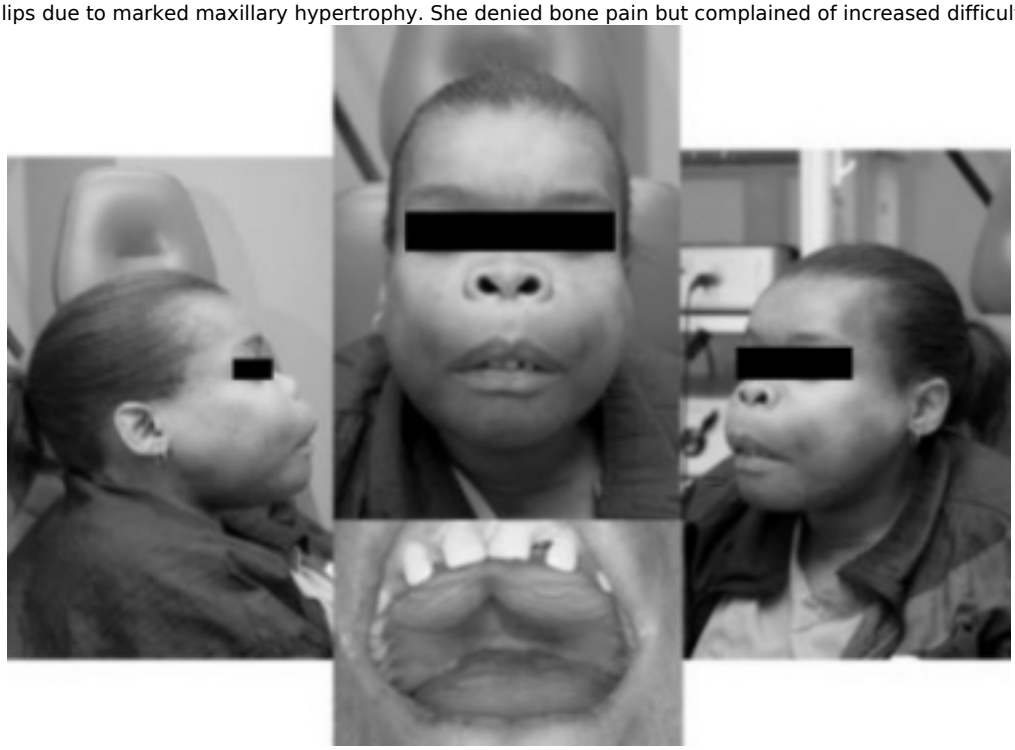
P2-223

Symmetric Craniofacial Hypertrophy in a Patient with Tertiary Hyperparathyroidism.

AW Gannon M.D.[1], JH Bain D.O.[1], T Fulop M.D.[1] and GI Uwaifo M.D.[1].

[1]Univ of Mississippi Jackson, MS.

A 29 year old African-American woman with end-stage renal disease and tertiary hyperparathyroidism presented for parathyroidectomy and had diffuse bone disease characterized by leontiasis ossea, kyphoscoliosis with vertebral compression fractures and severe generalized osteopenia. Her face had marked frontal bossing and prominence of the maxilla and mandible that was symmetric and associated with severe dental derangement. She was unable to close her lips due to marked maxillary hypertrophy. She denied bone pain but complained of increased difficulty hearing.



She was noted to have hyperparathyroidism some years before and was treated with escalating doses of sevelamer, paracalcitol and cinacalcet. After several years of therapy she developed the facial changes above and her parathyroid hormone levels increased further so she was placed on supra-pharmacologic doses of cinacalcet – up to 270mg daily. The parathyroid hormone level fell from 3881 pg/mL to 8 pg/mL (11-72pg/mL) following near total parathyroidectomy. Alkaline phosphatase levels were 994 units/L (39-117 units/L) and further worsened post-operatively, rising to over 3000 units/L. Maxillary bone biopsy performed at the time of surgery demonstrated fibrosis and giant cells, compatible with Paget's disease and/or renal osteodystrophy. Bone scan demonstrated a marked increase in uptake in the skull, mandible, spine, sacroiliac joints and pubic rami. Serum IGF-1 level was 87.4ng/mL (117-329ng/mL). Unfortunately, she did not allow further workup and has not returned for repeat evaluation. This pattern has been seen in one other patient in our institution who was referred from the same dialysis center for the same features, also also on supra-pharmacologic doses of cinacalcet.

Discussion: Our patient's symmetric craniofacial bone disease could be compatible with Paget's disease and superimposed tertiary hyperparathyroidism –an association that has not been previously published. Since this pattern has been seen in two patients in our institution who received supra-pharmacologic doses of cinacalcet it is possible that cinacalcet doses may play a role in this bone disease syndrome.

Nothing to Disclose: AWG, JHB, TF, GIU

P2-224

Recurrent Hypercalcemia Preceded by an Elevation of Third Generation/Second Generation PTH Ratio in a Patient with Parathyroid Carcinoma.

Philippe Caron M.D.[1], Jean-Christophe Maiza M.D.[1], Tom Cantor M.D.[2], Louise Rousseau M.D.[3], Jean-Claude Souberbielle M.D.[4] and Pierre D'Amour M.D.[3].

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Background: Parathyroid carcinoma remains a rare cause of primary hyperparathyroidism. A high 3rd generation (G)/2nd G PTH ratio [due to the secretion of a N-terminal PTH form (N-PTH) mostly detected by 3rd G PTH assays] has been observed in about 50% of patients with hypercalcemia related to a parathyroid carcinoma. The evolution of this 3rd G/2nd G PTH ratio in relation to hypercalcemic recurrence has not been described so far. Patient and Methods: A 62 year-old woman presented with a 5th episode of hypercalcemia related to a parathyroid carcinoma. A new surgery permitted the ablation of a 2-cm nodule in the inferior left parathyroid area, and an anterior neck irradiation was performed in the post-operative period. Total serum calcium and PTH levels were measured with 2nd G assay of “Total” PTH and 3rd G assay of “Whole” PTH (Scantibodies Laboratory Inc., Santee, California, USA) before and after the 4th and 5th surgery as were PTH molecular forms separated by HPLC before and after her 5th surgery. Results: Before the 4th and 5th surgery, the patient had elevated total calcium at 3.8 and 3.2 mmol/L, 3rd G PTH levels were elevated (675 and 590 pg/ml) and higher than 2 nd G PTH results (229 and 163 pg/ml) with 3rd G/2nd G PTH ratio of 2.95 and 3.61 respectively. After the 4th surgery, she maintained a normal total calcium concentration during 18 months and the 3rd G/2nd G PTH ratio remained normal for one year and then progressively increased to 3.6 over 15 months. The elevation of the ratio preceded the hypercalcemic recurrence by 6 months. After her 5th surgery, she became hypocalcemic, required calcium supplementation and 1alpha-vitamin D therapy, and both PTH levels and the 3rd G/2nd G PTH ratio remained normal over the next 6 months. Before the 5th surgery when the patient had recurrent hypercalcemia, HPLC analysis of circulating PTH molecular forms disclosed an overexpression of N-PTH (82.2%) relative to hPTH(1-84) (N = > 70%), which normalized after the last surgery. Conclusion: In this patient with parathyroid carcinoma, recurrent hypercalcemia was preceded by a high 3rd G/2nd G PTH ratio in relation with an overexpression of N-PTH relative to hPTH (1-84). A progressive increase in 3rd G/2nd G PTH ratio could be a useful tool to predict recurrence of disease after parathyroid surgery in patients with parathyroid carcinoma who initially presented with this abnormality.

Nothing to Disclose: PC, J-CM, TC, LR, J-CS, PD

P2-225

A Unique Case of Idiopathic Hypoparathyroidism and Prolactinoma Presenting in the Sixth Decade of Life.

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Background: Idiopathic hypoparathyroidism (IH) can present with varying neurological manifestations and this most commonly includes muscle cramps, paresthesia, seizures, and altered sensorium (1). However, extrapyramidal symptoms including choreoathetoid and hemiballismus are rarely reported (1,2). A unique case of untreated IH is presented.
Case: A 55 yo male with h/o of diabetes presented with progressively worsening left-sided uncontrollable movements of upper and lower extremities along with numbness and tingling in the lower extremities, and perioral area. On exam, patient was alert and oriented, but appeared uncomfortable secondary to this movement disorder. He displayed a resting tremor, 4/5 left-sided muscle strength, a positive Chvostek and Trousseau's sign. Laboratory results revealed a Calcium of 5.7 mg/dL (N: 8.5-10.3) (albumin 4.2g/dL) with a phosphorus of 4.8 mg/dL (N: 2.5-4.5) and a PTH of 3 pg/ml (N: 15-65). Patient was diagnosed with IH, and CT scan was obtained and showed diffuse symmetrical calcification of the subcortical white matter, caudate, thalamus, ventral posterior nucleus, and cerebellar white matter including the Dentate nucleus. On hospital day 1 (HD1) patient was started on calcitriol and calcium. On HD2, he developed delirium, disorientation, and so MRI was done which showed diffuse calcification, and an 11mm Sellar mass abutting the optic chiasm. Further workup showed a Prolactin of 322.3 ng/ml (N: 4-15.2), Testosterone of 142 ng/dL (N: 280-800), LH of 3.3 mIU/mL, (N: 1.7-8.6) and patient was started on Bromocriptine on HD 4. On HD6, he had Ca of 7.6mg/dL and choreoathetoid movements were virtually extinguished. On HD7 patient had Ca of 8.9 mg/dL, a normal neurological exam, and was discharged home with calcitriol and calcium with a goal Ca 8.0-8.5 mg/dL and Bromocriptine.
Conclusions: Choereoathetoid movements were likely due to hypocalcemia secondary to hypoparathyroidism. Untreated hypoparathyroidism can produce severe neurological and physical impairment. Correction of serum calcium levels is likely responsible for resolution of symptoms (1). This is a unique presentation of a patient with hypoparathyroid and incidental prolactinoma. There are only a handful of cases reported in the literature showing unilateral choreoathetoid movement disorder as a presenting symptom of hypoparathyroidism (2) but no case reports which also include a prolactinoma.

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Nothing to Disclose: MJD, PS

P2-226

Intranasal Calcitonin as an Adjunctive Therapy in Recurrent Central Giant Cell Tumor of the Mandible.

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Central giant cell tumor (CGCT) is a rare benign osteolytic lesion, usually presenting in the mandible or maxilla[1]. The first-line treatment ranges from intralesional steroid, calcitonin or α interferon to surgical treatment. However, the recurrence rates with surgical management alone remain high. Calcitonin has been suggested as a therapeutic option based on the inhibiting effect on osteoclast activity[2]. We present a case of recurrence CGCT, treated with intranasal calcitonin, as an adjunctive therapy.

A 23 year-old female referred to maxillofacial surgery with pressure like symptom and left jaw swelling for 3 months duration. Imaging studies showed destruction of the anterior left mandibular ramus. Incisional biopsy confirmed CGCT. She underwent enucleation and curettage. Laboratory data revealed normal calcium and parathyroid hormone. Postoperatively, she received 100 IU of subcutaneous calcitonin, which subsequently reduced to 50 IU due to nausea, vomiting and lightheadedness. The side effects from calcitonin made it difficult for her to remain with this regime. Two months later, there was an evidence of recurrence. She underwent marginal mandibular resection and reconstruction of left mandible. The histopathology revealed 3.2 x 2.5 x 2.0 cm central giant cell granuloma with fibrous tissue. She was referred to endocrinologist and intranasal calcitonin was prescribed as an adjunctive therapy to control the recurrence of the disease and avoid the intolerance to subcutaneous form. She was started on intranasal calcitonin 200 IU daily without adverse effect. CT scan of mandible at 3 months after the second surgery showed no evidence of recurrent tumor.

This case reinforces the therapeutic effect of calcitonin as an adjunctive therapy of CGCT. The major function of calcitonin by inhibiting the osteoclast activity results in stimulating the osteoblast. Calcitonin has been suggested for CGCT treatment based on the presence of osteoclast in multinucleated giant cell, detected by osteoclast-specific monoclonal antibodies. Since 1993, 34 reported cases were treated as adjunctive and exclusive therapy with complete resolution. Fifteen of the cases used 200-400 IU daily dose[3]. Although, the bioavailability of nasal spray is limited, the major advantages are patient compliance and minimal side effects[4]. Intranasal calcitonin should be considered as a combination or alternative therapy for the patients who are unable to tolerate the side effect of subcutaneous form.

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3)Allon DM et al.,Oral Surg Oral Med Oral Pahol Pral Radiol Endod 2009;107:811-818
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Nothing to Disclose: TH, DK, RC

P2-227

Interferon-Induced Sarcoidosis Presenting as Hypercalcemia without Apparent Organ Involvement.

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Background: Treatment with Interferon-alpha for HCV infection has been shown to cause sarcoidosis in a number of case reports. However, the initial presentation of interferon-induced sarcoidosis is usually multiorgan involvement. Hypercalcemia is only present in 5-10% of patients with sarcoidosis and is rarely the initial presentation.
Clinical Case: 57-year old Caucasian male admitted with hypercalcemia (Ca 14.1mg/dL; n 8.4-10.5 mg/dL), nausea, polyuria, polydipsia, anorexia, and fatigue. His past medical history is significant for HCV treated with Interferon-alpha. Laboratory evaluation revealed normal TSH, FT4, PTH-rP, and Vitamin A values. A UPEP and SPEP were unremarkable. The patient's ACE level was elevated at 168mcg/L (n<62mcg/L), as was the 1,25-OH Vitamin D level at 79pg/mL (n 16-65pg/mL). The 25-OH Vitamin D level was low at 6ng/mL (n 20-100 ng/mL). The creatinine was noted to be elevated at 1.4mg/dL (n 0.5-1.1mg/dL), and the PTH was noted to be within the normal range at 13.06pg/mL (n 10-65pg/mL). Radiologic evaluation revealed a normal CXR and Whole Body Bone Scan. A CT of the chest, abdomen, and pelvis showed no lymphadenopathy or signs of granulomatous disease. A Parathyroid Sestamibi Scan showed no suggestion of a parathyroid adenoma. A colonoscopy three months prior was negative for nodular disease.
Given the elevated ACE and 1,25-OH Vitamin D levels, interferon-induced sarcoidosis was suspected as the cause of the patient's hypercalcemia. With all other causes of hypercalcemia eliminated, no imaging evidence of sarcoidosis, and no obvious organ involvement from which to obtain a definitive pathologic diagnosis, the decision was made to empirically treat with oral Prednisone 20mg daily.
The patient remained on steroid therapy for four weeks. Follow-up laboratory evaluation showed a calcium of 9.4mg/dL with normalization of his creatinine (0.8mg/dL), ACE level (51mcg/L), and 1,25-OH Vitamin D level (21pg/mL). His PTH also appropriately rose to 46.60pg/mL.
Conclusion: Interferon-induced sarcoidosis after treatment for HCV infection can present as clinically significant hypercalcemia and may not have obvious organ involvement. These findings may make sarcoidosis difficult to diagnose, thus requiring an empiric trial of glucocorticoids to establish the diagnosis.

Nothing to Disclose: MMM, CME

P2-228

An Unusual Recurrence of Hypercalcemia Due to Concurrence of Parathyroid Adenoma and Parathyroid Sarcoidosis.

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Objective: To describe a patient presenting with the rare constellation of synchronous parathyroid adenoma and parathyroid sarcoidosis.
Methods: We describe the clinical history, physical examination, laboratory values, imaging findings and pathologic data of a man who developed recurrent severe hypercalcemia after a successful parathyroidectomy.
Results: Initial biochemical findings were: calcium 11.1 mg/dl (reference range 8.5-10.6), albumin 4.0 mg/dl (reference range 3.2-5.2), intact parathyroid hormone (iPTH) 166 pg/ml (reference range10-69), creatinine 1.9 mg/dl, 25(OH)D 15 pg/ml (reference range 30-80) and 1, $25(OH)_2D$ 44 pg/dl (reference range 16-72). The chest x-ray was normal and delayed images from a Tc-99m sestamibi scan showed increased activity in the right lower pole of the thyroid. Two months after successful parathyroidectomy the patient was admitted to the hospital with serum calcium of 17 mg/dl. Pathology of the resected gland confirmed the diagnosis of parathyroid adenoma and subsequent review disclosed the presence of non-caseating granulomas within the adenoma.
Conclusion: Sarcoidosis with parathyroid involvement causing severe hypercalcemia is unique to this case. Recurrent hypercalcemia after successful resection of a parathyroid adenoma may require consideration of potential causes other than the initial diagnosis.

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Nothing to Disclose: LC, SC, AG, AS, VM

P2-229

Sporadic Adult-Onset Hypophosphatemic Osteomalacia Caused by a Rib Tumor Producing FGF23.

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Background: Tumor-induced osteomalacia (TIO) is 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 Case:** A 51 year-old woman presented to our department for evaluation of skeletal pain and inability to walk. She had a 6-year history of multiply fractures and progressive weakness. Laboratorial tests showed: hypophosphatemia (1.2-1.6 mg/dL, N 2.7-4.5), high alkaline phosphatase (374-384 U/L, N 25-104) and low TmP/GFR (0.95-1.6, N 2.5-4.2). Renal tubular acidosis was ruled out. These data were consistent with hypophosphatemic osteomalacia that was confirmed by iliac crest bone biopsy. Oral therapy with phosphate and calcitriol improved her symptoms. An oncogenic origin was suspected due to late onset and the absence of familial cases. Localization exams (sinus MRI, chest X-ray and abdominal ultrasound) were performed but they failed in finding the tumor. Just after octreotide scan was carried out, a left infraclavicular region was identified. Chest CT scan disclosed an osteolytic heterogeneous lesion at the anterior left first rib. Medical treatment was interrupted as soon as the tumor was resected. Histologic analysis was compatible with mesenchymal tumor and the immunohistochemistry was positive for FGF23. Moreover, the patient had high FGF23 presurgical levels (316-458 pg/mL; N 10-50) which rapidly decreased to undetectable range after surgery. One week after tumor removal, phosphate metabolism and FGF23 levels were restored and have remained normal without further phosphate therapy 15 months later. **Clinical lessons:** Despite fact of TIO in a rare disorder, it should be suspected in patients with hypophosphatemia, hyperphosphaturia and metabolic bone disease or unexplained bone pain and/or muscle weakness even if bone deformity is absent. The causative tumor is often small and difficult to locate even on thorough clinical examination and may not be evident for a long time after the onset of symptoms. Because the tumor removal is curative, its localization is mandatory. Somatostatin receptors on mesenchymal tumors have supported the use of octreotide scan and it should be considered as the initial imaging study in the assessment of patients with TIO. 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: GAV, LZB, FGG, CRGCMO, RMAM, VJ, PHSC, RMM

P2-230

A Rare Case of Primary Hyperparathyroidism Due to Clear Cell Parathyroid Adenoma.

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Background: The most common cause of primary hyperparathyroidism is parathyroid adenomas, most of which contain a mixed cell population, predominantly chief cells[1]. Clear cell parathyroid adenoma, composed exclusively of tumor cells with abundant foamy to granular cytoplasm, is exceptionally rare. In medical literature, only 8 cases have been reported[2, 3]. We now present a patient with a very large clear cell parathyroid adenoma with exceedingly high PTH.
Clinical case: A 56-year-old African-Caribbean woman with schizophrenia for 23 years and primary hyperparathyroidism for 8 years complained of chronic generalized fatigue and bilateral knee pain but no history of peptic ulcer disease, nephrolithiasis or fracture. Serum Calcium ranged from 11 to 16 mg/dL (8.8-10.5mg/dL), PTH from 1500 to 2165 pg/mL (10-65pg/mL), serum phosphate- 2.1mg/dL (3-4.5mg/dL) with normal thyroid function tests, serum albumin and GFR. Knee X-rays showed osteopenia. Thyroid US and CT of neck and chest with contrast reported a large left parathyroid mass, normal thyroid gland, “brown tumors” in Right 7^{th} and 9^{th} ribs , multiple small lytic lesions throughout the skull, no cervical lymph adenopathy or focal abnormality in lung parenchyma. Sestamibi Scan revealed enlarged left parathyroid gland. ECG showed normal corrected QT interval. After parathyroidectomy (Left inferior and superior), serum Calcium and PTH normalized. She had uncomplicated recovery with no hungry bone syndrome. Pathology of left inferior gland showed a clear cell parathyroid adenoma (22 g; 5.0x3.0x1.5 cm) surrounded by a rim of normal parathyroid tissue, no capsular invasion, mitosis or necrosis. Immunostaining confirmed parathyroid origin. Left superior gland was a normal parathyroid gland without hyperplasia.
Conclusion: Since clear cell parathyroid adenoma is very rare, other etiologies such as parathyroid clear cell hyperplasia, parathyroid carcinoma, paraganglioma, metastases from clear cell thyroid cancer and renal cell cancer should be considered as differential diagnoses. Generally, large parathyroid tumors with very high PTH level are more likely to be malignant. However, this case was confirmed as a clear cell adenoma, supported by its indolent nature and lack of malignant features in imaging and pathology. Besides, resolution of hyperparathyroidism after surgery without recurrence further favored this diagnosis. However it is important to follow the patient to monitor long term for recurrence.

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Nothing to Disclose: KS, GB, SM, BI, AS, AG, ADN

P2-231

Refractory Hypoparathyroidism after Thyroidectomy in a Woman with Graves' Disease.

M Karegar MD[1], K Komorovskiy MD[1], CC Chang MD[1], BC Villafuerte MD[1] and SS Krishnasamy MD[1].

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Background: Postsurgical hypoparathyroidism (PSHP) occurs as a permanent or transient complication of total thyroidectomy. We present a case of PSHP with intractable hypocalcemia, which improved with teriparatide therapy.
Clinical case: A 36 year-old Caucasian woman who had Graves' disease for 2 years underwent total thyroidectomy due to opthalmopathy, large goiter and history of heavy smoking. Her preoperative serum calcium (Ca) was normal, and her serum alkaline phosphatase (AP) level was slightly elevated.Symptomatic hypocalcemia developed 6 hours postoperatively with serum Ca level of 5.9 mg/d (8.6-10.2), which responded to i.v. calcium gluconate infusion (9.3 mg/ml/h). When switched to oral Calcium and Calcitriol, she remained hypocalcemic (5.6-6.5 mg/dL) despite daily supervised regimen of oral elemental Calcium 30 g, 4-6 µg of Calcitriol and Hydrochlorothiazide 50 mg. She required continuous infusion of i.v. Calcium for more than 2 months in the hospital to maintain serum Ca above 7 mg/dL.On high dose oral Calcium and Calcitriol,labs included serum Phos of 8 mg/dl (2.5-4.5), intact PTH level of 5 pg/mL (10-65),25-OH Vitamin D of 35 ng/ml (20-100),1,25(OH)2 vit D of 29 pg/ml(18-72),24 h urinary Ca 33 mg (45-353 mg/24hr) and negative celiac screen. DEXA scan showed Z score of -1.9 in the Lumbar spine and -1.3 in the hip. After 1 month on above treatment, teriparatide (PTH 1-34 analog) 20 µg subcutaneous BID was added, and she was weaned off i.v.Ca over several weeks. After 82 days of hospitalization, she was discharged with serum Ca of 7.2-7.5 mg/dL.Her daily home medications include: teriparatide 20 µg, calcitriol 4 µg, elemental Ca 20 g, cholecalciferol 7500 IU, HCTZ 25 mg, levothyroxine 250 mcg, magnesium oxide 1600 mg, KCL 160 meq and ergocalciferol 50,000 units weekly.
Conclusion: Etiology of PSHP in hyperthyroidism may include intraoperative damage, devascularization of parathyroid glands, uncontrolled hyperthyroidism associated with osteoclast activation and bone resorption (thyroid osteodystrophy), vitamin D deficiency and impaired intestinal Ca absorption. In our patient elevated AP and osteopenia suggested increased bone turnover.
It is imperative to recognize risk for hard-to-treat postoperative hypocalcemia in patients with uncontrolled hyperthyroidism. Particular attention to preservation of parathyroid function is indicated in these cases. Teriparatide is effective in the treatment of certain patients with intractable PSHP.

Nothing to Disclose: MK, KK, CCC, BCV, SSK

P2-232

Severe and Prolonged Hypocalcemia Following Parathyroidectomy: An Extreme Case of Hungry Bone Syndrome.

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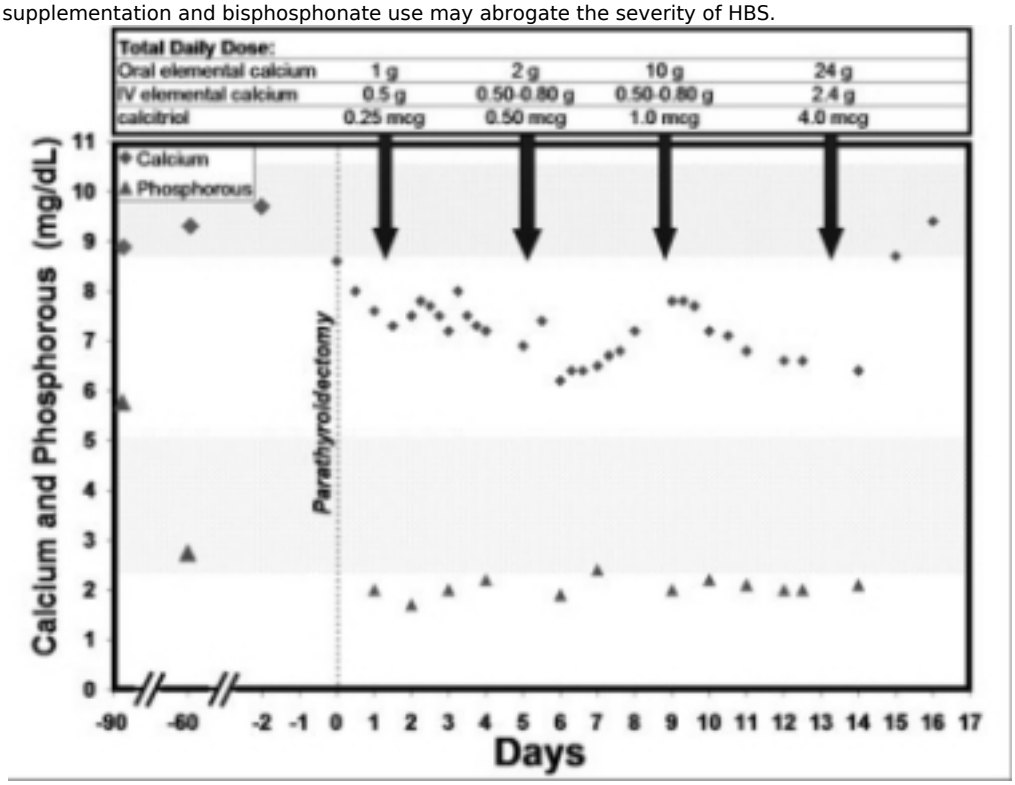
Background: Hypocalcemia secondary to hungry bone syndrome (HBS) can occur following parathyroidectomy.
Clinical Case: A 20 year old woman developed glomerulonephritis and fulminant renal failure. For 2 years she received hemodialysis (HD), calcitriol, and cinacalcet, but developed severe secondary hyperparathyroidism and renal calculi. During this time calcium (Ca) remained normal, but PTH was 800-6600 pg/mL (nl: 11-80) and alkaline phosphatase (AP) was 527-1013 U/L (nl: 36-118). A mandibular biopsy at age 22 for severe pain revealed osteitis fibrosa cystica. Renal transplantation was performed, but within months her graft rejected and she resumed HD with persistent normocalcemic hyperparathyroidism. Ultrasonography showed 4 parathyroid-gland hypertrophy.
She underwent 3.5 gland parathyroidectomy; pathology revealed parathyroid hyperplasia. Pre-operative laboratories included: Ca 9.7 mg/dL (nl: 8.8-10.5), phosphorous (Ph) 2.7 mg/dL (nl: 2.4-5.0), PTH 2798 pg/mL, AP 1013 U/L, and 25-vitamin D 11 ng/mL. Post-operatively, PTH fell to 50 pg/mL with profoundly low Ca and Ph, requiring increasing doses of oral and IV Ca with vitamin D therapy (Figure). She experienced peri-oral numbness and muscle spasms. Over the next 2 weeks, PTH rose to 166.1 pg/mL, but AP remained >1000 U/L, and hypocalcemia persisted until IV Ca was increased. On post-operative day 16 she left the hospital against medical advice on oral Ca, calcitriol, and HD. She returned 90 days later with facial tetany and Ca 4.2 mg/dL, AP 215 U/L, and PTH 330 pg/mL. She was treated with IV and oral Ca, and hemodialysate Ca concentration was maximized. One year post-operatively (while on 24 g of oral Ca, 4 mcg of calcitriol, 1000 IU of cholecalciferol daily) she is asymptomatic with continued hypocalcemia (6.8 mg/dL), elevated AP (165/L) and PTH (307 pg/mL), and 25-vitamin D of 33 ng/mL.
Conclusions: HBS can be severe and prolonged following abrupt PTH deficiency, especially in settings of renal osteodystrophy; it is distinguished from hypoparathyroidism by elevations in Ph and AP. Pre-operative vitamin D supplementation and bisphosphonate use may abrogate the severity of HBS.



Nothing to Disclose: AV, CBB

P2-233

Liver Mass Associated with Intractable Hypercalcemia: A Case of Ectopic PTH Producing Tumor?.

Efrem H Gebremedhin MD[1,2] and Elizabeth Batcher MD[1].

[1]Harbor UCLA Torrance, CA and [2]City of Hope Med Ctr Duarte, CA.

Background: Ectopic production of intact PTH from a solid tumor is a rare cause of hypercalcemia, in contrast to the commonly seen PTH related peptide, and its response to conventional treatment is largely unknown.
Clinical case: A 70 yo male with hepatitis C and alcohol abuse was admitted for severe hypercalcemia, confusion and vomiting. Serum calcium (Ca) was 15.8mg/dl (8.5-10.5mg/dl), Albumin 3.2mg/dl(3.5-4.8mg/dl), Phosphorous 2.1mg/dl(2.5-4.8mg/dl), Creatinine 1.8mg/dl(0.4-1.2mg/dl), iPTH >12,500pg/ml(10-65pg/ml), and PTHrp 34pg/ml(14-27pg/ml). He was treated with intravenous hydration, furosemide, pamidronate, and calcitonin. No abnormal parathyroid tissue was evident on whole body sestambibi, neck US or CT neck/chest. CT abdomen showed a 3.7cm x 4.1cm mass in the dome of the liver. The serum Ca normalized only temporarily and zoledronic acid and cinacalcet were administered. When Ca reached 19mg/dl hemodialysis was initiated. Given the degree of PTH elevation and aggressive nature of his illness a malignancy related process was suspected. The most likely cause was hepatocellular carcinoma (HCC) given a liver mass in the setting of hepatitis C, alcohol, and elevated alpha fetoprotein level, 42ng/ml(<9ng/ml). There are only two reports in the literature of HCC producing iPTH, one of which was successfully treated with chemoembolization. Ectopic secretion of iPTH has also been reported with nasopharyngeal rhabodmyosarcoma, neuroendocrine tumor, thymoma, Wilm's tumor, lung carcinoma and ovarian tumor. However, the patient became more confused and developed respiratory failure. Continuous veno-venous hemodialysis (CVVHD) was initiated and Ca improved to high normal. Interventional Radiology and General Surgery services were consulted for biopsy of the liver lesion and neck exploration but both procedures deemed too risky. Despite maximal supportive treatment the patient passed away. Autopsy to determine the diagnosis and source of PTH was declined by the family.
Conclusions: While typically associated with benign parathyroid disease, PTH dependent hypercalcemia can also be due to parathyroid carcinoma or ectopic production of iPTH and resistant to conventional therapies. Cinacalcet and CVVHD can be temporizing adjunctive measures. Identification and treatment of the underlying disorder is critical for the patient's recovery.

Nothing to Disclose: EHG, EB

P2-234

The Primary Hyperparathyroidism Accidentally Revealed in Pregnant Woman.

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Background. A high level of suspicion of primary hyperparathyroidism should be maintained if hypercalcemia is detected during pregnancy.
Clinical case. We report a case of 38 years old woman with gestational diabetes, arterial hypertension and pregnancy 22-23 weeks. She was admitted in our clinic with thirst, frequent urination, sleeplessness. On the diet therapy her glycemia became about 5.7 mmol/L, HbA1c 5.4%. She was prescribed Methyldopa 600 mg daily and BP has achieved 120/80 mm Hg. The hypercalcemia has been suspected because of EGG abnormalities (PR interval was prolonged). Laboratory investigations have been done and revealed serum calcium level 3.05 mmol/l (normal range 2.1-2.6),calcium ionized 1.85 mmol/l (normal range 1.08-1.31), phosphorus 0.85 mmol/l (normal range 0.87-1.45), parathyroid hormone (PTH) - 377 pg/ml (normal range 11-62).
US of neck, abdomen, kidney has not shown any abnormalities. CT with IV contrast has been performed and multinodular formation 14*10*5 mm have been found between trachea and esophagus. No abnormalities on fetus and placenta have been found. It was decided to perform the surgical removal of the parathyroid adenoma. Adenomectomy was perfomed on 27 week of gestation. The adipose adenoma was found by histological assessment. On the 2hd day after surgery the Cvosteck's sign and paresthesia have appeared. The level of calcium -2.15 mmol/l, calcium ionized - 1.2 mmol/l, PTH - 12 pg/ml. We prescribed calcicum at dose 1500 mg and vitamin D 100 mg per day. She is on the 29 week of pregnancy now. She has no paresthesia, but the Cvosteck's sign still persists. The level of calcium became 1.34 mmol/l, PTH -50 pg/ml and daily dose of calcium was reduced to 1000 mg per day. The fetus grows in accordance with gestation age. Delivery is due to April 2010.
Conclusion. Hypercalcemia is associated with a high complication rate in both mother and fetus. Such patients have to be on closed observation of endocrinologist, surgeon and obstetrician team.

Nothing to Disclose: AVD, IVK, OAN, TAB, VP, FFB, SSG, AVM, TPS, IDC

P2-235

Treatment of Tibial Nonunion Fracture Using Recombinant Human PTH 1-34 (Teriparitide): A Case Series.

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Introduction:
Tibial shaft fractures, particularly open fractures, continue to be associated with a high degree of morbidity despite recent advances in surgical techniques and fixation. Treatment of these fractures is often complicated by delayed union and nonunion despite bone grafting and local application of recombinant human bone morphogenetic protein-2 (BMP2). Parathyroid hormone has attracted considerable interest as a bone anabolic agent. Recent animal studies have suggested that PTH can also enhance bone repair after fracture and distraction osteogenesis.
Clinical Cases:
Two patients presented to the Center for Bone Health at The Children's Hospital of Philadelphia with closed tibial midshaft nonunion fractures. Both patients were treated by insertion of an intramedullary rod followed by daily subcutaneous teriparatide. Informed consent for use of teriparatide was obtained from both patients. Patient A, a 19 year old female, had fracture nonunion after two previous insertions of intramedullary rods. She was treated by intramedullary reaming and rod insertion with application of recombinant human BMP2. Immediately after surgery she began treatment with intermittant teriparatide, 20 mcg daily. Radiographs demonstrated progressive healing and complete union by six months, with absence of any visible callus at that time. Patient B was an 11 year old female with an osteogenesis imperfect-variant. She had a closed tibial fracture and after many months of conservative treatment she underwent intramedullary rod insertion. Seven months after the procedure there was no evidence of fracture healing, and at that point teriparatide 20 mcg daily was initiated. Four months after beginning teriparatide treatment, radiographs demonstrated progressive healing with the presence of callus formation. Both patients tolerated the teriparatide injections without untoward effect.
Conclusion:
We describe two pediatric patients with tibial nonunion who improved after treatment with daily teriparatide. In Patient A, fracture healing was accelerated markedly and remodelling was complete at six months, with no callus observed. In Patient B, callus formation occurred soon after initiation of treatment. We believe that these results justify a randomized clinical trial of intermittent teriparatide for fracture nonunion.

Nothing to Disclose: JLB, SM, MAL

P2-236

Hypercalcemic Crisis Secondary to De Novo Parathyromatosis Masquerading as Parathyroid Carcinoma.

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A 66-year-old previously healthy female was admitted to an outside hospital with 2 weeks history of flu-like symptoms. On admission she had orthostatic hypotension and a 2 cm palpable mass in her right neck. Laboratory results are shown in table.

Laboratory Values

Test	On Admission	On Discharge	Normal Range
Corrected Ca	**18.2**	9.4	8.9-10.2 mg/dl
Phosphorus	3.6	2.7	2.5-4.5 mg/dl
Albumin	3.5	2.6	3.5-5.2 g/dl
Creatinine	2.7	1.1	0.3-1.2 mg/dl
25 Vit D	16.2	-	15-50 ng/ml
1,25 Vit D	37	-	15-75 pg/ml
Intact PTH	**988**	42	12-88 pg/ml

She was treated with iv saline hydration and one dose of iv zolendronate. Ultrasound of her neck revealed a 2 cm complex cystic mass posterior to the inferior aspect of the right thyroid lobe. 99m Tc MIBI scan did not reveal any abnormalities. Fine needle aspiration (FNA) of the mass identified parathyroid cells with no cytological evidence of malignancy. She was then transferred to our hospital.
Due to the concern for parathyroid carcinoma, she underwent right hemithyroidectomy, en-bloc dissection of the right superior parathyroid mass, right inferior parathyroid adenoma and right central neck dissection. The surgery was done 1 week after the FNA of her neck mass.
Surgical pathology showed hypercellular changes with fibrosis, hemorrhage and necrosis in the right superior mass and hypercellular changes in the right inferior adenoma without any evidence of local invasion or lymph node pathology. Nodules of benign parathyroid tissue were present in the surrounding adipose tissue consistent with parathyromatosis. She needed calcium supplements post-operatively. She had normal serum calcium levels two months post discharge.

Conclusion: Parathyromatosis is a rare cause of hypercalcemia and is difficult to diagnose [1]. It usually occurs after parathyroidectomy or in association with secondary hyperparathyroidism in the setting of chronic kidney disease [2]. Our patient had de novo parathyromatosis although her initial presentation was more suggestive of parathyroid carcinoma. Parathyromatosis has a high rate of recurrence of hypercalemia and therefore needs long term clinical follow-up.

(1) Fernandez-Ranvier GG et al., Cancer 2007; 110(2):255-64
(2) Lentsch EJ et al., Arch Otolaryngol Head Neck Surg; 2003; 129:894-96.

Nothing to Disclose: JU, CGL, AC, AD

P2-237

Calciphylaxis: Clinical Consequence of Rapid Weight Loss.

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Background: Calciphylaxis is a rare, life-threatening entity characterized by cutaneous vascular calcification resulting in skin ulceration, ischemia, and infarction. It is typically observed in patients with end-stage renal disease in association with a raised calcium-phosphorous product (CPP). Malignancy, hypercoagulable state, warfarin use, obesity, and female gender have all been independently associated with a higher incidence of calciphylaxis(1). Both deficiency and excess vitamin D and PTH states have been linked to vascular calcification. Here, we present a case of calciphylaxis precipitated by weight loss.
Clinical Case: A 60 year-old female with a past medical history significant for painful skin ulcerations after weight loss induced by gastric bypass surgery over 20 years ago presents to the hospital with a recent history of a thirty pound weight loss and necrotic abdominal skin ulcers. She underwent surgical debridement of the ulcers and pathology was consistent with calciphylaxis. Her medical history was significant for a large ovarian mass suspicious for malignancy, rectal cancer, deep vein thrombosis on warfarin, and obesity. Laboratory evaluation revealed normal renal function; however PTH was elevated (128 pg/mL, n 10-65) secondary to low vitamin D levels (15.7 ng/mL, n 30-100). Corrected serum calcium was 9.8 mg/dl (n 8.6-10.5), serum phosphorus level was 3.1 (n 2.7-4.8), and CPP was 30 mg/dl (n <70).
Conclusion: Weight loss is associated with higher systemic levels of matrix metalloproteinases, a family of zinc-dependent enzymes that are produced by endothelial cells. Elastin is a prominent component of vessel walls. It has been hypothesized that metalloproteinase digestion of elastin serves to enhance calcium deposition, resulting in calciphylaxis (2). In this patient with other risk factors for calciphylaxis such as malignancy and warfarin use, weight loss may have precipitated calciphylaxis on two occasions. Considering the high mortality associated with calciphylaxis, this case illustrates the need for risk factor modification especially when instituting weight loss strategies like gastric bypass surgery.

(1) Couto FM, Chen H, Blank RD, Drezner MK. Calciphylaxis in the absence of end-stage renal disease. Endocr Pract. 2006 Jul-Aug;12(4):406-10.
(2) Munavalli G, Reisenauer A, Moses M, Kilroy S, Arbiser J. Weight loss-induced calciphylaxis: Potential role of matrix metalloproteinases. J Dermatol. 2003 Dec;30(12):915-9.

Nothing to Disclose: AC, MS, GG

P2-238

Is Serum Parathyroid Hormone a Useful Biomarker for Bone Health?.

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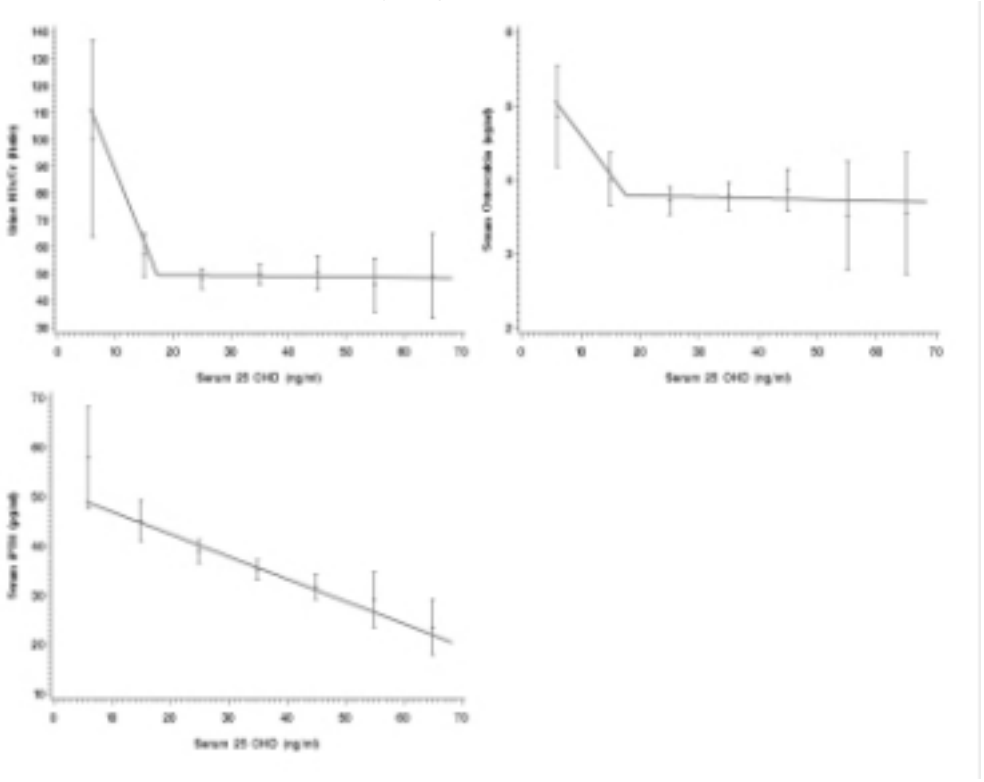
Background: Vitamin D deficiency, defined as serum 25OHD<10ng/ml is associated with osteomalacia and osteoporosis and Vitamin D insufficiency is associated with osteoporosis. There is disagreement about the definition of Vitamin D insufficiency; according to the WHO definition it is a serum 25OHD < 20ng/ml, however others suggest < 30ng/ml because serum parathyroid hormone (PTH) which is inversely associated with serum 25OHD reaches a plateau at a serum 25OHD < 30ng/ml. We examined the relationship between serum 25OHD, PTH and bone markers in elderly women

Methods: 489 postmenopausal women with osteopenia and osteoporosis, mean (SD) ages 71.5(3.6) were enrolled in an interventional treatment study (STOP IT). These results are from the baseline data. Serum PTH was measured by an intact assay (Nichols), serum 25OHD by competitive protein binding assay (Chen Haddad), 24h urine N-telopeptides (NTx) by an Elisa assay and serum osteocalcin by an Elisa assay. The relationship between 25 OHD and PTH was examined using linear regression and piece-wise regression was used for serum osteocalcin and Urine NTx/Cr.

Results: Serum 25 OHD was inversely correlated with serum PTH (r = -0.32, p < 0.001) and NTx/Cr (r = -0.12, p = 0.006).

There was a continuous decrease in mean (±SE) serum PTH from 57.9 (±5.1) pg/ml to 23.4 (±2.8) pg/ml corresponding to serum 25 OHD levels increasing from < 10 ng/ml to > 60 ng/ml respectively (p<0.0001); there was no sign of a plateau effect. Bone markers, serum osteocalcin and urine NTx, decreased with increasing serum 25OHD but reached a plateau at serum 25OHD levels of ~20 ng/ml (p= 0.0001).



Conclusion: The data suggests that Vitamin D insufficiency contributes to increased bone resorption when serum 25OHD < 20 ng/ml. A decrease in serum PTH at serum 25 OHD levels > 30 ng/ml is not an indication of secondary hyperparathyroidism related to vitamin D insufficiency. The continuing decrease in serum PTH at serum 25OHD > 30ng/ml probably reflects the pharmacologic effect of vitamin D on the PTH gene. The appropriate treatment for bone health using vitamin D is to increase serum 25OHD to >20ng/ml.

Sources of Research Support: NIH Grants AG28168, UO1-AG10373 and RO1-AG10358.

Nothing to Disclose: AJS, JCG, XF, CS

P2-239

Relationships between Age, Sex, Vitamin D, and Bone Mineral Density in Primary Hyperparathyroidism.

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Previous studies have shown that patients with primary hyperparathyroidism (PHPT) have disproportionately decreased bone mineral density (BMD) in the forearm (FA) and lumbar spine (LS).The relationships between age, sex, 25-hydroxyvitamin D (25OHD) and BMD in PHPT have not been established.
Objective: We hypothesized that in PHPT, BMD at all sites were equally affected and the degree of bone loss may be influenced by age, sex, and 25OHD.
Design: We performed a retrospective review of patients, referred to our clinic for evaluation of hyperparathyroidism (HPT) between 1/2005 and 6/2009. We excluded patients with incomplete data, previous parathyroidectomy, lone secondary HPT, normocalcemic HPT, secondary and tertiary HPT due to end-stage renal disease, and those on lithium treatment. We arbitrarily selected 50 years (yrs) as the cut-off age between pre- and post-menopausal women.
Results: There were 5 men under 50 yrs of age, 15 men over 50, 7 women under 50, and 49 women over 50. Baseline characteristics are shown in Table 1.

Table 1: Baseline Characteristics of the Cohort (n = 76)

	Mean and SD	Normal Range
Age (yrs)	63.8 +/- 12.5	
PTH (pg/mL)	109 +/- 52	15 - 65
Ca (mg/mL)	10.65 +/- 0.48	8.4 - 10.2
25OHD (ng/mL)	29.1 +/- 12.2	30 - 80
LS BMD (g/cm2)	0.970 +/- 0.184	
FN BMD (g/cm2)	0.723 +/- 0.133	
FA BMD (g/cm2)	0.678 +/- 1.05	

BMD was preserved in all men and women 50 years of age and younger. In the over 50-years-of-age groups, 80% men and 76% women had low BMD (T-scores <-1). Further stratifying the older cohorts based on areas of reduced BMD, we found that 42% men had lowest T-scores in the LS, 42% had lowest T-scores in the FA, and 16% had lowest T-scores in the femoral neck (FN). Among the older female cohort, 32% women had lowest LS T-scores, 46% had lowest FN T-scores, 14% had lowest FA T-scores, and 8% had equally reduced T-scores in the LS, FN, and FA. In multiple regression analysis, only age was associated with BMD reduction in the FN and FA.
Conclusions: BMD was preserved in the younger-than-50 yrs cohorts with PHPT. In the older cohorts, equal proportions of males and females have reduced BMD. In the over-50- yrs male cohort, BMD in the LS and FA were affected most often whereas the FN was least affected. In the over-50-yrs female cohort, the FN was most often affected, followed by the LS and FA, respectively. There was no association between 25OHD and the degree of BMD reduction.

Nothing to Disclose: HDN, TDH, VQM, PWC, BCG, KMMS

P2-240

Hyperparathyroidism Secondary to Hypovitaminosis D Depends on Serum Levels of Magnesium.

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Context. Vitamin D deficiency in adults may precipitate or worsen osteopenia and osteoporosis, cause muscular weakness and osteomalacia, and increase the risk of bone fractures (1). Some of these effects may be mediated by secondary hyperparathyroidism induced by low 25-hydroxyvitamin D (25-OHD) levels. It is not clear why some individuals do not develop elevation in parathyroid hormone (PTH) levels following vitamin D deficiency.
Objective. To estimate the prevalence of hypovitaminosis D and secondary hyperparathyroidism in a sample of adult ambulatory patients, and analyze possible causes of absence of correlation between 25-OHD and PTH levels.

Design and setting. Cross-sectional study of 91 outpatients of a tertiary care center in Southern Brazil (latitude 30^0S).
Patients. Adults aged 40 years or older.
Main Outcome Measures. Serum levels 25-OHD measured by chemiluminescence and serum levels of PTH measured by electrochemiluminescence.
Results. Mean age was 60.3 ± 12 years, 56 (61.5%) women, and 70 (76.9%) white. Hypovitaminosis D was found in 69 (75.9%), 11 (12.1%) had secondary hyperparathyroidism and none had hypomagnesemia (Mg <1.5 mEq/L). There was no significant correlation between serum levels of 25-OHD and PTH, but this correlation became significant when individuals with magnesium serum level > 2.1 mg/dl were considered in the analysis ($r = 0.37$, $p = 0.02$). There was a positive correlation between PTH and serum magnesium levels in the normal range ($r = 0.29$, $p < 0.01$).
Conclusions. The association between hypovitaminosis D and secondary hyperparathyroidism is dependent on serum levels of magnesium.

Figure 1. PTH concentrations according to 25-OHD levels in all patients (n=91)

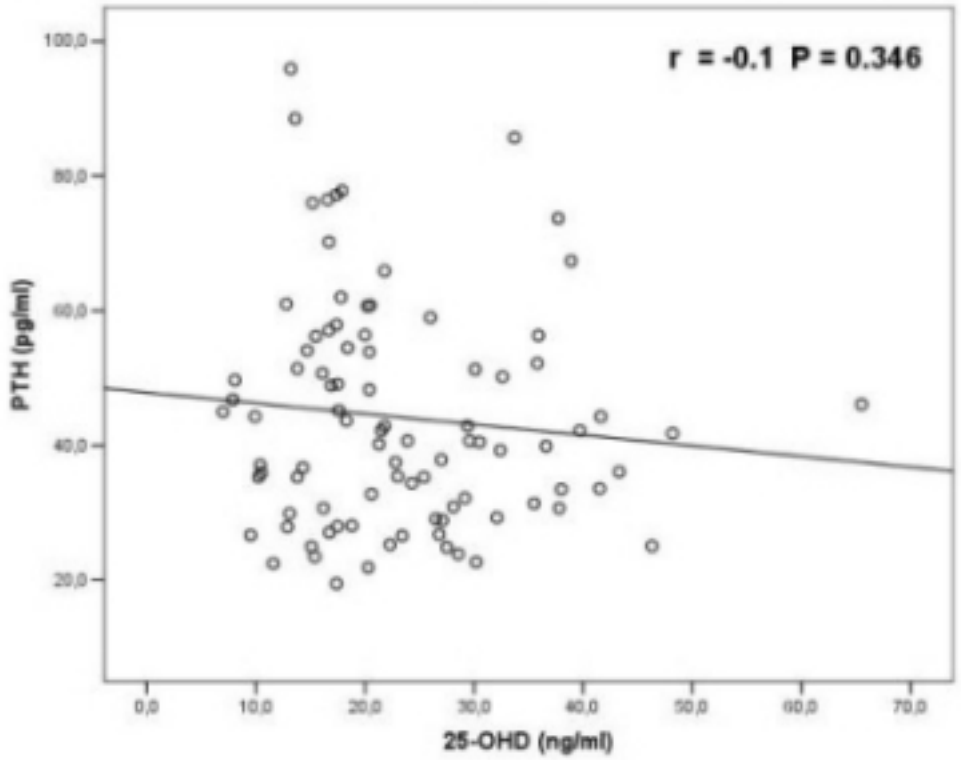
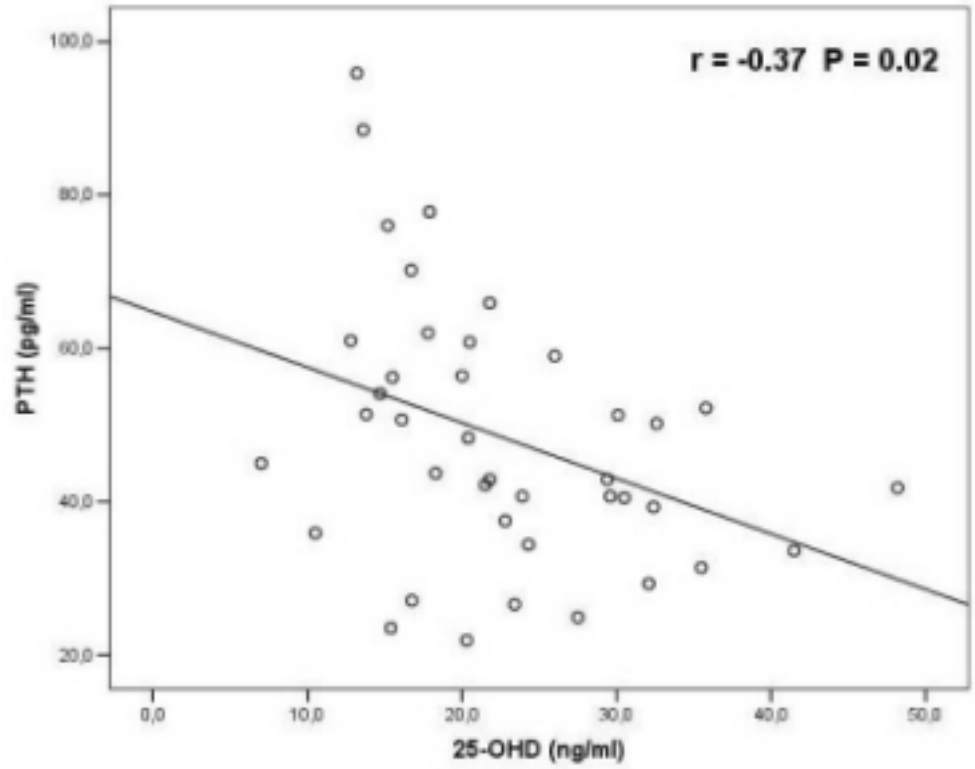


Figure 2. PTH concentrations according to 25-OHD levels in patients with serum Mg > 2.1 mg/dl (n=40)



(1) Holick MF, N Eng J Med 2007; 357:266

Nothing to Disclose: TS, LCFP, DVR, RS, JLG

P2-241
Renal Function in Primary Hyperparathyroidism.

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Introduction: renal insufficiency (RI) is a complication of primary hyperparathyroidism (PHPT) and it can negatively affect the clinical presentation of PHPT and increase the risk of mortality. In asymptomatic PHPT a Glomerular Filtration Rate (GFR) less than 60 ml/min represents the precise level below which surgery is recommended, however the prevalence of RI in asymptomatic PHPT is unknown. Thus we sought to investigate the prevalence of RI in a large case series of PHPT patients mostly asymptomatic.
Subjects and methods: in 294 consecutive PHPT patients (M/F=76/218; asymptomatic/symptomatic=151/143; age=59.1±13.7yrs; BMI=25.5±4.9kg/m^2; PTH=215.3±221.1pg/ml; ionized calcium=1.46±0.17mmol/l; serum creatinine=0.88±0.3mg/dl) renal function estimated by means of MDRD (Modification of Diet in Renal Disease) equation was evaluated. A GFR <60ml/min represent the threshold of moderate-to-severe RI definition .
Results: In the whole group mean (±S.D.) GFR was 92.3±31.6ml/min, with a RI prevalence of 17.4 %. Patients were subdivided according to their median age (60 years): younger patients showed higher GFR than older ones (98.7±32.1 vs 85.5±29.6ml/min, respectively, p<0.0003) with a RI prevalence of 11.2% vs 23.9% (p<0.00001). Asymptomatic patients compared to symptomatic did not differ both for mean GFR (92.1±31.3 vs 92.5±31.9ml/min, respectively, p=n.s.) and for RI prevalence (14.7% vs 17.9% , p=n.s.). Patients with kidney stones, also, did not differ from those without in terms of GFR (93.1±31.3 vs 91.5±31.7m l/min, p=n.s.) and for RI prevalence (16.7% vs 17.9%, p=n.s.). Males showed a lower GFR compared to females (59.4±17.4 vs 103.8±27.0ml/min, p<0.001), and also higher RI prevalence (56.6% vs 3.7%, p<0.00001). These findings persisted also adjusting for age, serum calcium and PTH levels. In the whole group GFR was negatively associated with age (R=-0.25, p<0.00002) and with ionized serum calcium levels (R=-0.13, p<0.04).
Conclusions: in a large contemporary PHPT case series a lower than previously reported prevalence of moderate to severe renal insufficiency was observed. No significant differences were found between asymptomatic and symptomatic patients and also between patients with kidney stones and those without. A sharp difference of RI prevalence was found between sexes (independently of the activity of the disease). Finally, the negative relationship of GFR with serum calcium would confirm the pathogenetic link between PHPT and RI.

Nothing to Disclose: GB, LG, CB, FC, GM, MP, VB, FT

P2-242

Low Urine Calcium Excretion in African Americans with Primary Hyperparathyroidism.

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[1]Wayne State Univ Detroit, MI.

Background: Primary hyperparathyroidism (PHPT) is a common disorder characterized by hypercalcemia and normal or elevated urinary calcium excretion (Ur Ca). Familial hypercalcemic hypocalciuria (FHH) is a rare genetic disorder characterized by hypercalcemia but low Ur Ca. FHH is a benign disorder that needs no interventions. In our endocrine practice, we have observed many African Americans (AA) with FHH range of Ur Ca despite features of PHPT.
Objective: To compare Ur Ca in AA patients with PHPT with that of non-AA and retrospectively evaluate the usefulness of Ur Ca to differentiate PHPT from FHH in AAs.
Methods: We conducted a retrospective cohort study. 291 patients were evaluated from a cohort of 1300 patients, who had 24 hour urinary excretion of calcium and creatinine (calculated UCa/Cr mg/gr) performed between January 2004 to December 2009. Inclusion criteria were hypercalcemia with albumin corrected serum Ca > 10.5 mg/dL with serum intact parathyroid hormone (PTH) >40 pg/mL. We excluded patients who were on agents that affect Ur Ca. Their urinary excretion of calcium was evaluated with regards to their urine creatinine.
Results: 98 patients satisfied the above criteria for PHPT. The AA (n=70) and non-AA (n=26) patients were not different in their mean age, body mass index, glomerular filtration rate, PTH and 25-hydroxy vitamin D values. The Ur Ca (mg/gr creatinine, means ± SEM) of AA was significantly lower than that of non-AA patients (173.9 ± 18.1 vs 302 ± 38.2 mg/gr, P=0.001). Ur creatinine excretion was not different between AA and non-AA (0.97 ± 0.05 Vs 1.05 ± 0.08 gr/day, respectively, p=0.4). Out of 70 AA patients, 44% (n=31) had low UCa/Cr $\leq$100 mg/g, whereas only 7% of non AA had this value (p=0.001). There was no significant gender difference in Ur Ca in each group.
Conclusions: In AA patients with hypercalcemia, evaluation of Ur Ca to rule out FHH could be misleading. Clinical criteria and other laboratory parameters shall be used to differentiate PHPT from FHH

Nothing to Disclose: WT, NS, FM, OA, ABA-S

P2-243

Characteristics of Lacunar Wall in Patients with Renal Hyperparathyroidism.

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Purpose: RANKL mRNA was expressed in the mouse osteocyte cell line, and osteocytic osteolysis has been proved in rats. In addition, both perilacunar mineralization and mineralization of the osteocyte itself at the point of cell death have been already reported. Therefore, the shape of lacunar wall were investigated to observe the results of bone resorption or formation by the osteocytes in patients with renal hyperparathyroidism.
Methods: Lacunae (Lc) was classified into the three groups according to its shape in 26 patients with renal hyperparathyroidism (Age; 56.8 ± 8.3 years, Duration of dialysis; 15.6 ± 7.8 years), namely, (1) Lc with predominant eroded surface (ES), (2) Lc with predominant quiescent surface (QS) and (3) Lc with predominant osteoid surface (OS). Bone volume (BV) referent number of Lc with predominant ES (N.ES.Lc/BV: N/mm^2), BV referent number of Lc with predominant QS (N.QS.Lc/BV: N/mm^2) and BV referent number of Lc with predominant OS (N.OS.Lc/BV: N/mm^2) were measured.
Results: N.ES.Lc/BV was greater than N.QS.Lc/BV (185.7 ± 61.5 versus 131.3 ± 51.8 N/mm^2, $p < 0.01$) and N.QS.Lc/BV was greater than N.OS.Lc/BV (131.3 ± 51.8 versus 21.4 ± 26.5 N/mm^2, $p < 0.01$). Perilacunar mineralization was observed in these patients.
Conclusion: Characteristics of the lacunar walls and perilacunar mineralization indicated that the osteocytes resorb and form the mineralized matrix in patients with renal hyperparathyroidism. Osteocytic osteolysis seems more active than perilacunar formation by the osteocytes in these patients.

Nothing to Disclose: AY, SO, AI

P2-244

Role of Selective Venous Sampling for PTH before Revision Surgery for Persistent Hyperparathyroidism Revisited.

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[1]Leiden Univ Med Ctr Leiden, Netherlands.

Introduction:
Localisation studies are mandatory prior to revision surgery for persistent hyperparathyroidism to reduce the risk of lengthy explorations and improve surgical outcome. However, noninvasive localisation studies have been reported to have a poor sensitivity in this case. The aim of our study was to determine the ability of selective PTH venous sampling (SVS) to correctly localize residual hyperactive parathyroid glands in patients with persistent or recurrent hyperparathyroidism.
Patients & Methods:
We retrospectively evaluated the localizing accuracy of 26 PTH SVS performed prior to revision surgery in 21 patients with persistent or recurrent primary hyperparathyroidism (n=14) or autonomous (tertiary) hyperparathyroidism (n=7). Operative and pathology data were compiled from hospital records. Data on other localisation studies, such as Tc99m-MIBI-SPECT (n=20), CT scans (n=7), US of the neck (n=5), MRI scan (n=5) and PET scan (n=3), were also available.
Results:
SVS was able to accurately localize 18 of the 24 pathological glands (75%) removed at revision surgery. No complications were reported. The sensitivity of SVS was significantly higher than that of the more widely used Tc99m-MIBI-SPECT, which was only 30% (*p=0,021*).
Conclusion:
Our findings demonstrate that SVS is an effective localisation study in patients with persistent or recurrent hyperparathyroidism, with a sensitivity significantly higher than that of Tc99m-MIBI-SPECT. This suggests that SVS represents a useful addition to the pre-operative work-up of patients with persistent hyperparathyroidism before revision surgery.

Nothing to Disclose: JEW, JK, JM, JAR, NATH

P2-245

Comparison of Preoperative Examinations in Patients with Primary Hyperparathyroidism: US, MIBI, SPECT and MRI.

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[1]Ministry of Hlth, Ankara Numune Res and Training Hosp Ankara, Turkey and [2]Duzce Univ, Fac of Med Duzce, Turkey.

Objective: In recent years minimally invasive parathyroidectomy (MIP) has been preferred to conventional parathyroidectomy. Accurate preoperative localization is important to successful MIP. The purpose of this investigation was to directly compare the accuracy of ultrasonography (US), 99mTc-sestamibi (MIBI), single photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI) for localization of parathyroid adenomas.
Methods: Ninety-eight patients with primary hyperparathyroidism (pHPT) who underwent parathyroidectomy were included in the study. In all patients, serum total calcium (Ca^{+2}), ionized Ca^{+2}, phosphate (P), albumin, creatinine, parathormone (PTH), and 25-hydroxyvitamin D3 and 24-hour urinary Ca^{+2}, P and creatinine levels were measured preoperatively. Ultrasonography, MIBI, SPECT and MRI were performed in all patients.
Results: Of the 98 patients, who underwent surgery, 86 had adenoma, 6 had hyperplasia and another 2 had carcinoma while 4 cases had no lesions. Two cases had bilateral adenoma. Abnormal parathyroid gland could be localized preoperatively in 82 of the cases with US (83.7%), 66 of the cases with MIBI (67.3%), 71 of the cases with SPECT (72.4%), while only in 60 cases with MRI (61.2%). While there was a statistically significant difference among cases in terms of adenoma volume by MIBI + (1.30 ± 1.51 vs 0.58 ± 0.91, $p < 0.05$), no difference was established in adenoma volume among cases who were localized and could not be localized by US, SPECT and MRI. Sensitivity, specificity and diagnostic accuracy values were 87.2%, 25.0%, and 83.0%; 70.2%, 50.0%, and 69.4%; 75.5%, 50.0%, and 74.5%; 63.8%, 50.0%, and 63.3% for US, MIBI, SPECT and MRI respectively. Sensitivity, specificity and diagnostic accuracy were found as 94.2%, 25.0% and 91.1% when US was combined with MIBI. Two of the patients who could not be scanned by US had parathyroid adenoma localized in the mediastinum. While adenoma in one of those patients was localized by MIBI, we were able to localize by SPECT and MRI in both patients.
Conclusion: The combination of US and MIBI is mostly adequate for preoperative imaging of primary hyperthyroidism. While the success of US is low in ectopic localization, the success of MIBI is associated with the size of lesion.

Nothing to Disclose: GA, DB, YA, SI, DC, IP, YT, SG

P2-246

4D Neck CT Versus SPECT/CT Using Tc99m Sestamibi in the Localization of Parathyroid Adenomas: Which Study and When?.

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BACKGROUND: Accurate preoperative localization of a parathyroid adenoma provides valuable information to the endocrine surgeon. Localization rather than mere lateralization can minimize dissection by allowing the surgeon to place the incision in a precise location. SPECT/CT using Tc-99m sestamibi(sestamibi) has historically been the localizing study of choice,but recently, 4-dimensional neck computed tomography (4D-CT) has become an emerging technique that assesses parathyroid anatomy. **OBJECTIVE:** To evaluate and compare 4D-CT with sestamibi imaging techniques as tools for localization of hyperfunctioning parathyroid tissue in the surgical naïve neck.**METHODS:**A prospectively maintained surgical endocrine database was queried to identify patients who underwent initial parathyroidectomy after preoperative localization using 4D-CT and sestamibi. Reoperative cases were excluded. Sensitivity and specificity were calculated for each of the modalities as compared to operative & pathologic findings. Logistic regression analyses were then used to calculate other factors that may influence the sensitivity and specificity of each of these imaging techniques. Risk-benefit analysis was also performed for each of these modalities.**RESULTS:** 356 patients were identified from the database. 4DCT and sestamibi had comparable sensitivity of 89%(85-92%) to identify any hypercellular gland as compared with pathology. However, when using the techniques to specifically localize the location of the gland, 4DCT was more sensitive in identifying the superior glands(65-70% 4DCT vs 36-38% sestamibi)and the 2 techniques were comparable for the localization of the inferior glands. Sestamibi had a higher specificity in the superior glands, however, and 4DCT had higher specificity in the inferior glands. 4DCT's false positive rate was 9-11%, and false negative rate was 28-30%. Risk factors such as higher BMI and older age are associated with higher false positive 4DCT readings; other risk factors (pre-existing thyroid disease & tumor size)that make one technique superior to the other are discussed.**CONCLUSIONS:**4DCT and SPECT/CT using sestamibi are comparable modalities for parathyroid adenoma identification but 4DCT is more sensitive for precise gland localization, particularly with adenomas originating within the superior glands. Body habitus, age and coexisting thyroid disease are factors that may be important in decided which one modality to obtain for preoperative purposes.

Nothing to Disclose: NLB, TV, EG, DU, EK, CL, CJ, MIH, SGW, HG, NDP

P2-247

A Humanized Monoclonal Antibody to the FGF Receptor 1c Reverses Obese Phenotypes Caused by a High-Fat Diet and in ob/ob Mice, Independent of Food Intake, Associated with Increased Hypothalamic Expression of Glucose Transporters.

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A humanized monoclonal antibody against the FGF Receptor 1c (FGFR1c) receptor, initially developed as a possible therapy for cancer, was previously shown to reduce food intake and body weight in healthy mice, rats, and monkeys. In the present study we examined if the antibody would reverse obese phenotypes, and if so to begin to assess the mechanisms mediating these effects. Male C57Bl/6J mice were made obese by access to a high-fat (35%) diet. Within one day of i.p. injection of the FGFR1c antibody (either 1 or 10 mg/kg), food intake and body weight was significantly reduced, and stayed reduced for 7 days after a single i.p. injection. Thereafter the antibody was injected once per week. By the second week food intake returned to normal levels observed in mice injected with control antibody, but body weight continued to stay reduced throughout the 8-week study. To assess potential mechanisms by which the antibody produced these effects, we examined the effect of the antibody on nutrient-sensitive hypothalamic gene expression. The antibody induced hypothalamic expression of GLUT1 and GLUT4, and consistent with enhanced glucose transport, responses to fasting and hypoglycemia were attenuated. These data suggest that the anti-obesity effects of the FGFR1c antibody are associated with, and may be mediated by, enhanced hypothalamic sensitivity to glucose.

Disclosures: HS: Collaborator, Lilly USA, LLC.
Nothing to Disclose: FI, MP, CVM

P2-248
Novel Anorexigenic Effects of Vaspin.

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Vaspin is a newly-discovered adipokine that belongs to the serine protease inhibitor (serpin) family. Vaspin has glucose-lowering and insulin-sensitizing effects by acting in the periphery. In the present study, we have investigated the regulatory role for vaspin in central metabolism. Here we found that intracerebroventricular administration of vaspin caused potent and prolonged anorexia and weight loss in normal and diet-induced obese mice. Interestingly, hypothalamic expression levels of vaspin are dependent on anorexigenic hormone leptin. Leptin-deficient ob/ob mice had lower hypothalamus expression of vaspin, which was increased by administration of leptin. Furthermore, similarly to leptin, vaspin activates the signal transduction activated transcript-3 (Stat3), stimulates proopiomelanocortin expression but suppresses *Agouti* related protein expression in the hypothalamus. Notably, the anorexigenic effect of vaspin was partly mediated through serpin activity. Mutation of serpin domain of vaspin significantly ameliorated the anorexigenic effect of vaspin. Tissue type-plasminogen activator (t-PA) is a serine protease that is abundantly expressed in the hypothalamus and involved in neuronal plasticity and synaptic reorganization. Central administration of t-PA decreased food intake and body weight. Interestingly, the feeding effect of t-PA was antagonized by coadministration of vaspin. Our findings suggest that hypothalamic vaspin is a novel anorexigenic neuropeptide that acts through Stat3 signaling and proteolytic pathway.

Nothing to Disclose: MSK, K-UL, J-YP, WJL, EHK, SAL, M-SS, JYH, G-MK, JYL, BSY

P2-249

The Novel Neuropeptide Alarin Stimulates Food Intake and the Reproductive Axis in Male Rats.

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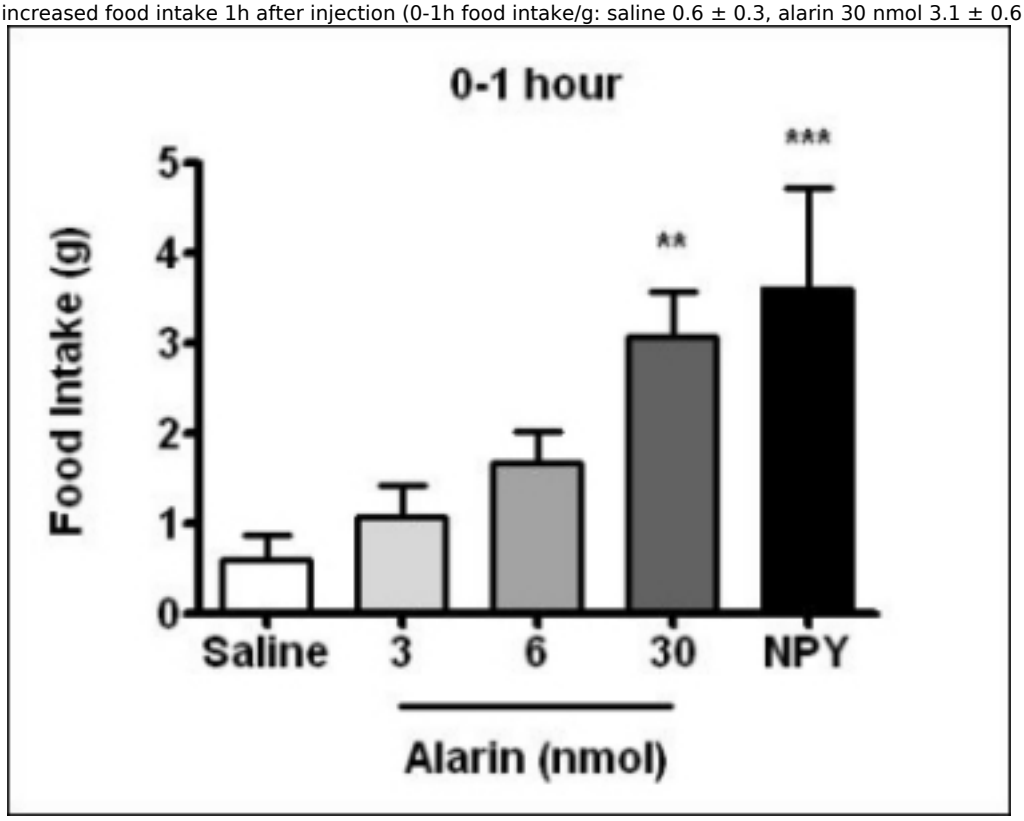
Alarin is the most recently discovered member of the galanin peptide family, and is encoded by a splice variant of galanin-like peptide (GALP) mRNA. Galanin and GALP are known to regulate energy homeostasis and reproduction. Galanin is thought to mediate its effects via the three galanin receptors. GALP also binds to these known galanin receptors, but evidence suggests that it may also act via an as yet unknown GALP specific receptor. The role of alarin in energy homeostasis and reproduction is currently unknown. The objective of these studies was thus to determine the effects of alarin on food intake and the hypothalamo-pituitary-gonadal axis.
Intracerebroventricular (ICV) administration of alarin (30 nmol) to unanaesthetised *ad libitum* fed male rats significantly increased food intake 1h after injection (0-1h food intake/g: saline 0.6 ± 0.3, alarin 30 nmol 3.1 ± 0.6 P<0.01).



ICV administration of alarin (6 nmol) to adult male rats significantly increased plasma LH levels 30 min after injection (plasma LH ng/ml; saline 0.7 ± 0.1, 6 nmol alarin 1.0 ± 0.1, P<0.05). *In vitro*, alarin stimulated Neuropeptide Y (NPY) and GnRH release from hypothalamic explants from male rats, and GnRH release from GT1-7 cells. *In vivo*, pre-treatment with the GnRH receptor antagonist cetrorelix blocked the alarin-induced stimulation of plasma LH levels in unanaesthetised adult male rats. Receptor binding studies confirmed that alarin does not bind to galanin receptors 1 or 2, and demonstrated that alarin does not compete with radiolabelled galanin for hypothalamic binding sites. Alarin immunoreactive cell bodies were detected within hypothalamic nuclei known to be involved in appetite regulation and reproductive function. These results suggest that alarin is a novel orexigenic peptide, and that it stimulates the HPG axis via hypothalamic GnRH. Further work is now required to determine the receptor that mediates the biological effects of alarin.

Nothing to Disclose: CKB, MP, JAT, GAB, JVG, MAG, SRB, KGM

P2-250

Refeeding-Activated Neurons in the Hypothalamic Paraventricular Nucleus (PVN) Mediate Effects of Melanocortin Signaling in the Nucleus Tractus Solitarius (NTS).

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Recently, we demonstrated that alpha-melanocyte stimulating hormone (α-MSH) derived from hypothalamic arcuate nucleus neurons densely innervate neurons in the ventral parvocellular subdivision of the hypothalamic paraventricular nucleus (PVNv), and that these neurons are activated when fasting animals are refed (1). As icv administration of the melanocortin receptor antagonist, AGRP, not only prevents cFos activation in PVNv neurons but also increases cumulative food intake after a fasting animal has been refed, we proposed that this subdivision of the PVN serves as important integrative center for melanocortin signaling in the forebrain and is involved in the regulation of satiety. The nucleus tractus solitarius (NTS) in the caudal brainstem has an important role as an integrative center for feeding signals relayed through the vagus nerve as well as some forebrain centers (2). We hypothesized, therefore, that refeeding-activated neurons in the PVNv may mediate satiety effects of melanocortin signaling through the NTS. To test this hypothesis, the retrograde neuronal tracer, cholera toxin b subunit (CtB) was iontophoresed into the NTS of adult, male, Sprague-Dawley rats. One week later, the animals were fasted for 2 days and then allowed to refed for 2h. Injections that filled the NTS resulted in retrograde accumulation into the medial, ventral and lateral parvocellular subdivisons in the PVN. As previously observed, cFos immunoreactive cells concentrated in the PVNv, but also were identified in the lateral parvocellular subdivision of the PVN (PVNl). Approximately 79.2 ± 5.7% CtB labeled cells in the PVNv and 62.3 ± 3.7 % in the PVNl contained cFos. CFos activation was also identified in the medial, dorsomedial, and lateral subdivisions of the NTS in the refed animals but no cFos activation was seen in the NTS during fasting. In response to a focal injection of α-MSH (0.6 nmol) into the PVN of fasting animals, however, cFos localized primarily to medial subdivision NTS neurons (NTSm). We conclude that melanocortin-responsive neurons in the PVNv and PVNl project directly to the NTS and during refeeding, may mediate its effects on satiety through the NTSm.

1. Singru PS et al., Endocrinology 2007;148:638-46
2. Blevins et al., Am J Physiol Regul Integr Comp Physiol 2009;296: R476-84

Sources of Research Support: Grant DK37021 from the National Institutes of Health.

Nothing to Disclose: PSS, GW, CF, RML

P2-251

Inhibition of Food Intake, Body Weight Gain and GH Release Via a Putative Ghrelin *O*-Acyltransferase (GOAT) Inhibitor.

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New approaches are urgently needed for the treatment of metabolic diseases and disorders such as obesity. The recent discovery of ghrelin *O*-acyltransferase (GOAT) that catalyses the addition of octanoate to Ser-3 of the ghrelin peptide, and is essential for ghrelin's stimulatory actions on appetite, food intake and GH secretion, offers an opportunity for novel strategies; i.e. to inhibit the synthesis of active ghrelin by a GOAT inhibitor. To this end, in the present study we designed and synthesized, within a series of ghrelin analogs, a duodecapeptide designated FI-I-GOAT (GOAT-12) and examined its effects on food intake, body weight gain and GH release via the CNS.
In the first set of experiments, food intake was monitored on an hourly basis for 5 hours during the light phase of the L-D cycle. Intracerebroventricular (icv) administration of 10 μg GOAT-12 to male rats significantly (P<0.02) inhibited cumulative food intake at 4 and 5 hours after injection compared with vehicle icv-injected controls; body weight gain over 24 h was also decreased during *ad libitum* food intake. In addition, the latency time to first meal was significantly (P<0.05) prolonged following icv GOAT-12 and the duration of that meal was decreased. Furthermore, the inhibition of 24-h body weight gain by GOAT-12 was reproduced in rats maintained on a reverse L-D cycle and icv-injected with GOAT-12 during the dark phase (P<0.01). In the second experiment, spontaneous 4-6 h plasma GH profiles were obtained from free-moving rats. Centrally administered GOAT-12 (10 μg) caused a 2-to 3-fold decrease in mean plasma GH levels compared with vehicle icv-injected controls (GH AUC: 29 ± 8 *vs.* 81 ± 26 ng.h/ml). Although activity of GOAT-12 has yet to be determined in a newly developed GOAT assay, the initial results reported here suggest that inhibiting and/or disrupting the endogenous GOAT-ghrelin system may provide novel strategies/useful drugs to regulate food intake and GH secretion in the treatment of metabolic disorders such as obesity and its complications.

Nothing to Disclose: GST, ZJK, J-KC, CYB

P2-252

The Effect of Exendin-4 on Appetite-Regulating Neuropeptides in the Mouse Hypothalamic Neurons.

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Exendin-4, a long-acting GLP-1 receptor (GLP-1R) agonist is used as a type 2 diabetes drug due to its insulinotropic and glucose-lowering actions. In addition to its glucoregulatory effect, exendin-4 acts as a potential regulator of feeding behavior by its ability to inhibit gastric emptying, reduce food intake and induce satiety. Many studies have revealed molecular targets of exendin-4, but the exact mechanisms by which it acts on the appetite-regulating nuclei of the hypothalamus and the neuronal cell types involved in feeding regulation are not completely known, primarily due to the lack of representative hypothalamic cell models. To address this issue, we used a clonal, immortalized hypothalamic cell line mHypoA-2/30 that has recently been generated from adult mouse hypothalamus. This cell model endogenously expresses GLP-1R and appetite-regulating neuropeptides such as orexigenic ghrelin and anorexigenic neurotensin and POMC. Using the mHypoA-2/30 cells, we studied the activation of signaling cascades triggered by exendin-4 and the changes in the mRNA transcript levels of ghrelin, neurotensin and POMC. We found that exendin-4 (10 nM) stimulated phosphorylation of ERK1/2 and CREB/ATF1, and significantly suppressed mRNA levels of ghrelin by 28% at 8h, and increased that of neurotensin by 48% at 6h and POMC by 67% at 24h time points in the mHypoA-2/30 neurons suggesting that ghrelin, neurotensin and POMC are the putative downstream modulators regulated by exendin-4. Furthermore, to assess which hypothalamic neuropeptidergic neurons are activated by exendin-4 *in vivo*, we intracerebroventricularly injected exendin-4 into mice and followed its effect on the activation of hypothalamic neurotensin, POMC and ghrelin neurons at 2h post-treatment. By double-label immunohistochemistry with c-Fos, a marker of neuronal activation, we demonstrated that central exendin-4 activated neurotensin-neurons in the paraventricular nucleus (PVN), POMC-neurons in the arcuate nucleus and ghrelin-neurons in the PVN and periventricular hypothalamic nucleus. Taken together, our findings from both *in vitro* and *in vivo* studies provide a previously unrecognized link between the GLP-1R activation and the hypothalamic neuropeptides involved in appetite-regulation. Specifically, the findings establish that neurotensin, POMC and ghrelin are regulated by exendin-4 in the hypothalamus and this regulation may play a role in the GLP-1R-mediated control of appetite and energy homeostasis.

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 Reseach Chairs (CRC) Program and the Ontario Graduate Scholarship (OGS) program.

Nothing to Disclose: PSD, MJP, DDB

P2-253

Chronic Subcutaneous Infusion of Nesfatin-1 Inhibits Food Intake and Alters Energy Expenditure in Fischer 344 Rats.

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[1]York Univ Toronto, Canada and [2]York Univ Toronto, Canada.

Nesfatin-1 is a recently discovered 82-amino-acid-peptide that inhibits food intake and exerts weight-reducing effects. Based on these observations, nesfatin-1 has been proposed to function as an anti-obesity peptide. However, studies to date merely focused on the acute satiety effects of nesfatin-1, without addressing other potential long-term metabolic effects of this peptide. Therefore, the main objective of this study was to characterize the short- and long-term effects of nesfatin-1 on whole-body energy balance and metabolic partitioning in male Fischer 344 rats. Short-term (24 h) subcutaneous infusion of nesfatin-1 (50 µg/kg body weight/day) using osmotic mini-pumps increased total spontaneous activity and whole-body fat oxidation during the dark phase. This was accompanied by decreased food intake, feeding bout size, and basal metabolic rate compared to saline infused controls. Cumulative food intake and total spontaneous physical activity during the dark phase remained significantly reduced and elevated, respectively, even after long-term (7 days) continuous nesfatin-1 infusion. However, no changes in body weight were found after either short or long-term nesfatin-1 infusion. As expected, increased serum nesfatin was found after long-term infusion of this peptide. Prepronesfatin mRNA expression in the brain and stomach, as well as serum nesfatin concentrations were significantly reduced under fasting conditions. A post-prandial increase in serum nesfatin-1 was also detected in ad libitum fed rats. Our results report for the first time the extensive metabolic effects of nesfatin-1 in rats. Collectively, our results indicate that chronic peripheral administration of nesfatin-1 at the dose tested is effective in modulating metabolic functions of rats.

Sources of Research Support: Canadian Institutes of Health Research (CIHR); Canada Foundation for Innovation (CFI) and Natural Sciences and Engineering Research Council (NSERC) of Canada.

Nothing to Disclose: SM, RGG, RBC, SU

P2-254

Molecular and Functional Evidences for Endogenous Nesfatin-1 and Its Physiological Actions in Non-Mammalian Vertebrates.

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Nesfatin-1 is a recently discovered peptidyl anorectic hormone encoded in the precursor peptide, nucleobindin 2 (pronesfatin). Studies to date reporting physiological actions of nesfatin-1 have used mammals. Is nesfatin-1 a physiological regulator in non-mammalian vertebrates? Our studies, for the first time, address this question. The major objectives of this study were to characterize prepronesfatin mRNA in non-mammalian vertebrates, identify the relative expression of prepronesfatin mRNA in tissues and test the effects of nesfatin-1 on food intake. Using RT-PCR, RACE or data mining, we identified prepronesfatin mRNA sequences from zebrafish, goldfish and frog. Prepronesfatin mRNA expression is abundantly expressed in the pituitary, hypothalamus, telencephalon, olfactory bulb, liver, and gall bladder of goldfish. Pronesfatin-like immunoreactivity was detected in the brain, specifically in the hypothalamus of goldfish. We hypothesized that if nesfatin-1 is an endogenous regulator of food intake in goldfish, we should see changes in prepronesfatin mRNA expression under altered nutritional conditions. Indeed, we observed an approximate 2-fold increase of prepronesfatin mRNA expression in the hypothalamus 1 and 3 hours after feeding compared to unfed control goldfish. Three or seven day fasting also induced an approximate 3-fold reduction in prepronesfatin mRNA expression in the hypothalamus of goldfish. In agreement with the mRNA expression data, a 3-fold decrease in circulating nesfatin-1 levels were detected in 24 h fasted goldfish (11.89 ng/mL) compared *ad lib* fed controls (36.17 ng/mL). These results suggest a potential anorectic role for nesfatin-1 in goldfish. In support of this notion, intraperitoneal injections of full-length goldfish nesfatin-1 induced approximately a 23% reduction of food intake compared to the saline treated control group. Collectively, our data illustrate a wide expression of nesfatin-1 in goldfish tissues, the possibility of a tissue-dependant multifunctional role of nesfatin-1, as well as an anorexigenic role for nesfatin-1 in goldfish.

Sources of Research Support: Natural Sciences and Engineering Research Council (NSERC) of Canada.

Nothing to Disclose: BEK, RGG, SU

P2-255

Knockout of the CaR in Neurons Produces a Dysregulated Hypothalamus, Panhypopituitarism, and Abnormal Energy Homeostasis.

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The hypothalamus (Hyp)regulates growth, skeletal development, and energy homeostasis by controlling autonomic activities and secretion of hormones in the pituitary gland (Pit). Changes in the extracellular [Ca2+] modulate ion channel activities and neurite outgrowth in neurons potentially by activating the extracellular Ca2+-sensing receptor (CaR). The CaR is expressed in different regions of the brain, including the Hyp. To determine whether neuronal CaRs are involved in maintaining metabolic homeostasis and skeletal development, we generated NeuronCaR-/- mice with CaR knockout (KO) targeted to neurons by breeding a floxed-CaR mouse with a transgenic nestin-Cre mouse line. The KO mice were born in normal Mendelian ratios, but had lower body wts (by ~20-30%), smaller skeleton, and reduced bone mineral content vs wild-type (WT) controls at 2 weeks and 3 months of age. In the KO mice, serum growth hormone and IGF-1 levels were decreased to <10% of WT controls along with reduced expression of GH and GH-releasing hormone RNA by >60% in the Pit and Hyp, respectively. DNA analysis confirmed the excision of CaR gene in the brain but not in liver, cartilage, and bones, suggesting that CaR KO in the Hyp might cause a dysregulated Hyp-Pit-GH/IGF1 axis in the mice. The expression of TSH, ACTH, FSH, and LH was reduced by 30-60% in the Pit of KO vs WT mice. RNA levels for TRH, CRH, and GnRH in the Hyp of KO mice were also decreased by 35-60%. These data suggest that CaR KO also impacted on the Hyp-Pit-thyroid/adrenal and Hyp-Pit-Gonadal axes and produced a panhypopituitarism in the KO mice. Furthermore, in the KO hypothalamus, the expression of agouti-related protein and neuropeptide Y, which enhance feeding and decrease energy expenditure, were increased by 70 and 25%, respectively, while pro-opiomelanocortin, which suppresses feeding and increases energy expenditure, was decreased by ~30%. These changes in gene expression were accompanied by a significant increase in the fat composition assessed by DEXA scan and weighing fat pads. Elevated serum leptin levels suggest an increase in leptin-resistance in the KO mice. In addition, intraperitoneal glucose tolerance testing revealed impaired glucose tolerance in the CaR KO animals. Together, our data demonstrate a critical role for the CaR in modulating the hypothalamic functions to control hormone secretion in the Pit, maintain energy homeostasis, and support growth and skeletal development.

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Vizard et al. Nat Neurosci. 11: 285-291, 2008.
Chang et al. Sci Signal. 1: ra1, 2008

Nothing to Disclose: JP, ZC, J-YK, HL, CD, VP, MY, WC

P2-256

Do Melanocortin-3 Receptors Mediate the Augmented Food Anticipatory Activity of Leptin Deficient *ob/ob* Mice?.

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Obese, leptin deficient mice and children have profoundly decreased activity and increased food seeking behavior. When food availability is temporally restricted, mice increase their activity in the 1-3 h period preceding food presentation. We demonstrated previously that food anticipatory activity (FAA), measured as activity in the 3 hours preceding food presentation over total 24 hr activity, is augmented in leptin deficient *ob/ob* mice compared to wild type mice. Leptin replacement abolished the augmented FAA in leptin deficient *ob/ob* mice, but did not reduce FAA in wild type mice.
Melanocortin-3 receptors (MC3-R) have been reported to mediate food anticipatory activity in mice. In this study, we tested the hypothesis that melanocortin-3 receptors mediate the augmented FAA produced by leptin deficiency. To test this hypothesis, we bred mice that are leptin deficient *ob/ob* and lack MC3-R expression (*ob/ob*-MC3-R -/-) and compared their FAA to ob/ob mice with functional MC3-R.
Locomotor activity, measured as running wheel activity (RWA) in the two groups of mice was not different. FAA was evoked by providing food for just 4 hours/day (for 10 consecutive days) or after marked food restriction (for 10 consecutive days). Total RWA during both protocols increased and surprisingly FAA (measured as RWA in the 3 hours preceding food presentation over activity in the 24 hours) was not reduced in *ob/ob*-MC3-R -/- mice.
In summary, in contrast to a previous report on the essential role of melanocortin-3 receptors (MC3-R) in mediating food anticipatory activity (FAA), we found that deletion of MC3-R did not abrogate the augmented FAA of leptin deficient *ob/ob* mice.

Nothing to Disclose: GC, AR, CD, DWP, JMF, ALM

P2-257

A Novel MC4R Agonist Reduces Body Weight and Improves Glucose Homeostasis in High Fat Diet Induced Obese Rhesus Macaques.

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The melanocortin-4 receptor (MC4R) plays a central role in the regulation of body weight homeostasis. Inhibition of this pathway results in severe obesity and conversely, activation results in decreased food intake and significant weight loss. As such, the MC4R has been a major target for drug development. Here we describe a new compound that specifically activates the MC4R. An 8-week treatment with BIM-22493 resulted in a 13% weight loss reduction in a diet-induced obese nonhuman primate model. Concomitantly, we observed decreases in food intake, fat mass, leptin levels and triglyceride levels while activity and energy expenditure were increased. Further, there was a marked improvement in glucose homeostasis with decreases in fasting insulin levels and HOMA-IR. More impressively, during a glucose tolerance test we demonstrated an increase in glucose clearance while total insulin secretion was reduced. Contrary to other studies with MC4R agonists, we noticed a decrease in heart rate and diastolic blood pressure after 8 weeks of treatment, likely due to an improvement in body weight. Further studies in clinical trials will determine the effectiveness of this compound as an anti-obesity drug in humans.

Sources of Research Support: Sponsored research agreement with Ipsen Biomeasure Inc, Milford, MA.

Disclosures: KLG: Consultant, Genentech, Inc., Ipsen, Novo Nordisk, Wyeth Pharmaceuticals; Principal Investigator, Ipsen, Genentech, Inc.
Nothing to Disclose: PK, HAH, LP, MAC, DLM, MDC

P2-258

The Role of Neuropeptide Y within the Hypothalamic Dorsomedial Nucleus in the Regulation of Energy Homeostasis.

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Neuropeptide Y (NPY), a 36 amino acid peptide and member of the PP-fold family, is a key hypothalamic neuropeptide involved in the regulation of energy balance, potently stimulating food intake following central administration. NPY mRNA is expressed in the arcuate nucleus (ARC) and dorsomedial nucleus (DMN) of the hypothalamus and studies suggest that NPY may be differentially regulated in these two nuclei. In diet-induced obesity and a number of genetic models of obesity, NPY mRNA is reduced in the ARC but increased in the DMN.
In order to study the role of NPY in the DMN in the regulation of energy balance, the gene transfer vector recombinant adeno-associated virus (rAAV) encoding NPY was administered by stereotactic microinjection into the DMN of male Wistar rats. Control animals were injected with rAAV encoding enhanced green fluorescent protein (EGFP). Body weight and food intake were monitored for 94 days.
Over-expression of NPY within the DMN resulted in a reduction in cumulative energy intake ((MJ) 41.8 ± 1.1 EGFP vs. 39.9 ± 1.7 NPY) and body weight gain ((g) 258.0 ± 9.8 EGFP vs. 233. 0 ± 12.8 NPY, n=10-11) compared with controls. This effect was accentuated in animals fed on a high fat diet (cumulative energy intake: (MJ) 41.2 ± 0.9 EGFP vs. 36.7 ± 1.1 NPY, $p<0.05$ n=10-11), (body weight gain: (g) 398.7 ± 20.4 EGFP vs. 332.2 ± 14.0 NPY, $p<0.05$, n=10-11). Plasma leptin was reduced in NPY injected rats compared to controls ((ng/ml) 51.5 ± 3.0 EGFP vs. 37.7 ± 4.2 NPY $p<0.05$ n=10-11), reflecting a decrease in adiposity. Plasma thyroid hormones, brown adipose tissue (BAT) weight and BAT uncoupling protein-1 mRNA expression were similar between both groups suggesting that the reduction in body weight gain was not due to an increase in energy expenditure.
To further investigate the effects of DMN NPY administration on energy balance, a single injection of NPY (0.1, 0.3 or 1nmol) was administered into the DMN of male Wistar rats at the onset of the dark phase. In the first hour post injection, there was a significant reduction in food intake in the 0.1nmol NPY group compared to controls ((g) 4.60 ± 2.3 saline vs. 2.60 ± 0.4 1nmol NPY, $p<0.05$, n=3-9).
These results suggest that in addition to the well-characterised orexigenic effect of NPY in the ARC, NPY may have a different, anorectic role within the DMN.

Nothing to Disclose: EML, JVG, ER, NWB, NMM, SRB, KLS

P2-259

Effects of Endomorphins on Feeding Behavior and Hypothalamic Peptide Gene Expression in the Rat.

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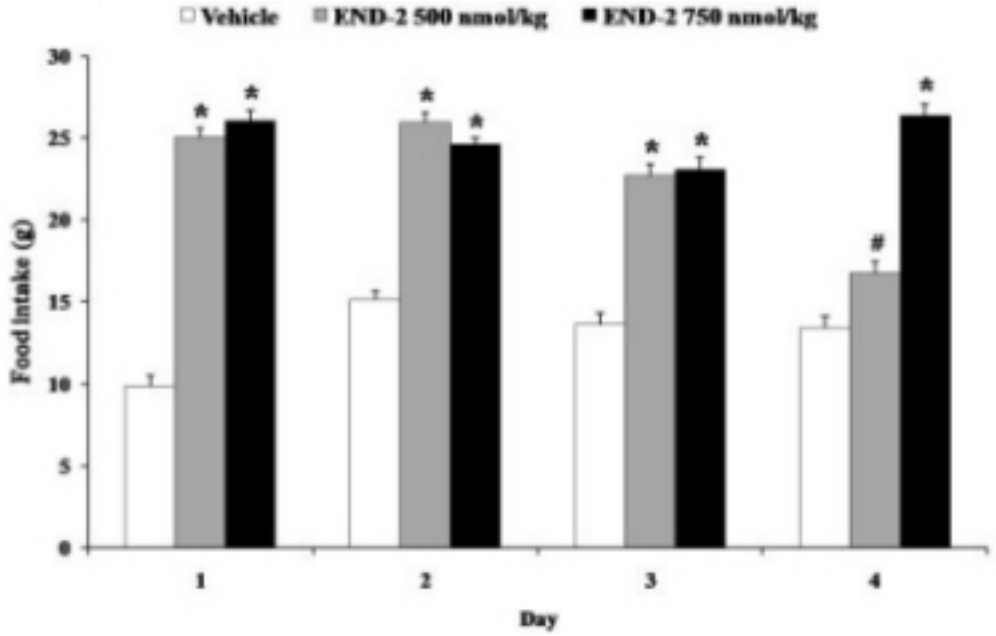
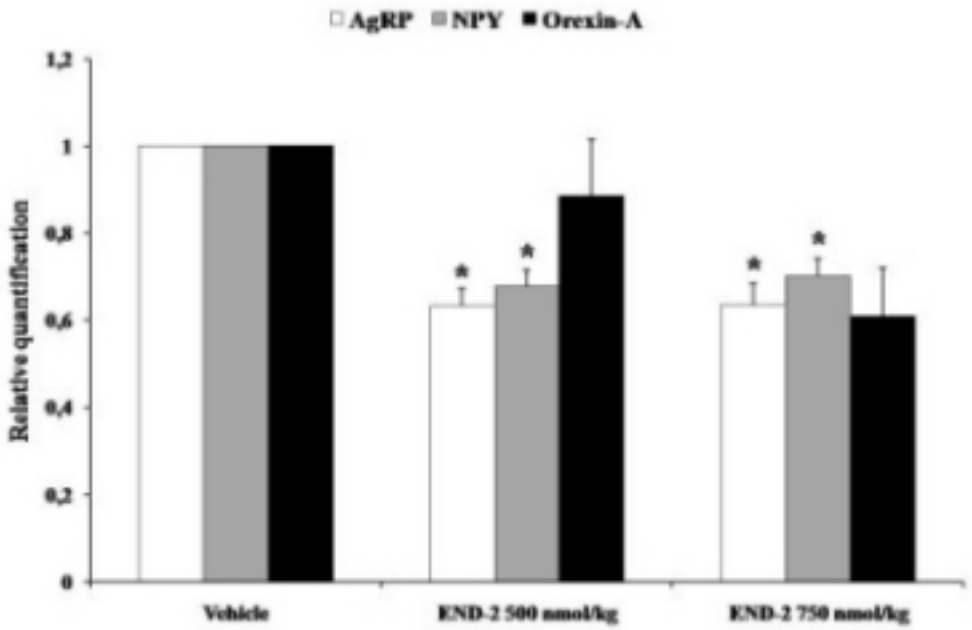
Endomorphin-1 (END-1) and endomorphin-2 (END-2) are opioid peptides which selectively bind to the μ-opioid receptor (1,2). We studied the effects of END-1 and END-2 injected into the arcuate nucleus (ARC) of the hypothalamus on feeding behavior and gene expression of orexigenic [agouti-related peptide (AgRP), neuropeptide Y (NPY) and orexin-A] and anorexigenic [cocaine and amphetamine-regulated transcript (CART), corticotrophin releasing hormone (CRH) and proopiomelanocortin (POMC)] peptides in male Wistar rats fed a standard laboratory diet.
48 rats (8 for each group of treatment) were injected into the ARC, at 9.00 am, daily for 4 days, with either END-1 or END-2 (500-750 nmol/kg), or saline.
Total RNA was extracted from hypothalami and reverse transcribed. Gene expression of hypothalamic peptides was determined by quantitative real-time polymerase chain reaction (PCR) using β-actin as the endogenous control. The comparative $2^{-\Delta\Delta Ct}$ method was used to quantify the relative changes in individual gene expression.
Results were analyzed by one-way analysis of variance (ANOVA), followed by Newman-Keul's multiple comparison test.
Both END-1 and END-2 significantly increased food intake compared to vehicle since day 1 of treatment (ANOVA, $P<0.0001$; *post-hoc* *$P<0.001$ vs. vehicle; $^{\#}P<0.05$ vs. vehicle)(Fig. 1).



We observed a significant reduction in gene expression of AgRP and NPY in rats injected with both 500 nmol/kg and 750 nmol/kg END-2, but not with END-1, compared to vehicle treated rats (AgRP: ANOVA, $P<0.0001$; NPY: ANOVA, $P<0.001$. *Post-hoc* *$P<0.001$ vs. vehicle)(Fig. 2).



Neither END-1 nor END-2 administration modified gene expression of orexin-A, CART, CRH, and POMC.
We can hypothesize that opioid-induced feeding by END-2 takes along a compensatory inhibition of the gene expression of the orexigenic peptides NPY and AgRP.

(1) Fichna J et al., Pharmacol Rev 2007; 59:88
(2) Zadina JE et al., Nature 1997; 386:499

Sources of Research Support: Italian Ministry of Education (60% funds).

Nothing to Disclose: CDN, LB, LR, GO, CF, SL, AC, RS, PDM, MV

P2-260

Deletion of Nhlh2 Impairs Transcriptional Regulation of the Melanocortin Pathway and Leads to Reduced Sympathetic Nervous System Tone in Adipose Tissue.

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Mutations in the melanocortin 4 receptor (MC4R) are the most frequent cause of monogenetic forms of human obesity. Despite its significant importance, MC4R signaling pathways and transcriptional regulation underlying the melanocortin pathway are far from being fully understood. Using a knockout mouse model which lacks Nescient Helix Loop Helix 2 (Nhlh2), transcription factor, as well as molecular gene regulation assays, we show that Nhlh2 plays an important role in transcriptional regulation of genes involved in melanocortin pathway. Electrophoretic mobility shift assay (EMSA) and transfection assays were used to show that Nhlh2 interacts with, and transcriptionaly regulates the mouse melanocortin 4 receptor (Mc4r) gene through three E-Box motifs (-555, -363, +59) located on its proximal promoter region. Transfection in presence of Nhlh2 along with leptin stimulation increases Mc4r expression in N29/2, neuronal cells. Site directed mutagenesis to disrupt each of the three E-Boxes individually significantly reduces the expression of the Mc4r reporter construct. EMSA results show strong binding of Nhlh2 to all three of the Mc4r E-Boxes. Deletion of Nhlh2 in mice (N2KO) leads to reduced melanocortinergic tone evident by the 5-fold reduction in Mc4r mRNA and protein levels in N2KO mice. Disrupted melanocortinergic tone in the N2KO is reflected in a reduction in peripheral insulin sensitivity and glucose tolerance levels. Decreased melanocortinergic tone in N2KO mice also results in a reduction in mRNA for β2-adrenergic receptor (β2AR; 4-fold), β3-adrenergic receptor (β3AR; 2-fold) and uncoupling protein-2 (UCP-2; 10-fold) in white adipose tissue and UCP-1 (4-fold), β2AR (5-fold), β3AR (2.5-fold) in brown adipose tissue. These results suggest that N2KO mice have an overall reduction in sympathetic nervous system tone. Whole body indirect calorimetry studies and fatty acid oxidation analyses of muscle and liver are ongoing and will help us to better understand the overall energy expenditure pattern in N2KO mice. Together these data link Nhlh2 with defects in sympathetic innervation to peripheral organs and identify Mc4r as a putative gene regulatory target of the bHLH transcription factor Nhlh2. The possibility exists that transcriptional control of hypothalamic Mc4r by Nhlh2 is necessary for normal peripheral metabolism and energy expenditure.

Nothing to Disclose: UDW, DJG

P2-261

AMPK and PI3K Signaling Are Required for the Anorectic Action of Leptin in a Novel, Murine, NPY-GFP Hypothalamic Cell Model.

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Accumulating evidence shows that neuropeptide Y (NPY) neurons are involved in mediating the anorexigenic action of leptin via neuronal circuits in the hypothalamus. However, studies have produced limited data on the cellular and molecular processes involved in the direct control of NPY secretion by leptin. We have therefore generated a novel NPY-expressing cell line derived from the hypothalamic primary culture of a transgenic mouse, in which green-fluorescent protein (GFP) is driven by the NPY gene promoter. Cells expressing GFP were selected by fluorescent-activated cell sorting (FACS) and immortalized through the retroviral transfer of the SV-40 T-antigen. To investigate the direct regulation of NPY-secretory responses to leptin, we used the newly generated NPY-GFP cell line. We report that 1-h leptin treatment directly decreased NPY secretion in the NPY-GFP cells by 20%. We hypothesized leptin may directly regulate NPY secretion through an AMP-activated protein kinase (AMPK) and/or a PI3K-dependent mechanism as suggested by in vivo studies in the whole hypothalamus. Western blot analysis indicated leptin inhibits AMPK activity and activates acetyl-CoA carboxylase (ACC) in the NPY neuron, supporting the hypothesis of an AMPK-dependent mechanism. However, AKT, a key signaling component of the PI3K pathway, was not significantly altered by leptin. Interestingly, inhibiting both AMPK with compound C or the PI3K pathway with LY294002 prevented the leptin-mediated decrease in NPY secretion. More so, we find NPY secretion was increased by the AMPK activator, 5-amino-imidazole-4-carboxamide riboside (AICAR) by 30%. These results were corroborated in the adult, murine, NPY-synthesizing mHypoA-59 cell line (Belsham et al, FASEB J, 2009). Our studies support the hypothesis that the AMPK and PI3K pathways are important for the anorectic action of leptin, particularly in the NPY neuron. Understanding the mechanisms of leptin signaling in the hypothalamus will better our understanding of the development of obesity.

Sources of Research Support: Natural Sciences and Engineering Research Council of Canada (NSERC), Canadian Institutes for Health Research (CIHR), Canada Foundation for Innovation (CFI), and the Canada Research Chairs (CRC) Program.

Nothing to Disclose: SSD, MLC, GLK, DDB

P2-262

Pharmacological Studies on Twenty Novel Naturally Occurring Melanocortin-4 Receptor Mutations.

Z-Q Wang PhD[1], H Huang BS[1] and Y-X Tao PhD[1].

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The melanocortin-4 receptor (MC4R) is a G protein-coupled receptor critically involved in regulating energy balance. MC4R activation results in decreased food intake and increased energy expenditure. Genetic and pharmacological studies demonstrated that the MC4R regulation of energy balance is conserved from fish to mammals. In humans, more than 150 naturally occurring mutations in the MC4R gene have been identified. Functional study of mutant MC4Rs is an important component in proving the causal link between MC4R mutation and obesity. In this study, functional characterization of twenty novel MC4R mutations was performed. The endogenous ligand α-melanocyte stimulating hormone (MSH) was used for ligand binding and signaling studies. The mutants studied were I69R, H76R, M79I, S94N, D126Y, D146N, I195S, Del170, I186V, I194T, F201L, G231V, F260Q, F280L, I289L, L300P, R30S, Q307X, Y332C, and Y332H. The mutant MC4Rs were generated by site-directed mutagenesis and transiently transfected into 293T cells. We showed that six mutants, I69R, D126Y, Del170, I194T, P260Q, and Q307X, had little or no binding to radiolabeled NDP-MSH (a superpotent analog of α-MSH that retains the ability to bind to receptor after iodination). Wild-type (WT) MC4R responded to α-MSH stimulation with and EC50 of 2.47 nM and maximal response of 2700 pmol cAMP per million cells. Four mutants, I69R, D126Y, I194T, and Q307X, had no response to α-MSH stimulation. Del170 had 17% of WT MC4R maximal response, but the EC50 was increased by 3567-fold. P260Q had 22% of WT MC4R maximal response and the EC50 was increased by 27-fold. The other mutants had normal signaling compared to the WT MC4R. Interestingly, three mutants, H76R, D146N, and F280L, were constitutively active. They have increased basal signaling which was decreased by an inverse agonist Ipsen 5i. Eleven mutants had decreased basal activities. In summary, we identified mutants that are defective in ligand binding with corresponding defect in signaling. Defect in basal signaling might be a common mechanism in obesity pathogenesis due to MC4R mutation. The mechanism for obesity caused by constitutively active MC4R mutants remains to be investigated.

Sources of Research Support: NIH R15DK077213.

Nothing to Disclose: Z-QW, HH, Y-XT

P2-263

Functional Characterization of Nine Naturally Occurring Melanocortin-3 Receptor Mutations.

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The melanocortin-3 receptor (MC3R) is a G-protein-coupled receptor expressed in brain including the cortex, thalamus, hippocampus, and hypothalamus as well as heart, gastrointestinal tract, and placenta. MC3R and the related meanocortin-4 receptor have non-redundant roles in regulating energy homeostasis. Melanocortin-4 receptor is a critical regulator of both food intake and energy expenditure. It is controversial whether the MC3R regulates food intake. Data from Mc3r knockout animals showed that Mc3r deficiency results in increased feed efficiency and adiposity. Recently, MC3R was also shown to be required for entrainment to meal intake. The relevance of MC3R mutations in human obesity pathogenesis was not clear and few studies were performed. Sixteen MC3R mutations and two polymorphisms have been identified. We have reported the functional studies of four MC3R mutations and the two polymorphisms. In this study, we performed functional studies on nine novel MC3R mutations recently reported. We generated the mutants by site-directed mutagenesis and transiently expressed these mutants as well as wild-type MC3R in HEK293T cells. Ligand binding and signaling were then studied. The mutants were S69C, A70T, I87T, M134I, L249V, A260V, M275T, T280S, and L297V. Of the nine mutants, four (S69C, T280S, M134I and M275T) had decreased maximal ligand binding. Two mutants (S69C and T280S) had significant impairments in responding to NDP-MSH stimulation with increased cAMP generation. The other mutants had normal ligand binding and signaling properties as the wild-type MC3R. Further studies are being performed to measure the cell surface expression of the mutant receptors using flow cytometry. In summary, we identified four loci at the MC3R that are important for MC3R function. Some of the MC3R mutations cause dysfunction in the mutant receptor and therefore could potentially contribute to obesity pathogenesis.

Sources of Research Support: NIH R15DK077213.

Nothing to Disclose: FY, Y-XT

P2-264

Tumor Necrosis Factor-α Increases Protein Tyrosine Phosphatase 1B Activity in Rat Hypothalamic Organotypic Cultures.

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Protein Tyrosine Phosphatase 1B (PTP1B) is widely expressed in the body, and has been shown to negatively regulate leptin signaling via direct dephosphorylation of Janus tyrosine kinase 2. It is also shown that reduction of hypothalamic PTP1B improves leptin sensitivity in diet-induced obese rats, and that high fat diet increases hypothalamic PTP1B expression levels accompanied by leptin resistance. On the other hand, tumor necrosis factor α (TNF-α) is well known to be elevated in adipose tissue or serum in obesity, and high fat diet reportedly increased TNF-α expression in the hypothalamus including the arcuate nucleus and lateral hypothalamus. In the present study, we examined if TNF-α regulates PTP1B activity in rat hypothalamic organotypic cultures. Sprague-Dawley pups of 15 days old were killed by decapitation, and hypothalamic tissues were sectioned at 350 μm thickness on a McIlwain tissue chopper. Eight coronal slices containing arcuate nucleus were cultured in the standard medium containing horse serum for 48 h, and the medium was changed to a defined serum free medium containing various concentrations of TNF-α (1, 25, 50 and 100 ng/ml) for 24 h while control slices were incubated with vehicle. According to company's manual, PTP1B antibody was added to and incubated with Dynabeads Protein G in advance. Cultures were homogenized with PTP lysis buffer and tissue lysates equivalent to 500 μg protein were incubated with PTP1B antibody. Immunoprecipitated PTP1B was incubated with PTP assay buffer containing epidermal growth factor receptor as a PTP1B substrate, and PTP1B activity was determined by measuring 620 nm absorbance after adding Biomol Green reagent to the assay buffer. TNF-α treatment increased PTP1B activity in a dose- and time- dependent manner compared to control. These data show that TNF-α acted directly on the hypothalamus and increased activity of PTP1B, suggesting that elevation of TNF-α in obesity may cause leptin resistance through PTP1B in the hypothalamus.

Nothing to Disclose: YI, RB, YO, HA, YO

P2-265

Hypothalamic Nitric Oxide Synthase and Energy Homeostasis in Mice.

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Introduction:The increasing incidence of obesity and diabetes places a huge burden on our society. The potential for therapeutic intervention has generated renewed interest in the underlying metabolic basis. Hypothalamic nuclei are implicated in energy homeostasis through a novel regulatory pathway involving malonyl CoA as a regulator. This molecule, in turn, is regulated through AMPK/ACC pathway(1). Recent studies have found elevated levels of neuronal nitric oxide synthase (nNOS) upon treatment of mice with NPY, an orexigenic peptide(2). We hypothesize that fasting alters nNOS levels in the ventromedial(VMH) and arcuate(AN) nuclei.
Methods: Two month old CD-1 mice were divided into 3 groups of 9 each. Group 1 had free access to food, group 2 was fasted for 16 hrs and then allowed to feed for 24 hrs and group 3 was fasted for 16 hrs. After that, VMH and AN nuclei were extracted. Samples were homogenized with Laemmli buffer with protease inhibitors, electrophoresed on 3-8% Tris-acetate reducing gels, transferred to PVDF membranes and probed with anti-nNOS and anti-actin Abs (Santa Cruz). Actin was used to correct for the loading efficiency. Results are given as mean +/- SEM
Results: In this study, consistent with the previous reports, pAMPK and pACC in the arcuate nucleus were elevated in the fasted state. Interestingly, nNOS in the fasted mice (93 ± 5) and fasted/fed mice (83 ± 12) were lower than the fed (102 ± 16). Although there was a trend, it was not statistically significant. In contrast to AN, the VMH nNOS was higher in the fasted (80 ± 10) compared to fed (64 ± 11) and fasted/fed (60 ± 7) at 90% confidence interval. The pAMPK and pACC values were lower in the fasted state in the VMH.

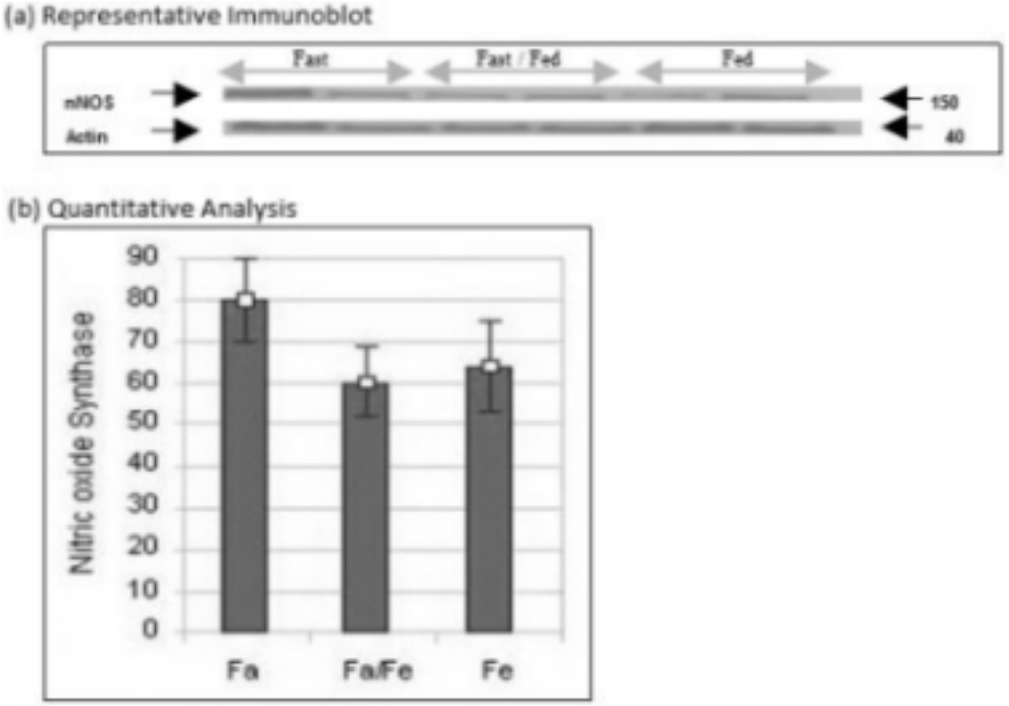

Figure 1: nNos expression in VMH

Discussion: A reciprocal relationship between the pAMPK, pACC and nNOS in the AN and the VMH suggest a role for nNOS in energy homeostasis through ancient AMPK energy sensor. Finding the role of nitric oxide could provide intriguing insights into the energy homeostasis and will provide effective therapeutic options.

(1) The role of hypothalamic malonyl-CoA in energy homeostasis. Wolfgang MJ, Lane MD J Biol Chem. 2006 Dec 8;281(49):37265-9. Epub 2006 Oct 3.
(2) Leptin and neuropeptide Y (NPY) modulate nitric oxide synthase: further evidence for a role of nitric oxide in feeding. Morley JE, Alshaher MM, Farr SA, Flood JF, Kumar VB. Peptides. 1999;20(5):595-600.

Nothing to Disclose: SA, PHP, SMC, WAB, JEM, GNS

P2-266

Gamble on a Full Stomach: Monetary Risk-Taking Is Altered by Metabolic State in Normal-Weight Human Male Subjects.

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Background
Metabolic signals regulate body weight by acting upon central appetite-regulating circuits. In addition, there is growing evidence that these metabolic signals also impact upon brain reward regions that are implicated in regulating not only food reward but also economic risk-taking. However, the effects of metabolic state manipulation on financial risk-taking in humans has not been evaluated.
Methods
To evaluate the effect of metabolic state on financial risk-taking, we evaluated monetary reward-seeking behaviour in normal weight subjects fasted for 14 hours and in the fed state, 1 hour post-ingestion of 2066 kcals. We employed a randomised crossover-study design and used a paired lottery task, where subjects had to choose between safe or risky options for monetary reward. Body composition was assessed using Tanita scales. Blood samples were drawn throughout the performance of tasks from a venous cannulae sited an hour prior to the commencement of the task period. Plasma acyl-ghrelin and leptin were measured using commercially available radioimmunoassay and ELISA (Millipore, UK), according to the manufacturer's instructions.
Results.
Fasting leptin concentrations associated positively with risk-taking (R^2 = 0.3, P<0.05): the higher the leptin the more risky choices the subjects took. Circulating acyl-ghrelin concentrations were high in the fasted state and reduced in response to nutrient ingestion. Risk-taking was altered by metabolic status with subjects making more risky choices in the fed state. Moreover we found that the degree of meal-induced acyl-ghrelin suppression correlated to the change in risk-taking behaviour (R^2 = 0.3, P<0.05): a large suppression of acyl-ghrelin led to more safe choices compared to a small suppression.
Conclusions:
An individual's energy reserves, indexed by circulating leptin and also acute nutrient intake, reflected in acyl-ghrelin levels, impacted upon their financial risk-taking behaviour.
Our results show that metabolic signals influence monetary decision-making and suggest that financial decisions are best made when satiated. Moreover, given that obesity is associated with both leptin resistance and abrogated post-meal ghrelin response the effect of obesity and its treatment on risk-taking behaviour warrants further study.

Sources of Research Support: MRC clinical research fellowship awarded to JE; Wellcome Trust Programme Grant to RJD; Funding from the Comprehensive Biomedical Research Centre to RLB.

Nothing to Disclose: JE, MS, MED, EK, RJD, DJW, RLB

P2-267

Identification of Novel Targets of Estradiol in the Anteroventral Periventricular Nucleus Transcriptome.

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[1]Univ of Massachusetts Amherst Amherst, MA.

Several microarray studies have identified targets of estrogen receptor (ER) in the preoptico-hypothalamus or neural cell lines. However, none has specifically focused on the anteroventral periventricular (AVPV) nucleus wherein ER-containing cells are highly concentrated. The AVPV is a critical region for the neuroendocrine control of ovulation in rodents. Comprised of dual-phenotypic GABA/glutamate/peptide-expressing neurons, this nucleus is necessary for mediating the estrogen-induced preovulatory surge of luteinizing hormone from the pituitary gland. These neurons synapse directly onto gonadotropin-releasing hormone neurons and possess ER-alpha and ER-beta, yet few gene targets of estrogen have been identified within this nucleus. In order to better understand the full functional scope of this nucleus, it is important to elucidate the estrogen-regulated transcriptome of the AVPV. We used expression microarrays to compare AVPV of adult ovariectomized C57Bl6 mice treated with stradiol (E2) or oil vehicle. The results of the gene ontology analysis identified multiple E2-regulated genes involved with cell cycle arrest and apoptosis (Mad2L1, PHLDA1, GADD45A, Trp53i11 and PDCD4), as well as genes related to feeding behavior and satiety (HCRTR1, CCKAR, NRip1 and NPY2R). These findings may have important implications for understanding how E2 links energy balance and reproduction and how both E2 exposure and unrestricted food intake advance the onset of reproductive senescence.

Sources of Research Support: NIH Grant HD027305 to SLP.

Nothing to Disclose: LKA, SLP

P2-268

Peripheral T_3 Administration That Maintains Somatic Growth and Thyroid Hormone Status in Methimazole-Treated Monkeys Corrects the Delay in the Initiation of Pubertal LH Secretion Observed in Globally Hypothyroid Monkeys.

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The objective of this study was to determine the impact of a selective central hypothyroid condition on the timing of the pubertal reactivation of LH secretion in the monkey (*Macaca mulatta*). Three juvenile male monkeys (17 mo of age) were orchidectomized and placed on the antithyroid drug methimazole at a dose previously established to induce a profound hypothyroidism (meth; 0.025% in drinking water). All three animals were immediately placed on triiodothyronine (T_3) replacement therapy (0.75 to 3 µg/kgBW/day) using sc implanted osmotic minipumps (Alzet, model 2ml4). As expected, plasma T_4 decreased to undetectable levels that were sustained while T_3 dose was adjusted to maintain peripheral euthyroid status and normal somatic development. Plasma LH levels were monitored weekly to assess changes in the activity of the GnRH pulse generator that drives pubertal development. The onset of pubertal LH secretion was defined using a previously described algorithm i.e., a sustained rise in plasma LH to 1.0 ng/ml or greater. Results from the present study were compared to historical data for euthyroid and for globally hypothyroid (meth-treated, no thyroid hormone [TH] replacement) animals from our laboratory. The highest dose of T_3 replacement (2-3 µg/kgBW/day) generated plasma T_3 levels that were higher than euthyroid levels, but heart rate and body temperature indicated that peripheral thyroid status remained within the euthyroid range. Plasma TSH rose initially at the start of meth treatment and then declined as the T_3 dose was adjusted but remained well above euthyroid levels. T_3 treatment supported progressive linear growth and bone maturation, and corrected the delay in the pubertal rise in LH secretion observed previously in untreated hypothyroid monkeys. Also, bone age and BMI at puberty did not differ between euthyroid and T_3-treated meth monkeys. The data suggest that peripheral administration of a T_3 dose sufficient to maintain normal somatic development (but supposedly not sufficient to cross the blood-brain-barrier and correct central hypothyroidism) normalized the age of onset of the pubertal LH rise and therefore the timing of the reactivation of the GnRH pulse generator at this stage of development. This being the case it would appear that the effect of thyroid hormone on the timing of primate puberty is mediated indirectly via its effects on somatic development rather than directly on hypothalamic mechanisms that govern this event.

Sources of Research Support: NIH grants: HD41749, RR03034, RR18386, and HD08610.

Nothing to Disclose: DRM, SR, CDS, TMP

P2-269

Interactions between Kisspeptin and Neurokinin B in the Control of GnRH Neurons in the Female Rat.

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Neurokinin B (NKB) is a member of the tachykinin family and has emerged as a pivotal player in the neuroendocrine control of reproduction. Humans bearing loss-of-function mutations of either NKB or its receptor, neurokinin 3 (NK3), exhibit hypogonadotropic hypogonadism and infertility. NKB is co-expressed with dynorphin (Dyn) and kisspeptin (Kiss1) genes in neurons of the arcuate nucleus (Arc), which also play key roles in the regulation of GnRH and gonadotropin secretion. However, despite the apparent significance of NKB in the control of reproductive function, its mechanisms of action as a cotransmitter with kisspeptin and dynorphin remain poorly understood. To explore the role of NKB in the control of LH secretion in the rat, first, we examined the effect of an NKB agonist (senktide, 600 pmol, ICV) on LH secretion in the adult female rat and observed that senktide induced a dramatic increase in LH secretion within 20 min of treatment ($p < 0.05$ vs control). We also found that senktide evoked a 10-fold increase in the number of Kiss1 neurons expressing c-fos in the Arc, suggesting an autosynaptic action of NKB in these cells, which express NK3. Second, we studied the effect of estradiol (E2) on the expression of NKB and NK3 in the Arc and found that E2 inhibits the expression of both genes ($p < 0.01$), as shown previously in the mouse. We also observed that the expression of NKB and NK3 is at its nadir on the afternoon of proestrus (when E2 is high), suggesting a role for NKB/NK3 in the negative feedback regulation of GnRH secretion by ovarian hormones. Furthermore, we detected expression of NK3 in other hypothalamic regions, including the lateral hypothalamic area (LHA), periventricular nucleus (PVN), ventromedial nucleus, and supra-optic nucleus, while the expression of NKB was also observed in the LHA. Paradoxically, E2 induced the expression of NK3 in the LHA and PVN and increased expression of NKB in the LHA (in contrast to the inhibitory effect of E2 on NK3 and NKB in the Arc), suggesting that NKB/NK3 signaling plays a complex role in neuroendocrine processes, involving several NK3-expressing neuronal phenotypes. In summary, we present evidence for a stimulatory role of NKB on GnRH/LH release in the Arc of the rat, as well as for the regulation of expression both NKB and NK3 by E2. These observations are consistent with a model of pulsatile release of GnRH/LH secretion, ultimately driven by kisspeptin/NKB/dynorphin neurons that reside in the Arc.

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Nothing to Disclose: VMN, JMC, SMM, RP, FR-P, LP, DKC, MT-S, RAS

P2-270

AGRP Plays a Critical Role in Leptin's Effects on Puberty and Reproduction.

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Deficient leptin signaling causes infertility via central hypofunctioning of GnRH neurons and thus a hypothalamic hypogonadal state in both rodents and humans. Due to a lack of leptin receptors on GnRH neurons, leptin's effect on GnRH must be indirect. AGRP/NPY neurons are a known primary target of leptin within the hypothalamic arcuate nucleus, an area which has projections to the preoptic area where GnRH neurons are present. Restoration of fertility in NPY knockout leptin-deficient mice is associated with diminished obesity and diabetes. We have observed that a loss of AGRP in leptin receptor deficient females results in complete restoration of fertility and lactation despite persistence of the obesity and diabetes phenotypes. We hypothesize that overexpression of AGRP as a result of leptin signaling deficiency is a major inhibitor of pubertal development and reproduction. Our goal is thus to ascertain whether the timing of pubertal development in db/db AGRP knockout females parallels the development of wild type females. We will compare three groups of mice: wild type, db/db, and db/db AGRP knockout. Mice will be followed from weaning (18-21 days) until 8 weeks of age and monitored for age at vaginal opening and onset of first estrous on vaginal wash cyclicity. Specimens of mammary glands, uteri, and ovaries will be examined for morphological differences at prepubertal, peripubertal and postpubertal stages. Our preliminary observations show that AGRP deficiency in db/db females have a timing of vaginal opening similar to wild type females, normal size and morphology of the ovaries and uteri along with near normal mammary gland ductal development. In contrast, the db/db females did not develop a vaginal opening by 8 weeks of age, had small ovaries with irregular histology, hypoplastic uteri, and incomplete mammary gland development at all time points. Further studies are being pursued regarding hormonal status and biomarker expression in the reproductive organs. We conclude that the restoration of normal puberty in the db/db Agrp KO is due to the loss of AGRP's central role as an inhibitor of GnRH release.

Nothing to Disclose: SS-B, DDI, SL, SCC

P2-271

Neurokinin B Receptor (NK3R) Activation Stimulates Acute GnRH Dependent LH Release in the Juvenile Male Rhesus Monkey (*Macaca Mulatta*) but, in Contrast to Repetitive Kisspeptin Receptor Activation, Does Not Sustain LH Secretion.

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The recent discovery that inactivating mutations in neurokinin B (NKB, encoded by *TAC3*)-neurokinin B receptor (NK3R, encoded by *TAC3R*) signaling results in a phenotype (hypogonadotropic hypogonadism with delayed or absent puberty) similar to that reported earlier for mutations in the kisspeptin receptor (GPR54) is intriguing. This is because studies in sheep indicate that kisspeptin and NKB are expressed by the same neurons in the arcuate nucleus, and lesioning experiments in the monkey indicate that this hypothalamic area is essential for pulsatile GnRH release and for initiation of the estrogen induced pre-ovulatory LH surge. The purpose of the present study was to begin to examine, in a primate, the interaction between kisspeptin and NKB that appears obligatory for generating pulsatile GnRH release that is required to drive the pituitary-gonadal axis. Four bilaterally castrated juvenile male rhesus monkeys (2.3-2.6 kg body wt; 13-14 mo of age), in which pituitary responsiveness to GnRH had been heightened with a pulsatile iv infusion of the synthetic decapeptide, were used to indirectly monitor endogenous hypothalamic GnRH release by tracking LH secretion. Animals were implanted with indwelling venous catheters and housed in remote infusion withdrawal cages that enable moment to moment changes in LH levels to be followed without sedation and with minimal restraint. Bolus single iv injections of either NKB (100 µg) or the NK3R agonist, Senktide (50 µg), elicited an LH discharge with a magnitude similar to that evoked by iv administration of 2 µg human kisspeptin-10 and comparable to those observed spontaneously in post pubertal monkeys. Pre-treatment with a GnRH-receptor antagonist (acyline, 60 µg/kg) abolished both NKB and Senktide induced LH release indicating that the site of action of the 2 peptides was supra-pituitary. While repetitive iv injections of kisspeptin (2 µg/pulse every h) produced, as previously established, a sustained train of GnRH discharges, the same mode of Senktide administration at 50 µg/pulse resulted in a progressive decrement of the LH response. Kisspeptin induced LH release was intact after 24 h of intermittent Senktide administration. These findings are consistent with the human genetics indicating that NK3R signaling is involved in activating the neuroendocrine axis governing reproduction. Whether this pathway and that of kisspeptin operate in parallel or are hierarchal remains to be determined.

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Nothing to Disclose: TMP, SBS, SR

P2-272

Early Metabolic Programing of Puberty Onset: Impact of Changes in Perinatal Feeding on the Timing of Puberty and the Development of Hypothalamic Kisspeptin System.

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Persistent energy unbalance, as in severe undernutrition or obesity, is frequently linked to alterations in the timing of puberty. This phenomenon is likely to involve the adipose hormone, leptin, whose actions on the reproductive axis are mainly conducted at the hypothalamus, where leptin operates on GnRH neurons via an indirect pathway. Kiss1 neurons in the arcuate nucleus have recently emerged as major conduit for the metabolic gating of puberty; leptin being a regulator of hypothalamic Kiss1 expression. Intriguingly, early perturbations in the metabolic/nutritional state are known to predispose to different metabolic disorders later in life. However, whether such early metabolic programming influences also the timing of puberty, and the potential underlying mechanisms, remain ill defined.
We evaluate herein the influence of changes in the pattern of postnatal feeding (under- and over-feeding) on the timing of puberty, and characterize hormonal (leptin) and neuropeptide (Kiss1/kisspeptin) responses linked to such early nutritional manipulations. To this end, female rats were raised in litters of different size: small (SL: 4pups/dam), normal (NL: 12pups/dam), and large litters (LL: 20pups/litter). At the time of puberty, hypothalamic RNA and peptide expression was analyzed, and leptin levels assayed. Gonadotropin responses to kisspeptin (Kp-10) administration were also monitored in the different groups.
Postnatal overfeeding (SL) resulted in persistently increased body weights (BW), and earlier age of vaginal opening (as external sign of puberty); phenomena that were linked to elevated levels of circulating leptin and hypothalamic Kiss1 mRNA. Conversely, postnatal underfeeding (LL) caused a persistent reduction in BW, lower ovarian and uterus weights, and a delay in the age of vaginal opening; changes that were paralleled by similar modifications in leptin and Kiss1 mRNA levels. Kisspeptin-immunoreactivity (IR) at the hypothalamus followed an identical trend, with significantly decreased numbers of kisspeptin-IR neurons at the arcuate in LL animals. Yet, hormonal (LH and FSH) responses to kisspeptin were grossly similar in all groups, except for a detectable increase in responsiveness to low doses of Kp-10 in LL rats. In conclusion, our data point out that body weight and the timing of puberty are sensitive to early changes in postnatal feeding, and that alterations in hypothalamic expression of Kiss1/kisspeptin may underlie such a programming phenomenon.

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Nothing to Disclose: JMC, AHB, MAS-G, FR-P, MR, RP, DG-G, EA, CD, LP, JDM, MT-S

P2-273

Impact of Hypothalamic Astrocytes on GnV-3 GnRH Secreting Cells: Functional and Transcriptomic Analysis.

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In recent years compelling evidence has been provided that astrocytes of the neuroendocrine brain play a critical role in regulating the function of the hypothalamic neurons releasing GnRH, the neurohormone that controls both sexual development and adult reproductive function. Whether astrocytes also contribute to early maturational events in GnRH neurons as previously shown for other brain neuronal populations (e.g., synaptic contact formation, synapse efficacy,…) is not yet known but is amenable to experimentation using the conditionally immortalized GnRH-expressing GnV-3 cells. Here we report that exposure of differentiated GnV-3 cells to astrocytes induces neurite extension and expression of markers of neuronal differentiation like MAP2 and TUJ. In addition, GnV-3 cells co-cultured with astrocytes acquire electrophysiological characteristics of mature neurons and a well-synchronized pattern of spontaneous pulsatile GnRH secretion. Parallel transcriptomic analyses demonstrated that 1024 genes participating in 10 different regulated pathways are specifically regulated in GnV-3 cells cultured in astrocyte-conditioned medium. These data promise to provide new insights into the molecular switches prompted by astrocytes to promote the maturation and function of GnRH neurons.

Nothing to Disclose: VM, SG, JPR, PP, MG, MG, AD, VP, FPP

P2-274

Voltage-Gated Potassium Currents Are Targets of Estradiol Feedback Regulation and Kisspeptin Action in Gonadotropin-Releasing Hormone (GnRH) Neurons.

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GnRH neurons centrally control fertility and are in turn regulated by steroid feedback. Estradiol exerts negative and positive feedback on GnRH neurons and also regulates expression of the excitatory neuromodulator kisspeptin. Kisspeptin can activate GnRH neurons via both transsynaptic and direct mechanisms. The latter involves, in part, changes in potassium currents. Estradiol was previously shown to alter two components of voltage-gated potassium currents in GnRH neurons; transient IA and a sustained IK. Here we examined how estradiol, kisspeptin and time of day interact to regulate these conductances in GnRH neurons from mice that exhibit daily switches between estradiol negative (AM) and positive feedback (PM). Whole-cell voltage-clamp recordings were made from GnRH neurons in brain slices from ovariectomized (OVX) and OVX mice treated with estradiol (OVX+E) for 2-4 days. To isolate potassium currents, sodium and calcium channels and ionotropic neurotransmitter receptors were blocked. Membrane potential was manipulated to reveal different current components. From a holding potential of -60mV, channel inactivation was first removed by a 500 ms step to -100mV. This was followed by 500 ms prepulse to either -100 mV or -30 mV (the latter of which inactivates IA) and then a test pulse to -10 mV to measure current. There were no diurnal changes in either IA or IK in GnRH neurons from OVX mice ($p>0.5$). In contrast, in GnRH neurons from OVX+E mice IA and IK were significantly greater during the AM and smaller in the PM hours ($p<0.03$), consistent with the decreased/increased GnRH neuronal activity observed at these two times of day, respectively. Estradiol significantly increased IA during the AM hours relative to that in OVX mice ($p<0.01$), but there was no effect of estradiol on either IA or IK during positive feedback compared to OVX mice ($p>0.5$). Kisspeptin significantly reduced IA in all groups studied regardless of time of day or estradiol status ($p<0.0001$, for all groups). Series resistance, capacitance and input resistance were not different between the groups; further, in cells not exposed to kisspeptin but recorded for the same duration, no changes in potassium current were observed with time ($p>0.6$). Together these data suggest that estradiol feedback and time of day interact to modulate voltage-gated potassium currents of GnRH neurons, and that the mechanism by which kisspeptin increases GnRH activity involves reduction in IA potassium current.

Sources of Research Support: NIH HD41469.

Nothing to Disclose: JP-F, SMM

P2-275

Prenatal Androgenization of Female Mice Causes an Increase in Firing Activity of Gonadotropin-Releasing Hormone (GnRH) Neurons That Is Reversed by Metformin.

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Prenatal androgenization (PNA) of female mice with dihydrotestosterone recapitulates some aspects of the fertility disorder polycystic ovary syndrome (PCOS). Similar to women with PCOS, PNA mice can exhibit elevated androgen and luteinizing hormone (LH) levels, irregular reproductive cycles, and impaired glucose disposal. Previous electrophysiological studies in these mice demonstrated increased frequency and amplitude of gamma-amino-butyric-acid (GABA)ergic postynaptic currents in GnRH neurons, which may stimulate activity of these neurons. The aims of this study were to evaluate activity parameters of GnRH neurons from PNA mice, and to test how they are altered by in vivo treatment with the AMP-activated protein kinase (AMP)-activating drug metformin, which can improve menstrual cyclicity in women with PCOS. First, estrous cycles were monitored in PNA and control (CON) mice, and then a subgroup of each was treated with metformin. Prior to metformin treatment, estrous cycles were longer in PNA mice, and percent of time in estrus lower ($n=10$ per group, $p<0.05$); after eight weeks on metformin, estrous cyclicity was significantly improved in PNA mice such that there were no longer differences between PNA and CON. One-hour extracellular recordings were used to monitor firing activity of GnRH neurons in brain slices from diestrous mice. Mean firing rate was higher and percent quiescence and maximum duration of quiescence were lower in GnRH neurons from PNA than CON mice ($n=17$ per group, $p<0.05$); these findings suggest increased GnRH neuron activity, which is consistent with elevated LH in PNA mice. Concomitant with improved estrous cyclicity, LH levels and firing rate and quiescence measures of GnRH neurons in metformin-treated PNA mice were restored to control levels ($p>0.8$). To assess whether activation of brain AMPK may contribute to the reduction in GnRH neuron activity by metformin, the AMPK antagonist Compound C (CC) was acutely applied to cells. CC stimulated a robust increase in firing in cells from metformin-treated, but not untreated PNA and CON mice, suggesting AMPK is activated in GnRH neurons or their afferents in metformin-treated mice. These studies indicate that prenatal androgenization of female mice causes enhanced firing activity of GnRH neurons and increased LH levels that are reversible by treatment with metformin, and that activation of brain AMPK by metformin may play a role in its restoration of normal reproductive cycles in PCOS.

Sources of Research Support: NRSA F31 NS62646 awarded to AVR; NIH Specialized Cooperative Centers Program in Reproduction and Infertility Research U54 HD28934.

Nothing to Disclose: AVR, SMM

P2-276

Selective Deletion of ERK1, 2 in GnRH Neurons Does Not Alter Neuronal Survival, Targeting or Reproductive Function.

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Gonadotropin releasing-hormone (GnRH) neurons that control the pituitary-gonadal axis originate from progenitor cells in the nasal compartment then migrate along vomeronasal nerve fibers across the cribriform plate into the forebrain. GnRH neuronal survival is critical to appropriate targeting as is appropriate GnRH gene expression to normal reproductive competence. Prior studies have shown the critical role of ERK MAPK at the level of the pituitary to mediate LHB gene expression and female fertility. Our studies showed the importance of ERK 1,2 MAP kinase downstream of Axl and Tyro3 receptor tyrosine kinase to mediate GnRH neuron survival and inhibition of gene expression. Thus, we tested the hypothesis that deletion of ERK 1 and 2 in GnRH neurons would alter the survival or targeting of GnRH neurons in the brain and/or GnRH expression levels and thus alter reproductive function. GnRH-cre +/wt mice (provided by C Dulac, Harvard) were crossed with ERK1-/-ERK2fl/fl mice to create mice with a systemic deficiency of ERK1 and a GnRH neuron specific loss of ERK2. GnRH-cre +/wt ERK1-/- ERK2 fl/fl mice (GnRHERK1,2 null) had similar numbers of GnRH neurons along the migratory route at E15 compared with GnRH-cre wt/wt ERK1-/- ERK2fl/fl mice (GnRHERK1,2 control) (1198 ± 65 neurons, n = 4, compared with 1160 ± 80, n = 4). Analysis of GnRH neuron populations in adult mice showed similar numbers and distribution compared with controls (741 ± 157 neurons, n = 3, in GnRHERK1,2 nulls, compared with 756±7 neurons, n = 2, in controls). Consistent with no change in GnRH neuron number, GnRH neuronal function was normal as assessed by similar age at vaginal opening [GnRHERK1,2 null, 32.6 ± 0.6 d (n = 11) and control, 32.8 ± 0.9 d (n=5), and age at first estrus, 38.2 ± 1.2 d (n = 11) vs 37.4 ± 1.9 d (n=5)], indicating that onset of sexual maturation was unaffected by the selective loss of ERK function. In adults, estrus cycle length was similar in both mutant and control mice (4.9 ± 0.2 d, n = 8) compared with 5.3 ± 0.7 d, n = 3). Together these data suggest that ERK 1,2 MAPK is not a central integrator of signaling in the GnRH neuron and that other intracellular pathways compensate to ensure neuronal survival, GnRH gene expression and normal reproductive function.

Sources of Research Support: HD31191 (MEW) and HD034722 (MSR).

Nothing to Disclose: MEW, AP, BB, MX, SB, MR

P2-277

Genetic Profile of a Large Cohort of Women with GnRH Deficiency Reveals Genetic Heterogeneity and a Role for *KAL-1*..

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Context: GnRH deficiency or idiopathic hypogonadotropic hypogonadism (IHH) is a rare genetic disorder caused by defective hypothalamic GnRH secretion or action with anosmia (Kallmann Syndrome [KS]) or normosmia (nIHH) and other non-reproductive phenotypes. The sex ratio of this condition is 4-5:1. Since few studies have focused on the genetics of women with GnRH deficiency, we examined the genotypic characteristics of a large cohort.
Methods: 220 women >17 years old with primary amenorrhea and hypogonadotropic hypogonadism without an anatomic or functional cause were studied. Subjects were genotyped for *KAL1, FGFR1, FGF8, NELF, PROKR2, PROK2, GPR54, TAC3R, TAC3, GNRH1*, and *GNRHR*. Mutations were defined as rare sequence variants that were: 1) within coding regions ± 5 bp; 2) nonsynonymous; and 3) present in <1% of control women (n=150). Women were classified as KS or nIHH based on report and olfactory testing (51% of subjects).
Results: Mutations were identified in 42% of women with IHH. Of these, 80% were familial, 85% were monogenic (82% heterozygous, 12% biallelic), 14% digenic, and 1% trigenic with the majority being missense mutations. 39% were loss of function mutations (frameshift or *in vitro* testing). The prevalence is indicated in Table 1. Notably, 8 *KAL1* mutations (7 missense, 1 nonsense) were identified in 12 women (11 heterozygous, 1 biallelic) and *GNRHR* mutations were demonstrated in both KS and nIHH women.

Table 1. Frequency of mutations in 11 genes expressed as % of women tested for each gene

Gene (# of women tested)	*KAL-1* (160)	*FGFR1* (202)	*FGF8* (185)	*NELF* (144)	*PROKR2* (187)	*PROK2* (183)
All women	6.9%	16.8%	2.2%	1.4%	7.0%	2.2%
nIHH women	4.8%	13.2%	3.0%	0%	6.0%	2.0%
KS women	9.1%	20.8%	1.2%	3.0%	8.1%	2.4%
Gene (# of women tested)	*GPR54* (145)	*TACR3* (78)	*TAC3* (79)	*GNRH1* (85)	*GNRHR* (167)	
All women	2.8%	6.4%	5.1%	3.5%	10.2%	
nIHH women	5.1%	6.6%	4.0%	3.9%	17.2%	
KS women	0%	0%	33%	0%	2.5%	

Conclusion: 42% of women with IHH have mutations in the spectrum of genes known to be associated with GnRH deficiency including: 1) *KAL-1*, which has not previously been linked to the female IHH phenotype; and 2) *GNRHR*, which was found in both nIHH and KS women. Despite the quoted higher prevalence of GnRH deficiency in men, the genetic load appears to be similar in GnRH deficient men and women, suggesting that GnRH deficiency may be underdiagnosed in women.

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Nothing to Disclose: NDS, SBS, CKW, KAM, MGA, LP, VAH, WFC, NP, JEH

P2-278
SEF Is a Novel Locus for GnRH Deficiency.

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Background: The fibroblast growth factor (FGF) signaling pathway plays a critical role in GnRH neuron ontogeny. Mutations in both *FGF receptor 1* (*FGFR1*) and *FGF8* underlie human GnRH deficiency, i.e. Kallmann syndrome (KS) or normosmic idiopathic hypogonadotropic hypogonadism (nIHH). SEF (Similar Expression as FGF) is a conserved transmembrane protein which potently inhibits FGF8 signaling. We hypothesized that germline mutations in *SEF* could underlie GnRH deficiency in humans.
Methods: The *SEF* gene was sequenced in 443 IHH subjects (225 KS, 218 nIHH). Identified mutations were studied in transfected cells using an AP1-luciferase reporter assay as readout for FGF signaling. Expression of Sef was documented in the embryonic olfactory system and adult hypothalamus of mice by RT-PCR and immunohistochemistry.
Results: Six missense *SEF* mutations were found in respective KS probands and were absent from 200 healthy controls. In addition to anosmia, 5 out of 6 patients had hearing loss, while missing teeth, bilateral ptosis, osteopenia, and synkinesia were seen sporadically. Two mutations were homozygous (p.P306S and p.P577Q) and four heterozygous (p.K162R, p.Y379C, p.S468L, p.A735V). The K162R, S468L and A735V mutations decreased FGF signaling in transfected cells, whereas P306S increased signaling; p.P577Q and p.Y379C had no effect. The cell surface expression of K162R, S468L and A735V was decreased. Consistent with a role in GnRH biology, Sef was expressed in olfactory and hypothalamic regions where GnRH neurons are present.
Conclusion SEF, an inhibitor of FGF8 signaling, is a novel gene implicated in KS and associated hearing loss.

Nothing to Disclose: BF, WCC, MGA, LP, AD, RQ, SBS, JPC, GPS, YS, PT, NP

P2-279

Cell-Specific Expression and Transcriptional Regulation of the Mouse GnRH Gene.

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Introduction: Appropriate tissue-specific expression of GnRH is critical for establishing and maintaining reproductive competence. GnRH is synthesized and secreted from specific hypothalamic neurons. Additionally, an extra-pituitary role for GnRH has been appreciated in peripheral reproductive tissues including the ovary. A common element in the mouse GnRH (mGnRH) promoter, between -2806 bp and -2078 bp, was shown to mediate differential regulation of hypothalamic and ovarian mGnRH expression. In addition, in vitro studies have identified several transcription factors that interact with this region to regulate GnRH expression.
Objectives: 1) To further define the mGnRH promoter and 2) To determine transcription factors that bind to the mGnRH DNA fragment from -2444 to -2078 bp.
Materials and Methods: We performed a yeast one-hybrid screen using the -2444 to -2078 fragment. cDNA libraries were then constructed from GnRH neuronal (GT1-7, Gn11) and ovarian granulosa (KK) cell lines. Over 25% of the clones that we obtained from the GT1-7 cell library contained the DNA binding domain of Oct-1 and about 15% contained the DNA binding domain of FoxJ1. We also obtained both Oct-1 and FoxJ1 from the Gn11 cell library in the clones screened. In contrast, over 30% of the clones from the KK library were FoxJ1, and we did not obtain Oct-1 in the screen. About 70% of GnRH neurons were found to be immunopositive for FoxJ1. FoxJ1 mRNA (by RT-PCR) was not ubiquitously expressed as is Oct-1 mRNA; it was found only in the brain, reproductive tissues and lung. We performed a chromatin immunoprecipitation assay (ChIP) to determine the binding characteristics of Oct-1 and FoxJ1. Oct-1 bound to, or nearby, the region between -2444 and -2087 bp of the mGnRH prompter in GT1-7 and Gn11 cells, but not to a more upstream DNA control fragment. In contrast, we have not been able to detect binding of Oct-1 to this region in KK cells. However, we have determined that all cell lines contain Oct-1 mRNA. The ChIP assay confirmed binding of FoxJ1 on the GnRH promoter in GT1-7, Gn11 and KK cells.
Conclusion: The studies identified and characterized cis-regulatory DNA elements involved in cell-specific expression of hypothalamic and ovarian GnRH. Oct-1, although a ubiquitous transcription factor, may be critical in determining the expression of GnRH in the hypothalamus. FoxJ1, a more anatomically restricted protein, may be important in expression of GnRH in the hypothalamus and ovary.

Nothing to Disclose: HJN, MY, SR

P2-280

Developmental Changes in Primate GnRH Neurons: Epigenetic Contribution.

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Pulsatile release of GnRH from neuroterminals into the portal circulation is essential for maintenance of the ovulatory cycle and the pulsatile application of GnRH agonists and antagonists is a standard clinical treatment for precocious and delayed puberty and infertility in human patients. Therefore, understanding the mechanisms underlying pulsatile GnRH release is important in reproductive neuroendocrinology. To study cellular and molecular mechanisms regulating GnRH neurons, we previously developed GnRH neuronal cell cultures derived from the nasal placode of rhesus monkeys at embryonic days 35-37. At this stage the GnRH neurons are functionally immature, i.e., at the beginning of cultures GnRH release is very low and no intracellular calcium, [Ca2+]i, oscillations are observable. However, GnRH neurons gradually mature over the next 2-weeks. By 3-weeks neurons are fully mature with GnRH release at ~60 min intervals and periodic [Ca2+]i oscillations, which also synchronize at ~60 min intervals. A similar study in mouse nasal placode culture also indicates that GnRH mRNA increases during GnRH neuronal maturation. In the present study we hypothesized that GnRH gene expression changes during GnRH neuronal development and that an epigenetic mechanism controls this maturational process. To test this hypothesis we measured GnRH mRNA and assessed the DNA methylation status of the GnRH gene in rhesus monkey embryonic placode cultures at 0, 14 and 20 days in vitro (div). Specifically, using quantitative RT PCR we found that GnRH mRNA expression increased through culture days with expression highest at 20 div. We then analyzed gene promoter CpG methylation status from the same samples. Using the methylation-sensitive restriction enzyme/real time PCR technique, we found the CpG methylation status at the -1983 CpG site reciprocally mirrored the mRNA expression pattern in cultured GnRH neurons. Moreover, by bisulfite sequencing across the 5' CpG island (-2126 to -1861) of the GnRH gene, we found that methylation at 8 of 14 CpG sites significantly ($p<0.05$) decreased during in vitro development. These data indicate that GnRH mRNA expression increased, while CpG methylation status decreased during neuronal maturation. Our data further suggest that an epigenetic mechanism regulates GnRH gene expression and that manipulating the epigenetic structure of this gene can impact GnRH neuronal function.

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Nothing to Disclose: JRK, ET

P2-281

The Functional Role of the First Intracellular Loop of Prokineticin Receptor 2.

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Background: Physiological activation of the prokineticin pathway plays a critical role in olfactory bulb morphogenesis and GnRH secretion. Inactivating mutations in prokineticin 2 (*PROK2*), and its G protein-coupled receptor (GPCR), *PROKR2*, have been identified in patients with Kallmann syndrome (KS) and normosmic isolated hypogonadotropic hypogonadism (IHH). We recently reported a PROKR2 mutation localized in the first intracellular loop (ICL) of PROKR2, R80C, in a patient with KS. Additional mutations in the first ICL of PROKR2, R85C and R85H, have also been reported. Little is known about the function of the first ICL of GPCRs. In this study, we explored the effects of these mutations on PROKR2 function, to determine whether these amino acid substitutions contribute to the patient phenotypes and to elucidate the mechanisms by which mutations in this domain affect receptor function. **Methods:** The new PROKR2 mutant, R80C, was studied in functional assays, in parallel with the two other reported mutations in the first ICL of PROKR2, R85C and R85H. Expression vectors encoding wild type (WT) or R80C, R85C, or R85H mutant PROKR2 were transiently transfected into HEK293 or COS-7 cells. The ability of PROK2 to activate signaling cascades through Gαq coupling was evaluated by measuring inositol phosphate (IP) accumulation and using an Egr1-Luc reporter assay to measure MAPK activation. The effects of the mutations on ligand binding were studied using ^{125}I-MIT, a homologue of PROK2, together with increasing concentrations of unlabeled PROK2 to produce competition binding curves. **Results:** R80C PROKR2 significantly impaired PROK2-stimulated IP accumulation and MAPK activation, with both right-shifted dose-response curves and reduced maximal responses, whereas R85C and R85H PROKR2 showed only small differences in IP or MAPK activation compared to WT. Binding studies showed reduced maximal binding of R80C PROKR2 to its ligand, PROK2 (i.e., reduced Bmax), consistent with reduced cell surface expression. **Conclusions:** These findings indicate that the R80C mutation in the first intracellular loop of PROKR2 impairs cell surface expression, ligand binding and ligand-stimulated activation of intracellular signaling pathways. These data implicate a role for the first ICL of GPCRs in receptor cell surface expression and suggest that the arginine located at position 80 in the first ICL of PROKR2 is important for the proper folding or trafficking of PROKR2 to the plasma membrane.

Nothing to Disclose: APA, SDN, SX, EBT, CM, YS, NP, RSC, BBM, ACL, UBK

P2-282

TACR3 Mutations Identified in Patients with Idiopathic Hypogonadotropic Hypogonadism Interfere with NK3R Function through Several Distinct Mechanisms.

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Mutations in TAC3, the gene encoding neurokinin B (NKB), and in TACR3, encoding its receptor, NK3R, have been identified in patients with idiopathic hypogonadotropic hypogonadism (IHH). Both NKB and NK3R have been implicated in the regulation of GnRH release. We recently screened 345 probands in a cohort of patients with IHH by DNA sequence analysis and identified 5 missense mutations that each resulted in an amino acid substitution in NK3R (G18N, I249V, Y256H, R295S, and Y315C). We have investigated the mechanisms through which these identified mutations affect NK3R function. Expression vectors encoding wild type (WT) or mutant NK3R were transiently transfected into COS-7 cells and NKB-stimulated inositol phosphate (IP) accumulation was measured. The mutant G18N and I249V NK3Rs had IP dose-response curves similar to WT NK3R, indicating that these two mutations did not interfere with receptor signaling in this assay. In contrast, the Y256H, R295S, and Y315C NK3R mutants had markedly reduced IP accumulation, with both right-shifted dose response curves and reduced maximal responses compared to WT NK3R. Next, the effects of mutations on cellular receptor protein expression were determined in COS-7 cells transfected with WT or mutant NK3R by Western blot analysis. Both Y256H and Y315C NK3Rs had a decrease in protein expression compared to WT NK3R. Finally, to assess effects of these mutations on ligand binding to the receptor, binding studies were performed in WT or mutant NK3R transfected COS-7 cells, using ^{125}I-labeled NKB together with increasing concentrations of unlabeled NKB to produce competition binding curves. R295S NK3R bound ^{125}I-labeled NKB similarly to WT NK3R, but both Y256H and Y315C NK3R failed to show appreciable binding to ^{125}I-labeled NKB, in agreement with their reduced expression levels. This study demonstrates that three missense mutations in TACR3, identified in our cohort of IHH patients, lead to impaired NK3R signaling. The R295S NK3R mutant, in the third intracellular loop of NK3R, is expressed and can bind NKB, but has reduced ability to activate intracellular signaling pathways, consistent with the known role of the third intracellular loop of G protein-coupled receptors in interacting with G proteins. The Y256H and Y315C NK3R mutants, located in the 5th and 6th transmembrane domains respectively, have reduced cellular expression, indicating a role for these domains in receptor folding and trafficking to the plasma membrane.

Nothing to Disclose: SDN, APA, SX, EG, CT, ACL, SBS, RC, UBK

P2-283

Genotype Prediction Based on Clinical Features of GnRH Deficiency.

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Background: Idiopathic hypogonadotropic hypogonadism (IHH) due to congenital GnRH deficiency is remarkable for its clinical and genetic heterogeneity. The significant overlap of reproductive features associated with *KAL1* and *FGFR1* mutations (1-5) makes it difficult to prioritize these major IHH loci for genetic screening. This study tested the hypothesis that non-reproductive phenotypes, response to GnRH, and/or reversibility of hypogonadotropism in IHH patients can predict the presence of *KAL1* or *FGFR1* mutations.
Methods: 178 IHH patients were classified into 3 groups based on the presence of mutations in *KAL1* (n=35), *FGFR1* (n=71), or neither gene (n=72). Each patient underwent detailed characterization, including non-reproductive phenotyping [low bone mass (T-score <-1.5), cleft lip/palate, high-arched palate, renal agenesis, synkinesia, bone abnormalities, and dental agenesis]; favorable response to ≥12 mo. GnRH therapy (serum T>270 ng/dL and sperm in the ejaculate); and reversal of IHH. To identify clinical predictors of genotype, the incidence of each of these phenotypes was compared among the 3 groups using Fisher's exact test.
Results: Data are summarized in Table 1. Renal agenesis, synkinesia, and lack of response to GnRH predicted *KAL1* mutations, while dental agenesis and reversal of IHH predicted *FGFR1* mutations.

Clinical course and non-reproductive phenotypes of subjects.

	FGFR1 (n=71)	KAL1 (n=35)	Negative controls (n=72)	p-value
Demographics				
Total IHH	100%	100%	100%	
Males	70%	89%	69%	
Females	30%	11%	31%	
KS	62%	100%	61%	
nIHH	38%	0%	39%	
Clinical Course				
Favorable Outcome to GnRH Therapy	100% (11/11)	55% (6/11)	93% (25/27)	p=0.01
Reversal	26% (5/18)	0% (0/11)	5% (1/22)	p=0.05
Non-Reproductive Phenotypes				
BMI	26.4±0.8	28.3±7.7	25.6±6.5	ns
Low bone density	84% (21/25)	86% (6/7)	87% (20/23)	ns
Cleft Lip/Palate	13% (6/47)	0% (0/14)	3% (1/31)	ns
High-arched palate	9% (4/47)	29% (4/14)	11% (4/38)	ns
Renal agenesis	0% (0/38)	18% (3/17)	3% (1/32)	p=0.01
Synkinesia	5% (2/39)	42% (10/24)	23% (7/30)	p=0.001
Bone abnormalities	59% (23/39)	63% (5/8)	47% (16/34)	ns
Dental agenesis	41% (16/39)	0% (0/8)	6% (2/34)	p<0.001

Conclusion: Specific non-reproductive phenotypes, clinical response to GnRH, and reversibility of IHH are valuable for prioritizing *KAL1* or *FGFR1* for genetic screening.

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Nothing to Disclose: KWK, XHH, LP, AAD, MGA, GPS, AT, TR, EJ-D, RQ, VAH, SBS, FJH, JEH, WFC , NP

P2-284

Effects of Estrogen on Temporal Patterns of GPR54 Expression in Gonadotropin-Releasing Hormone (GnRH)-Secreting GT1-7 Cells.

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Ovulation in females requires gonadotropin-releasing hormone (GnRH) surges released from specialized hypothalamic neurons. Surge regulation is mediated by ovarian estradiol (E2) feedback, acting as a negative signal until early afternoon of proestrus, at which point it stimulates robust increases in GnRH release. In rodents, GnRH surges are temporally confined to the late afternoon of proestrus, demonstrating the influence of a circadian clock. Endogenous clocks are comprised of transcriptional feedback loops in nearly all cells: BMAL1 and CLOCK heterodimers stimulate transcription of *Period (per)* and *Cryptochrome (cry)* genes, proteins of which dimerize and inhibit their own transcription. Using microarrays, real-time qRT-PCR and western blotting, we have investigated expression patterns of channels and receptors in cultured GnRH neuronal cells that oscillate with an approximate circadian periodicity, but only in the presence of elevated E2. Kisspeptin (Kiss1), first identified for anti-metastatic properties, has been revealed as an essential part of the reproductive axis, stimulating GnRH secretion through its receptor, GPR54. Kisspeptinergic neurons synthesize more Kiss1 in response to E2 and synapse on GnRH neurons, making Kiss1 a prime GnRH surge-generating signal. However, it is unclear if simple tonic increases in Kiss1 release are sufficient to initiate the surge, or if increases in sensitivity are required. Kisspeptin administration alone to GT1-7 cells does not stimulate GnRH neurons to secrete at levels observed during preovulatory surges. We have observed, however, that GPR54 expression levels in cultured GT1-7 cells oscillate over time, but only robustly in the presence of elevated E2, and that clock gene oscillations can be modulated by E2. This provides for a mechanism whereby Kiss1 receptor expression levels, and thus GnRH sensitivity to Kiss1, may change dramatically over the proestrus day. We have observed high-amplitude patterns of GPR54 expression oscillating with an approximate circadian period in GT1-7 cells, but only during “surge-level” estrogen treatment, lending credence to the hypothesis that estrogen positive feedback may induce dynamic changes in patterns of GPR54 expression by coupling to an endogenous oscillator. In this way, elevated ovarian E2 may increase kisspeptidergic tone while simultaneously increasing GnRH neuronal sensitivity to this neuropeptide for maximal surge release.

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Nothing to Disclose: KJT, CPG, KLL, PEC

P2-285

Transcriptional Regulation of the Mouse Gonadotropin-Releasing Hormone (GnRH) Gene by Kisspeptin.

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Introduction: Kisspeptins, products of the KiSS-1 gene, as well as their G-protein coupled receptor 54 (GPR54), have been shown to be key components in the regulation of gonadotropin-releasing hormone (GnRH) secretion in several mammalian species, including humans. In addition, *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 by 2-fold when cells were treated with 10^{-9}M kisspeptin for 4h (n=5, $p \leq 0.01$), thus localizing a kisspeptin-response element between -3446 bp and -1750 bp of the mGnRH gene upstream from the transcription start site. *In vitro* studies, however, are unlikely to reflect intricate signaling pathways regulating GnRH gene expression *in vivo*. Therefore our laboratory studied transgenic mice containing sequential deletions of the mGnRH gene promoter linked to the luciferase reporter. Kisspeptin (1nM via IP) treatment increased LUC activity in the hypothalamus of mice by 2-fold (n=3, $p \leq 0.01$) and a kisspeptin-response element was located between -3446 bp and -2078 bp of the mGnRH gene. Interestingly, this region had previously been identified to contain a GnRH enhancer element. In addition, 10^{-9}M kisspeptin treatment of GT1-7 and Gn11 cells increased mRNA levels of OCT-1, FOX-J1 and OTX-2 in a time-dependent manner.
Conclusion: This work demonstrates that the elements between -3446 bp and -1750 bp in GT1-7 and Gn11 neuronal cells, and -3446 bp to -2078 bp in transgenic mice play a significant role in positive regulation of GnRH by kisspeptin. In addition, we show for the first time that OCT-1, FOX-J1 and OTX-2 protein levels are induced by kisspeptin in GnRH-neuronal cell lines, and these transcription factors may mediate the transcriptional response of mGnRH to kisspeptin.

Nothing to Disclose: HJN, DF, SR

P2-286

The Premamillary Nucleus as a Site of Leptin Action on GABA Neurons.

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Gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus respond to signals about energy availability from the brain and periphery. Leptin is a peripheral signal that circulates in the blood in proportion to the amount of body fat. With no leptin signalling in the brain, individuals are obese and infertile. We have previously shown that leptin does not signal directly to GnRH neurons in the septal preoptic area, and thus must signal through a forebrain neuronal mediator (1). In this study we investigated the inhibitory neurotransmitter gamma-aminobutryric acid (GABA) as a possible intermediary of leptin's actions on GnRH and thus fertility.
GABA is synthesized directly from glutamate by glutamic acid decarboxylase (GAD), of which there are two isoforms: GAD-1 and GAD-2. The key enzyme in GABA degradation is GABA-transaminase (GABA-T). To test the hypothesis that GAD gene expression will increase in a state of leptin deficiency, ob/ob mice (mutants carrying a defective leptin gene) were used. Estrogen levels were normalised in ob/ob and wild-type littermate (control) mice by ovariectomy and low-dose estradiol replacement via s.c. implants. A range of different leptin-responsive brain structures known to be important to the GnRH neuronal network were micropunched from frozen brain section, including the septal preoptic area, anteroventral periventricular region and arcuate nucleus where kisspeptin neurons are located, and the ventral premammillary nucleus (PMV) where lesions have been shown to disrupt fertility (2). Gad1, Gad2 and GABA-transaminase (GABA-T) mRNA levels were measured in these distinct areas using quantitative RT-PCR (normalised to RPII). The expression of all three GABA mediators in PVN was twice as high in the leptin-deficient female mice (~100% upregulation) ($p<0.05$); a similar trend was observed in the other regions but this was not statistically significant.
These data suggest there is greater synthesis and turnover of GABA in the PMV of female leptin-deficient mice. This is consistent with the idea that leptin suppresses the activity of GABA neurons at this site, leading to dis-inhibition of GnRH function in the septal preoptic area of the hypothalamus.

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Nothing to Disclose: WZ, GMA, JHQ

P2-287

Functional Characterization of Human Prolactin Receptor Variants Identified in Breast Tumors.

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Although the involvement of the prolactin (PRL) system in promoting mammary tumorogenesis has been widely documented using animal models, extrapolation of these findings to humans remains inconclusive. One of the reasons is that mutations in human PRL or PRL receptor (hPRLR) genes in breast cancer patients are yet to be identified, while common genetic variations (polymorphisms) of these genes showed only limited correlation with breast cancer risk. We recently investigated a cohort of 95 women presenting with multiple breast fibroadenomas, a rare form of benign breast disease (1). Fifteen percent of patients were found to harbor one of the three germline, heterozygous nonsynonymous single nucleotide polymorphisms (SNP) that we identified in the hPRLR gene. Two of them involve the extracellular domain (exon 5/I76V, exon 6/I146L), and one the intracellular domain (E554Q/exon 10). Although the two former were already known, their impact on functional receptor properties had never been investigated. Each variant was stably expressed in human embryonic kidney (HEK 293) cells and in mouse lymphoid Ba/F3 cells. Ongoing analyses suggest E554Q is a loss-of-function variant. In contrast, both hPRLR-I146L and hPRLR-I76V were shown to exhibit constitutive activity, as reflected by basal activation of Erk1/2 and Stat5, and ligand-independent growth-promoting and anti-apoptotic effects (compared to cells expressing WT PRLR). These results suggest that I76V and I146L substitutions induce conformational changes leading to spontaneous receptor triggering. In all assays, the constitutive activity of PRLR-I146L was the most pronounced, although both variants remained sensitive to PRL stimulus (substitutions do not affect binding affinity)(1,2). As PRLR-I146L was earlier reported in breast cancer patients (3), it was stably introduced into MCF-7 breast cancer cells. Even in this heterozygous context mimicking that found in patients (MCF-7 endogenously express WT PRLR), PRLR-I146L conferred autonomous cell proliferation. Micro-array studies indicate that expression of a large set of genes is altered. Ongoing analyzes are aimed at identifying the multiple cascades and key target genes modulated by constitutive PRLR signaling in breast cancer cells.

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Nothing to Disclose: SB, IF, RLB, PT, VG

P2-288

Experimental Validation of a Nuclear Jak2/RUSH Signaling Pathway.

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Jak2/Stat-mediated prolactin (PRL) signaling culminates in the sequence-selective binding of Stat5a. However, in the absence of Stat-binding sites, RUSH mediates the ability of PRL to augment progesterone-dependent transcription of the SCGB1A1 gene. RUSH, the rabbit ortholog of human HLTF, is a SWI/SNF chromatin remodeling complex that uses the energy of ATP hydrolysis to change the structure of chromatin. RUSH is also a phosphonuclear protein whose ability to regulate transcription by sequence-specific DNA-binding is mediated by tyrosine-phosphorylation. Speculation about a Jak2/RUSH pathway compelled us to test the hypothesis that Jak2 phosphorylates RUSH. Western analysis and confocal immunofluorescence microscopy with chemically distinct Jak2 inhibitors - AG490, TG101209, Staurosporine, Jak inhibitor 1, Jak 2 inhibitor 2 and Tyrene CR4 - in conjunction with the PI-3 kinase inhibitor, Wortmannin, and the MAP kinase inhibitor, PD98059, showed that RUSH is rapidly phosphorylated by Jak2 as a direct consequence of PRL treatment. PRL augmented progesterone-dependent transcription of a RUSH construct (-712/+90) in transient transfection assays with HRE-H9 cells. The PRL effect was blocked either by Jak2 inhibitors or mutation of the RUSH (-616/-611) site. The construct was devoid of Stat5 binding sites. Subcellular fractionation and Western analysis demonstrated a nuclear pool of active Jak2 in endometrium and HRE-H9 cells. Co-immunoprecipitation of phospho-Jak2 with phospho-RUSH confirmed a physical interaction between the two proteins in the nucleus. Jak2 inhibitors abolished the nuclear pool of phospho-RUSH without altering the nuclear content of RUSH. Nucleolin (C23), a major nucleolar protein that is also found in the nucleoplasm, was unambiguously identified in MS-MS analysis of nuclear proteins that co-immunoprecipitated with both RUSH and GST-RING. Confocal immunofluorescence images of HRE-H9 cells showed an even nuclear distribution of RUSH and phospho-Jak2. RUSH lacks a nucleolar localization signal, and did not co-localize with fibrillarin to the nucleolus. The proteasome inhibitor, MG-132, failed to promote nucleolar accumulation of RUSH, and it had no effect on the degradation of nuclear phospho-RUSH. Collectively, these data authenticate a Jak/RUSH pathway in which phosphorylation of RUSH by Jak2 occurs in the nucleus where nucleolin might act via its histone chaperone activity to enhance RUSH's chromatin remodeling efficiency.

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Nothing to Disclose: BSC, RAH

P2-289

Prolactin Activation of Jak1 Signaling Pathways in Breast Cancer: Modulation by Her Receptors.

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Prolactin (PRL) is a pituitary hormone that acts through the prolactin receptor (PRLR) and is a critical regulator of normal growth, development, and differentiation of human breast epithelia. In addition, accumulating evidence implicates PRL in breast cancer initiation and progression. Despite this fact, one of the main signaling pathways of PRL, Jak2-Stat5, is a marker of favorable prognosis in primary human breast cancer. However, other signaling pathways activated by PRL include ERK1/2, c-Src, Akt, and Stat3, which have all been implicated in oncogenesis. This demonstrates the complexity of the PRL signaling in breast cancer, since PRL appears to have opposing roles in breast cancer depending on which signaling pathways are activated. Adding to the complexity of PRL signaling, our laboratory has recently reported that PRLR signals through a Jak1 pathway in a panel of breast cancer cell lines regardless of estrogen receptor status. PRL activation of Jak1 was dependent on activation of Jak2, and PRL mediated Stat3 and ERK1/2 signals were particularly dependent on Jak1. We now report that Her receptors may modulate PRLR-Jak1 signaling. Treatment of T47D and SKBR3 cells with the dual EGFR/Her2 kinase inhibitor Lapatinib decreased the PRL-Jak1 signal. Knockdown of Her2 but not Her4 by specific shRNA abolished the PRL-Jak1 signal but not the Oncostatin M-Jak1 signal in T47D cells. These data indicate that PRL activates the Jak1 pathway through a mechanism involving a signaling complex with Her receptor members. Furthermore, since oncogenic signals such as Stat3 and ERK1/2 activation by PRL is dependent on activation of Jak1, we hypothesize that this aberrant pathway promotes breast cancer growth and progression. This novel PRLR/Her Receptor-Jak1 pathway could be a separate pro-invasive pathway that offsets the pro-differentiation PRLR-Jak2-Stat5 pathway.

Nothing to Disclose: TS, HR

P2-290

The Effect of Acidic pH on Prolactin Signaling in Breast Cancer Cells.

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Prolactin (PRL), a polypeptide hormone, binds to the extracellular domain (ECD) of prolactin receptors (PRLRs) and activates Janus-activated kinase (JAK) 2/signal transducer and activator of transcription (STAT) 5. PRL signaling is best known for its indispensable role in terminal differentiation of mammary epithelium during pregnancy. The role of PRL signaling in breast cancer is more complex. While PRL has been implicated as promoting tumorigenesis in mouse models, multiple lines of evidence have also suggested PRL and JAK2/STAT5 signaling in suppression of breast cancer progression. Importantly, levels of active STAT5 is frequently lost during breast cancer progression and correlates with poor prognosis, despite continued expression of STAT5 protein (1). However, the causes for inactivation of STAT5 are unknown. Reduced pH is a frequent feature of the tumor microenvironment presumably due to increased glycolytic metabolism in cancer cells and poor perfusion of tumors. The pH of interstitial fluid of tumors often range between 6.5-6.9 while normal tissue pH is around 7.4. A previous report identified a 500-fold increase in the dissociation rate of human PRL and PRLR ECD between pH 8.3 and pH 5.8 (2), indicating that PRL binding to PRLR is disrupted at reduced pH. In this study, we tested whether acidic pH in tumor microenvironment affected PRL signaling. We found that PRL signaling was dramatically reduced at pH 6.8 compared to pH 7.4 in breast cancer cell lines, suggesting significant resistance to PRL at acidic pH commonly seen in tumors. PRL-induced gene transcription was also severely dampened under acidic pH conditions. Importantly, the effect of low pH on signaling was specific to PRL receptor, as EGF-induced activation of ERKs and AKT was not affected. The resistance to PRL at pH 6.8 could be rapidly reversed by restoring medium to pH 7.4. To address clinical relevance of these observations, we quantified levels of STAT5 phosphorylation and glucose transporter (GLUT) 1, a marker of elevated glycolytic metabolism and thus a proximate marker for acidic pH, in human normal and malignant breast tissues. A subgroup of high grade malignant cases showed elevated levels of GLUT 1, all of which displayed low levels of STAT5 activation, consistent with the results of our *in vitro* study. These data suggested that acidic microenvironment in breast cancer may cause PRL insensitivity and contribute to loss of STAT5 activation during disease progression.

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Nothing to Disclose: NY, AR, CL, THT, FEU, HR

P2-291

Immuno-Modulatory Role of Prolactin in Immune Tissues Driven by the Alternative Promoter.

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The pituitary is the main source of circulating prolactin (PRL), however it is also expressed in humans at extra-pituitary sites including immune tissues and endometrium where expression is regulated by an alternative promoter located 5.8kbp upstream of the pituitary transcription start site. Using a Bacterial Artificial Chromosome recombineering approach, we previously generated PRL-reporter transgenic rats that express luciferase (Luc) under the control of 160kbp of the human PRL gene locus. *In vivo* bioluminescence imaging of the anaesthetised intact animals following inflammatory stress showed a dramatic induction in luciferase expression in extra-pituitary tissues, in particular the thymus and spleen, which was shown to be driven by the alternative PRL promoter.
To explore further the regulation of extra-pituitary PRL gene expression and its association with inflammatory response we carried out an *ex vivo* study to assess the effect of a range of inflammatory stimuli on PRL expression in immune-related organs. Dispersed cell preparations from the spleen and thymus of PRL-Luc transgenic rats were treated with stimuli and assayed for luciferase activity. Stimuli included lymphocyte activators, growth factors, cytokines and LPS, all of which have a potential role in either the inflammatory or stress response. These stimuli were able to induce PRL expression in both male and female splenic and thymic cultures, with the greatest induction following LPS treatment (~3-fold). Induction in luciferase expression was confirmed to be driven by the alternative promoter by RT-PCR. These results support the potential role of PRL as an immune-regulator in addition to its endocrine function.
Characterising the immune cell populations expressing PRL following inflammatory stress is essential to the understanding of the immuno-modulatory role of the hormone. Using fluorescence and bioluminescent imaging we found that FITC-labelled Cd11b-positive macrophage/monocytic cells from PRL-Luc transgenic rat peripheral blood displayed luminescent signals, indicating PRL promoter activation in these cells. Further studies are in progress to compare this to the regulation of endogenous hPRL expression in peripheral blood mononuclear cells (PBMCs) isolated from normal human subjects. In summary, this heterologous transgenic rat model reveals new insights into regulation of the human PRL gene, and informs future studies of the role of PRL in inflammatory response in man.

Sources of Research Support: The Wellcome Trust.

Nothing to Disclose: AVM, RA, CVH, KF, SS, DGS, JJM, JRED, MRHW

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A Non-Prolactin Receptor (PRLR), High Affinity Prolactin Binding Protein on Human Milk Fat Globule Membranes.

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In order to study the types and amounts of prolactin (PRL) found in mammary gland ductal fluids and milk, we developed a highly sensitive ELISA that recognizes all forms of the hormone. While this assay detected PRL in whey (higher in early lactation), no PRL was detectable in whole milk. Further, when milk was added to standard curves of human PRL or pseudo-phosphorylated PRL (0.1 to 5 ng), 50% inhibition of assayability was observed at 0.5 µl milk. This effect indicated the presence of a PRL binding protein. Use of the ELISA to characterize the effect showed inhibition of PRL assayability by milk was rapid (detectable within 5 min), time and temperature dependent, and not due to protease digestion of PRL. Further, when milk was titrated against PRL, the steepness of the curve suggested high affinity and cooperativity between two or more interacting moieties. The binding protein was not a form of the PRLR because there was no competition between PRL and $\geq$ 20 fold excess human placental lactogen or human growth hormone in the absence or presence of 50 µM zinc. Pre-clearing of milk with anti-PRLR was also without effect. By contrast, addition of cyclosporine A showed a dose-related reversal of the milk inhibition of PRL assayability. Partial reversal was also achieved by pre-clearing milk with anti-cyclophilins. Neither cyclosporine A nor cyclophilin A or B affected the assay in the absence of milk. When whey and milk fat globule fractions were assayed separately, no inhibition of assayability was seen with whey, but potent inhibition was seen with milk fat globule membranes. Collectively, these data support the presence of a novel, cyclophilin-dependent binding protein on the milk fat globule membrane that is distinct from the previously described PRLR-related binding protein in whey and skimmed milk. Because of its high affinity, it may be more functionally significant than the soluble PRLRs in either buffering local concentrations of PRL within the mammary gland or in the delivery of PRL to the neonate.

Sources of Research Support: Grant from the Susan Love Foundation.

Nothing to Disclose: MYL, AMW

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Effect of Prolactin Proteolytic Fragments, Vasoinhibins, on Anterior Pituitary Cell Proliferation and Apoptosis.

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The anterior pituitary gland is under a constant cell turnover. In female rats, this process appears to be coordinated by the circulating levels of gonadal steroids. During proestrus, when the apoptosis rate of anterior pituitary cells is the highest, a peak in circulating levels of prolactin occurs. This hormone may be cleaved to different fragments (vasoinhibins) and their production varies along the estrous cycle. Proteolysis of rat prolactin by cathepsin D produces a single 16-kDa vasoinhibin (Vi) whith antiangiogenic actions, inhibiting proliferation and inducing apoptosis of endothelial cells.

The aim of this study was to investigate the effects of Vi on apoptosis and proliferation of anterior pituitary cells. In primary cultures of anterior pituitary cells we observed by Western Blot the presence of Vi in both media and cell extracts. A recombinant preparation of Vi (10 and 100 nM) induced apoptosis (determined by FACS) of anterior pituitary cells from ovariectomized rats (apoptotic cells, media ± SE. Control (C): 16.7 % ± 2.0; Vi 10 nM 24.1 % ± 1.5, $p<0.01$; Vi 100 nM 23.4 % ± 0.3, $p<0.05$, Dunnet). This effect was not observed when the cells were cultured in the presence of estradiol (E2, 10^{-9} M).

In addition, Vi (10 nM) decreased forskolin-induced proliferation (evaluated by BrdU incorporation) in total anterior pituitary cells and lactotropes (% of BrdU positive cells) when the cells were cultured without E2 (Total: C: 5.0 %; Vi 2.2%; Lactotropes: C: 8.1 %; Vi 2.9 %, $p<0.01$, $\chi 2$) or with E2 (Total: C: 5.4 %; Vi 0.8 %. $p< 0.01$. Lactotropes: C: 8.3 %; Vi 1.3 %, $p<0.01$; $\chi 2$).

These results suggest that vasoinhibins are present in anterior pituitary gland and that they could be involved in the control of anterior pituitary cell population by inducing apoptosis and inhibiting proliferation.

Nothing to Disclose: MJF, DBR, SZ, GJ, VB, GE, LM, VZ, AS, CC, DP

P2-294

Activation of ERK and Induction of MKP-1 by Perifused TRH Stimulation; Possible Role for Prolactin Gene Expression in Rat Pituitary GH3 Cells.

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The role of extracellular signal-regulated kinase (ERK) in mediating the ability of thyrotropin-releasing hormone (TRH) to stimulate the prolactin gene has been well elucidated. ERK is inactivated by a dual specificity phosphatase, mitogen-activated protein kinase phosphatase-1 (MKP-1). We investigated the pattern of ERK phosphorylation and the induction of MKP-1 by TRH under various stimulation conditions in pituitary GH3 cells. In static culture, ERK activation by continuous TRH was maximal at 10 min and persisted for up to 60 min. Stimulation with continuous TRH in perifused cells resulted in a similar level of ERK phosphorylation. MKP-1 was expressed 60 min following either static or perifused, continuous TRH stimulation. When cells were stimulated with pulsatile TRH every 30 min, ERK activation was maximal 10 min and returned to its baseline level by 30 min. ERK was phosphorylated again with each subsequent pulse. Pulsatile TRH did not induce MKP-1. Prolactin promoter activity following continuous, static TRH stimulation was higher than perifused TRH stimulation. Changes in pulse frequency resulted in alterations in the level of prolactin promoter. Following static stimulation, a 10 min exposure to TRH was sufficient to obtain full activation of the prolactin promoter. Additionally, a 5 min exposure of TRH was sufficient for maintain ERK activation. A single 5 min pulse of TRH stimulation resulted in low activation of the prolactin promoter. Next, we investigated the possible role for MKP-1 in TRH-induced prolactin gene expression. The effect of TRH on MKP-1 expression was completely prevented in the presence of specific MEK inhibitor, U0126. MKP-1 induction by TRH was completely inhibited by triptolide, a blocker for MKP-1. TRH-induced ERK activation was significantly enhanced in this condition. Prolactin promoter activated by TRH was reduced in the presence of triptolide. Additionally, in GH3 cells which were transfected with MKP-1 specific siRNA, both the basal and TRH-stimulated activities of the prolactin promoter were significantly reduced compared to the cells transfected with negative control siRNA. Although ERK is obviously a key kinase for prolactin transcriptional activity, duration of ERK activation were not obligatory for full activation of prolactin promoters. In addition, MKP-1 induced by TRH functions not only as an ERK-inactivating phosphatase, but also as an important mediator in the regulation of prolactin gene expression.

Nothing to Disclose: AO, HK, IP, KM

P2-295

A Novel *STAT5b* Mutation in Two Male Siblings with Growth Hormone Insensitivity, Hyperprolactinemia and Immune Dysfunction.

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BACKGROUND: Few patients with GH insensitivity (GHI) due to *STAT5b* mutations were reported to date. Immune dysfunction was reported in 5 female patients, whereas it was absent in 1 male patient with *STAT5b* mutation. This is the first report about the growth response to recombinant human IGF-1 (rhIGF-1) in patients with *STAT5b* mutation.
CLINICAL CASE: Two male siblings born from healthy non-consanguineous parents were evaluated for severe short stature associated with GHI phenotype and immunological defects (atopic eczema and interstitial lung disease). At his first evaluation with 6 yr, Sibling 1 had 86 cm (-5.6 SD) and 10 kg (BMI SDS = -1.9) and was being treated with corticosteroids and oxygen due to lymphocytic interstitial pneumonitis. Sibling 2 was first evaluated at the age of 2 yr and his height was 76 cm (-3.0 SD) and his weight was 7.5 kg (BMI SDS = -3.9). At age of 4 yr, he developed thrombocytopenic purpura requiring the use of chronic glucocorticoid therapy until 8yr. Both siblings were treated with rhGH without improve in growth rate. Hormonal evaluation in both patients showed normal basal and stimulated GH levels (Sibling 1: basal GH = 1.7 µg/L, peak GH = 20.6 µg/L; Sibling 2: basal GH = 1.0 µg/L, peak GH = 14.2 µg/L). IGF-1 and IGFBP-3 levels were extremely low (<-3 SD), and IGF-1 did not increase significantly during the generation test or during rhGH treatment. Prolactin levels were mildly elevated (61 and 77 ng/mL in Sibling 1 and 2) and the presence of macroPRL was ruled out. Lymphopenia and reduced levels of NK cells were observed, with no immunoglobulin abnormalities.
STAT5b genes were directly sequenced and revealed a homozygous deletion of 4 nucleotides in exon 5 (c.424_427del), which results in a frameshift mutation (p.L142fsX161) in both siblings. Their parents are heterozygous for this same mutation.
The younger sibling (age of 9.9 yr - prepubertal) was treated with rhIGF-1 at the dose of 110 µg/Kg BID and his growth velocity increased from 2.3 cm/y at base line to 3.3 cm/yr after 1.2 yr of therapy.
CONCLUSION: We identified a novel *STAT5b* mutation (p.L142fsX161) in two male siblings with clinical and laboratorial features of GHI associated with immunological defects and elevated prolactin levels without macroprolactin. The rhIGF-1 therapy resulted in insignificant improve in growth rate in one of the siblings, but additional studies in *STAT5b*-mutated patients treated with rhIGF-1 are necessary before conclusions can be drawn.

Nothing to Disclose: PNP-P, CAT, JMD, PCAS, MC, GS, IJPA, AALJ

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Developmental and Functional Role of Calcium/Calmodulin-Dependent Protein Kinase II (CaMKII) Isoforms in Normal Human Fetal Pituitary, LβT2 Gonadotropes and Zebrafish Neuroendocrine Tissues.

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Calcium/calmodulin-dependent protein kinases (CaMK) are major effectors of calcium signalling, including the Wnt-calcium pathway. CaMKs have been implicated in regulating pituitary hormone transcription and as a pivotal protein in the satiety pathway in the hypothalamus. However, spatial and temporal expression of the CaMKs is poorly documented and the role of CaMKs in endocrine development is unknown. Therefore, we have examined CaMKII expression in normal human fetal pituitaries (from 14-18wks old), and have used Zebrafish as a model for human neuroendocrine development to examine the expression and function of CaMKII in neuroendocrine tissues. Analyses of CaMKII isoform expression in the human fetal pituitary samples (n=8) revealed uniform expression of CaMKIIα, β and δ, but not γ. In contrast, LβT2 cells, which are embryonically-derived gonadotrope cells, expressed CaMKIIα, β and γ, but not δ. Extensive bioinformatic screening of the zebrafish genome revealed six isoforms of CaMKII (α, β, δα, δβ, γ1 and γ2) which were subsequently cloned from Zebrafish larvae (24hpf to 72hpf). Wholemount in situ hybridization (ISH) revealed hypothalamic and/or pituitary expression of CaMKIIα, β, δα, δβ and γ2, but no γ1. However, only CaMKIIγ2 expression was found in the presumptive pineal gland. In order to establish the functional requirement for CaMKII in Zebrafish pituitary development, larvae were exposed to 10μM W-7 or KN-62 (calmodulin and CaMKII-selective inhibitors, respectively) for up to 72hpf. ISH for pituitary markers revealed that inhibition of calmodulin with W-7 dramatically reduced pituitary expression of *prl* but not *pomc*, indicating a role for CaMK's in regulation of prolactin expression in Zebrafish. In summary, expression profiling of CaMKII isoforms in human fetal pituitaries and in Zebrafish larvae appears tightly conserved, which may indicate an important role for these protein kinases in the appropriate development of anterior pituitary cell lineages and pituitary gene expression.

Sources of Research Support: Small Grants Program award from the *Society for Endocrinology*.

Nothing to Disclose: ACC, IRT, SEA, ST, RAA, ASM, IMM, RCF

P2-297

Dependence of the Pacemaking on the Background TRP Channel Activity in Spontaneously Firing Pituitary Cells.

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All secretory anterior pituitary cells fire action potentials spontaneously, but the channels involved in the pacemaking have not been identified. Here we show that replacement of bath sodium with organic cations, but not blockade of voltage-gated sodium influx, led to an instantaneous hyperpolarization of cell membranes that was associated with a cessation of spontaneous firing in gonadotrophs, lactotrophs, somatotrophs and immortalized lacto-somatotrophs. When cells were clamped at -50 mV, which was close to the resting membrane potential from which the slow depolarization and firing of action potentials occurs in these cells, replacement of bath sodium with organic cations resulted in an outward-like current, reflecting a decrease in the holding membrane current and indicating a loss of inward-depolarizing sodium conductance. In intact cells, replacement of bath sodium with organic cations also abolished voltage-gated calcium influx in lactotrophs and somatotrophs, as well as basal prolactin release, whereas blockade of voltage-gated sodium channels did not affect basal prolactin release. Lithium substituted for sodium in electrical activity and prolactin secretion. 2-APB, a blocker of TRP channels, inhibited basal prolactin release in a concentration-dependent manner, with an EC50 value of 38 μM. Two other blockers of these channels, FFA and SKF-96365, also inhibited spontaneous voltage-gated calcium influx and basal prolactin release in a concentration dependent manner. Quantitative RT-PCR analysis revealed the high expression of mRNA transcripts for TRPC1 in both normal and immortalized pituitary cells and low expression of TRPC6 in both cell types. These results raised the possibility that constitutive activity of TRPC channels accounts for the background sodium conductance and firing of calcium-dependent action potentials in endocrine pituitary cells.

Sources of Research Support: Intramural Research Program of the National Institute of Child Health and Human Development, NIH.

Nothing to Disclose: MK, KK, MT, SSS

P2-298

Cyclic Nucleotides and Pacemaking in Anterior Pituitary Cells.

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Previous studies indicate that spontaneous generation of action potentials in rat lactotrophs is independent of the adenylyl cyclase signaling pathway, but that cAMP facilitates pacemaking, voltage-gated calcium influx (VGCI), and prolactin release. Here we show that activation of adenylyl cyclases by forskolin also stimulates VGCI in gonadotrophs, somatotrophs and other unidentified rat and mouse pituitary cells. In order to clarify whether cAMP directly stimulates electrical activity, we initially studied the role of cyclic nucleotide-modulated CNG and HCN channels in forskolin action. Single cell electrophysiological experiments confirmed the expression of HCN but not CNG channels in gonadotrophs, lactotrophs, somatotrophs, and other unidentified anterior pituitary cell types. However, the basal adenylyl cyclase activity in pituitary cells in vitro was sufficient to maintain intracellular level of cAMP high enough to fully activate native HCN channels, indicating that forskolin-stimulated electrical activity was not directly mediated by cAMP. To study the role of protein kinase A (PKA) in this process more directly, we used two mouse models of altered PKA signaling: Prkar1a-/+ and Prkar1a-/+ plus Prkaca-/+. The stimulatory effect of forskolin on cAMP productions was preserved in pituitary cells from both animals (over 30-fold in response to 5 μM) and the cyclic nucleotide transported activity was not affected. In contrast to rat pituitary cells, soluble guanylyl cyclase was practically silent in normal and knockout mice and cGMP content was at the level of detection limits of RIA. The stimulatory effect of forskolin on VGCI in pituitary cells from Prkar1a-/+ animals was practically abolished but the forskolin effect was preserved in pituitary cells from Prkar1a-/+ plus Prkaca-/+ knockout mice. The ongoing experiments are focused on the status of PKA activity in unstimulated and forskolin-stimulated cells from Prkar1a-/+ and Prkar1a-/+ plus Prkaca-/+ mice and on identification of channels involved in forskolin-stimulated pacemaking.

Sources of Research Support: Intramural Research Program of the National Institute of Child Health and Human Development, NIH.

Nothing to Disclose: MT, KK, MK, MN, CAS, SSS

P2-299

Synergy between Activin and Progesterone on the Follicle-Stimulating Hormone Beta Promoter Requires Foxl2.

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Follicle-stimulating hormone (FSH) is critical for normal reproduction in mammals and is produced in the gonadotrope cells of the anterior pituitary. Synthesis of the beta subunit of FSH has been shown to be the rate limiting step in the production of the mature hormone. FSHβ gene expression is modulated by hormones including gonadotropin-releasing hormone, activin and steroids. Activin is a potent inducer of FSHβ transcription. We and others have shown that the gonadal steroid hormone, progesterone, also induces FSHβ mRNA levels. Moreover, we have shown that synergy between activin and progesterone occurs directly on the FSHβ promoter and requires Smad and steroid receptor signaling, as well as DNA binding to the FSHβ promoter. This synergistic interaction between activin and progesterone may play an important physiological role by assisting in the generation of the secondary FSH surge that occurs during the early morning of estrus in rodents.
In this study, we have used the immortalized LβT2 mouse gonadotrope cell line to further examine the synergistic action of activin and progestins on the murine FSHβ promoter. In our previous work, we identified two elements critical for activin and progestin responsiveness at -381 and -267 of the FSHβ promoter, respectively, that are also required for synergy between the two hormones. Mutations in additional elements within the proximal promoter indicate that progesterone responsive elements at 273, 230, 197, and 139, as well as activin responsive elements at -153 and 120, also play a role. Since the elements between -381 and -267 are most critical for the synergistic response, we undertook a systematic analysis of this region. Deletions in this region from -360/-341 and -320/-311 resulted in significant reductions in FSHβ after activin, progestin and co-treatment. Mutation of a recently characterized Foxl2 site at -350 also decreased induction of FSHβ transcription by activin, progestin and co-treatment. Additionally, overexpression of Foxl2 in LβT2 cells resulted in an enhancement of activin and progestin responsiveness, as well as the synergy, suggesting that Foxl2 plays an important role in the interaction between activin and progesterone signaling pathways.
In summary, our data show that the Foxl2 transcription factor is not only critical for activin responsiveness of the FSHβ promoter, but may also play an important role in progesterone responsiveness and the synergy between activin and progesterone.

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Nothing to Disclose: YG, JKS, PLM, VGT

P2-300

AMPK Is a Key Intermediary in GnRH- and Insulin-Stimulated LHb Gene Transcription.

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Pulsatile GnRH regulation of pituitary gonadotropin gene transcription is required for fertility, and pituitary responses are influenced in conditions such as polycystic ovarian disease by increased levels of androgens and insulin. We found that insulin (Ins) stimulates LHβ gene transcription in LβT2 gonadotrope cells, and significantly enhanced GnRH stimulation. To understand the mechanism for the Ins effects, we examined the role of intracellular signaling pathways. Previous work showed that rapid GnRH stimulation of JNK specifically favors LHβ transcription, and stimulation of CAMKII contributes to transcription of α, LHβ, and FSHβ. GnRH-stimulated ERK1/2 favors FSHβ, but is also important for transcription of Egr-1, an early response gene transcription factor critical for LHβ gene expression. Ins treatment (15min,1h or 24h) of LβT2 gonadotrope cells had no effect on the time course (15 min to 1h) or extent of GnRH-stimulated CAMKII, JNK, or ERK1/2 activity. In contrast, Ins alone significantly stimulated Akt activity, with no influence by GnRH. Because inhibition of Akt in Ins + GnRH-treated LβT2 cells had little effect on LHβ promoter activity, we examined activity of AMP-activated protein kinase (AMPK), an enzyme that acts as an energy monitor in several cell types. Ins alone (1h or 14h) resulted in a 2-fold rise in phosphorylated and thus activated AMPKα (Thr172), which correlated completely with increased levels of total AMPKα protein. In contrast, GnRH rapidly (5 min) stimulated AMPK activity by 5- to-10-fold and maintained 3-fold stimulated AMPK at 1 and 4h; no changes in total AMPKα protein were observed. Cotreatment with GnRH plus Ins resulted in sustained 3-fold stimulation of total AMPK activity for up to 4h. Treatment with the AMPK inhibitor Compound C completely prevented both Ins- and GnRH-stimulated LHβ promoter activity, and transcription of the endogenous LHβ gene in LβT2 cells, suggesting that AMPK activity plays a significant role in both Ins- and GnRH-stimulated transcription of LHβ. In support of this hypothesis, Compound C also completely prevented Ins- and GnRH stimulation of Egr-1, which is required for LHβ expression. GnRH also stimulated AMPK activity in normal mouse gonadotropes (6-fold at 5 min; 3-fold at 1 h), and Compound C inhibited GnRH-stimulated gene expression, suggesting that AMPK may be a key intermediary of GnRH-stimulated transcription in gonadotropes.

Sources of Research Support: NIH U54-HD-289934 SCCPRIR program.

Nothing to Disclose: JA, JQ, ALR, MAS

P2-301

Progesterone Induction of Murine Follicle-Stimulating Hormone ß Subunit Promoter Activity Is Impaired by Introduction of a Single Nucleotide Polymorphism Observed in Humans with Low FSH Levels.

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Perturbations in FSH secretion or action are associated with infertility. Causes of elevated or suppressed FSH levels are often idiopathic. Recently, variability in serum FSH in men was associated with a single nucleotide polymorphism (SNP) in the human FSHβ subunit (FSHB) promoter (-211 G/T). Serum FSH levels in men homozygous for the T/T genotype at position -211 (relative to the start of transcription) are approximately 50% lower than in men with the more common G/G genotype [1, 2]. We previously identified this SNP in a man with isolated FSH-deficiency [3]. As we also observed the T/T genotype in a fertile man, it alone does not explain FSH-deficiency. We therefore examined additional 5' flanking sequence and found a second SNP (-938 C/T), for which the FSH-deficient patient was homozygous (T/T). In a cohort of 90 samples, this SNP occurred with the following frequencies: C/C (24%), T/T (17%), and C/T (59%). Because the FSH-deficient patient failed to release FSH in response to GnRH stimulation, we asked whether the SNPs affected GnRH-induction of FSHB promoter activity. We previously reported that GnRH stimulates human FSHB promoter-reporters in immortalized murine gonadotrope cells, LβT2 [4]. We therefore transfected these cells with reporters possessing different combinations of the two SNPs, but observed no differences in the fold GnRH response. Activin A, though largely ineffective on its own, potentiates the GnRH response; but, this effect was also unmodified by the SNPs. The -211 G/T SNP occurs at a conserved position across several species. In mouse, the corresponding base-pair, G-195, is located in a well-defined progesterone response element [5]. We asked whether the -211 SNP might therefore affect progesterone induction. Murine, but not human, Fshb/FSHB promoter-reporters were induced by the progesterone analog, R5020, in the presence of over-expressed human or rat progesterone receptors (PR). Introduction of the G to T substitution at -195 in the murine reporter almost completely abrogated the stimulatory effect of R5020. We are currently assessing PR binding to the common and variant sites in human and mouse. Collectively, the data suggest that the G to T transversion at position -211 (-195 in mouse) is sufficient to modify promoter activity in a manner consistent with observed alterations in FSH secretion in humans. Whether it impairs progesterone induction or some other aspect of promoter function in humans has yet to be determined.

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Nothing to Disclose: YW, MB, LP, DJB

P2-302

Orexins (Hypocretins) A and B Modify Orexin 1 Receptor Expression and Gonadotropins Secretion in Anterior Pituitary Cells of Proestrous Rats.

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Orexins A (OXA) and B (OXB) are neuropeptides controlling feeding, sleep, autonomic and neuroendocrine functions. Both are synthesized by neurons of the lateral hypothalamus with projections throughout the brain. They exert their actions interacting with orexin receptors 1 (OX1) and 2 (OX2). In previous works *in vivo,* we demonstrated that in the physiological neuroendocrine condition of proestrus leading to ovulation, information of the orexinergic system acts on the anterior pituitary (1, 2).
Here, we investigate whether OXA and OXB are involved in the modulation of the OX1 receptor expression at the mRNA level, and the effect on FSH and LH secretion, in an *in vitro* system using adenohypophyseal cell from proestrous rats. Regular cycling Sprague-Dawley rats were sacrificed in the morning of proestrus, a critical period according to our previous results (1, 3) and pituitary cell cultures done as described (4). After 72 h of incubations with OXA (10-9M) or OXB (10-9M) in absence (OXA-Ab or OXB-Ab), or presence (OXA-Pr or OXB-Pr) of the selective OX1 antagonist SB334867A (1mM, Tocris Bioscience; MO), cells were obtained and OX1 and OX2 receptors was determined by real-time RT-PCR as in (1, 3). Medium LH and FSH were determined by RIA (NHPP, NIDDK). Results were expressed as means ± SE and considered significant when $p<0.05$. Data were analyzed by one-way analysis of variance.
OXA decreased the expression of OX1 in absence or presence of SB334867A [C, control medium only, Arbitrary Units: 1 (n: 6) vs. OXA-Ab: 0.692±0.092 (n: 5); OXA-Pr: 0.706±0.046 (n: 6); $p<0.05$] and increased gonadotropins in medium, while SB334867A blocked this hormonal effect [LH (ng/ml); C: 19.04±1.86 (n: 7); OXA-Ab: 33.73±2.74 (n: 8); OXA-Pr: 20.38±1.59 (n: 8), $p<0.05$; FSH (ng/ml): C: 73.37±4.62 (n: 8) ; OXA-Ab: 146.67±17.54 (n: 7); OXA-Pr: 85.14±5.90 (n: 8); $p<0.05$]. OXB produced similar actions to OXA, i.e., decreased OX1 expression and increased LH and FSH release, while SB334867A blocked hormone release but not OX1 expression. SB334867A by itself did not alter the expression of OX1 or any hormonal parameter.
Both orexins decreased expression of the OX1 receptor in pituitary cell cultures while increasing gonadotropins release, this hormonal effect was blocked by SB334867A. The present *in vitro* results confirm previous *in vivo* studies about the participation of orexins on pituitary reproductive function, showing a direct effect of both neuropeptides on the gland.

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Nothing to Disclose: NIC, VAL-L, CL

P2-303

Somatostatin Subtype 2 Receptor (sst2) Overexpression in Gonadotroph Mice Cell Line: Characterization and Effect on Cell Proliferation.

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Introduction. Somatostatin receptor subtype 2 (sst2) is the main receptor mediating somatostatin (SST) action in most tissues and tumors. Gonadotroph adenomas express low levels of sst2, explaining limited efficacy of somatostatin analogs in controlling tumor growth. LbT2 mouse gonadotroph cell line express also low levels of sst2 and also dopamine receptor D2. We recently showed that sst2 overexpression in somato- and lactotroph adenoma cells was able to rescue the octreotide sensitivity of resistant tumors.
Material and methods:Stable expressing hsst2-GFP Lbt2 (hsst2_L) and control GFP Lbt2 (GFP_L) cell lines were obtained by lentiviral infection. Lentiviral infection efficiency was assessed by FACS (% fluorescent cells), confocal microscopy and real-time RTPCR (sst2). We compared mRNA expression for endogenous msst2, mD2DR, bLH, bFSH and Alpha subunit (alphaSU). Effect on cell proliferation was assessed by cell count and 3H thymidine incorporation.
Results: FACS analysis showed that more than 90 % of hsst2_L and GFP_L cells were efficiently infected. Immunohystochemistry showed an enhanced sst2 expression on hsst2_L cells as compared to control wild type LBT2 (WT_L) and GFP_L cells. QPCR showed that sst2 mRNA in sst2_L cells was ten times higher than in control cells (WT_L and GFP_L). Specific msst2 and mD2DR probes showed a similar endogenous mRNA expression for sst2_L and GFP_L cells. In basal condition, mRNA for aSU of glycoproteic hormones was significantly lower (ten times) in sst2_L cells than I control cells (WT_L and GFP_L). The follow-up of cell proliferation showed at 7 days a 50 % decrease in sst2_L cells.
Conclusion: Sst2 moderate overexpression in a gonadotroph cell line is associated with lower proliferation and decreased SUalpha mRNA expression suggesting an intrinsic activity of sst2 receptor. Characterization of pathways involved in this effect is underway.

Nothing to Disclose: TC, AM, CR, ST, RR, AE, TB, AB, AS

P2-304

GH Pulse Generation Involves Temporal Regulation of Oxygen Supply and Consumption In Vivo.

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Pituitary endocrine cells receive blood-borne stimuli, and deliver their hormonal outputs via a complex network of capillaries that pervade the gland. The pattern of release of many hormones, such as growth hormone (GH), is an important signal regulating the response of peripheral tissues. To understand how GH pulses are built-up in vivo, the close link between blood supply, metabolic requirements and functional activities of the GH cell network must be taken into account (1). Although O2 supply by portal vessels is essential for pituitary function, practically nothing is known about how incoming O2 is distributed within the gland and whether O2 intake and consumption are temporally regulated during hormone secretion. Recently, we have developed approaches to measure local blood flow, O2 partial pressure and cell activity at single-cell resolution in mouse pituitary glands in vivo (2). Here, we describe the relationship between GH-cell activity and O2 tension.
Network-driven GH responses, triggered by a GHRH bolus, were associated with both coordinated increases in blood flow and GH pulse generation in vivo. Similar, but inversely related bursts of O2 consumption (downward Ptiss,O2 deflections) occurred in acute pituitary slices stimulated with GHRH, and this was dependent on extracellular Ca2+. Both GHRH-induced bursts of O2 consumption and co-ordinated Ca2+ spiking activity (1) recurred with a similar frequency (~20 min). In vivo, GHRH-induced episodes of O2 consumption are likely compensated by O2 supplies from neighbouring capillaries since downward Ptiss,O2 deflections were much smaller and mixed with upward Ptiss,O2 deflections. Altogether, these data suggest that the GHRH response in vivo involves recurrent episodes of GH network cell activity, which are associated with periods of both O2 consumption by stimulated GH cell network motifs and enhanced O2 supply from neighbouring capillaries. These studies highlight the importance of the vascular microarchitecture and flow for the timing and delivery of input O2 that is required for the generation of GH pulses. Importantly, the cellular in vivo imaging techniques described hitherto can be used to investigate how both O2 supply and consumption are regulated in different pituitary cell types, not only during periods of normal physiological demand (e.g. the pre-ovulatory LH surge), but also when the endocrine tissue and microvasculature are altered due to pathology (e.g. tumours).

(1) Bonnefont X et al, PNAS 2005; 102:16880
(2) Lafont C et al., PNAS 2010; in press

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Nothing to Disclose: CL, TF, MD, DH, DC, PLT, ICR, PM

P2-305

Apoptosis of Lactotrophs Induced by Dopamine Is Mediated by the Short Isoform of D2 Receptor.

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Dopamine, by its interaction with D2 receptors in the pituitary gland, is the main regulator of lactotrophs function. We have described that dopamine induces apoptosis of lactotrophs in an estrogen-dependent manner. Also, we have observed in an in vivo model that cabergoline, a D2 receptor agonist, induces apoptosis in anterior pituitary gland of ovariectomized rats only when they were treated with estradiol. D2 receptor has two isoforms, long (D2L) and short (D2S). It has been demonstrated that both isoforms are expressed in lactotrophs. Although they have a similar pharmacological profile, both isoforms can interact with different Gi proteins, and differ in the mechanism that leads to MAPK activation. The aim of this work was to study the role of D2 receptor isoforms in the apoptosis of lactotrophs and the participation of p38 MAPK in the proapoptotic action of dopamine. Lactotroph-derived PR1 cells transfected with an empty expression vector, or with vectors containing cDNA encoding D2L or D2S receptors, were incubated with or without estradiol (10-9 M) for 48 h and with dopamine (10-6 M) for further 4 h. Apoptosis was determined by the quantitation of nucleosomes in an ELISA assay and by the TUNEL method. Dopamine, in the presence of estradiol, induced apoptosis only in cells expressing the D2S receptor. To study the role of p38 MAPK in the apoptotic action of dopamine, the cells were incubated in the presence of an inhibitor of p38 MAPK pathway (SB203850). SB203850 decreased dopamine-induced apoptosis in D2S expressing cells. Additionally, western blot analysis of the phosphorylated form of p38 MAPK indicates the activation of this MAPK by dopamine.
These data suggest that, in the presence of estradiol, dopamine induces apoptosis of lactotrophs by its interaction with D2S receptors. In addition, the p38 MAPK pathway may be involved in the proapoptotic action of dopamine.

Sources of Research Support: NIH R01 AA11591 grant (DKS) and ANPCyT (PICT 2006-092), Argentina (DP).

Nothing to Disclose: DR, JF, VB, AS, DKS, DP

P2-306

Effect of Thyroid Hormone and Dexamethasone on Human Growth Hormone Gene Expression in Primary Pituitary Cell Cultures from Transgenic Mice.

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Growth hormone (GH) production is regulated by both pituitary and hypothalamic factors. However, peripheral endocrine factors such as thyroid hormone/triiodothyronine (T3) and glucocorticoids (GLUC) can also regulate GH expression and contribute to effects at multiple levels on metabolism, growth and development. Studies on the regulation of human (h) GH have been limited, at least in part by species differences between GH genes and their response, in terms of production, to hormone treatment as, for example, in cases of hyperthyroidism or excess GLUC. Also, most in vitro studies of hGH-N gene regulation by T3 and/or GLUC, have been done with tumor cells without or with transfection with truncated hGH-N gene fragments and <1 kb of upstream flanking DNA. This is important as sequences located up to 30 kb upstream are required for appropriate pituitary-specific expression of hGH in vivo. Thus, transgenic (171hGH/CS-TG) mice were generated containing the intact hGH-N gene and locus control region in a 171 kb fragment of human chromosome 17. Pituitary expression of hGH was confirmed by RNA and protein analyses. Here, we have generated primary pituitary cell cultures from adult 171hGH/CS-TG mice and confirmed the presence of somatotrophs by assessing both mouse (m) GH and hGH expression by real time PCR (qPCR) and immunohistochemistry. Cultures were maintained for three days, grown in the absence of serum for one day and then treated without or with 0.1-10 nM T3 or 200 nM dexamethosone (DEX) in the absence of serum for two days, before isolation of RNA or (cellular) protein. In contrast to a lack of effect on mGH RNA, human GH RNA levels were decreased significantly (45%, n=6, $p<0.01$) by T3 in a dose-dependent manner as assessed by qPCR. In contrast, treatment with DEX increased hGH and mGH RNA levels 4.5 and 3.2 fold, respectively (n=6, $p<0.001$). This response to DEX treatment was muted significantly in the presence of 1 nM T3 for hGH (30%, n=6, $p<0.001$) but not mGH RNA levels. Further characterization is underway, however, the acute effects of T3 and DEX on hGH-N gene expression are consistent with previous studies using fragments of the hGH-N gene transfected into rat pituitary tumor cells. These observations suggest that 171hGH/CS-TG mice will provide a useful model as well as additional tools such as primary cell cultures, to investigate expression, regulation and function of hGH in a cellular, physiological and pathophysiological context.

Sources of Research Support: CIHR (MT-10853).

Nothing to Disclose: HV, YJ, JIN, PAC

P2-307

Characterization of Lactotropes Intracellular Calcium Response to TRH and DA in Acute Pituitary Slices from Mouse.

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Hypothalamic TRH and/or DA triggers or inhibit lactotrophs to synthesize and secrete prolactin, hormone that has an important role in lactogenic activity, lipidic metabolism and some effects in immune system. Different doses of TRH produce characteristics signalization patterns of intracellular calcium [Ca2+]i. It has been propose before that lactotrophs response to stimuli in an heterogeneous way, this variation depends on its localization in the pituitary. However, these studies were done in primary culture conditions, where the cells lose their interaction with others and their natural physiologic environment. In this work we use acute pituitary slices from adult male mouse and intracellular Ca2+ imaging to examine the spatial distribution of lactotrophs and their Ca2+ signaling patterns elicited by different TRH concentrations and dopamine.
Adult male Balb-C mouse were euthanized. Pituitary gland was removed and embedded in agar 3% and coronal slices of 130 µm of thickness were obtained. Slices were incubated with fluo-4 AM (22 µM for 30 min at room temperature and perfused (2 ml/ min)) with saline saturated with 95% O2 and 5% CO2. Image sequences at 510 nm emission were obtained (300 ms exposure; 250 ms interval) with a stereomicroscope (Leica MZ 16 Fluo Combi IIII) and cooled CCD. TRH was bath-applied for 30s at concentrations 0.01 nM to 100 nM, and washed for > 15 min between applications finally, separately dopamine 2 µM and high potassium solution were applied.
The responsive cells to TRH and DA were counted in two different regions, the central region and lateral region of the gland and their patterns of response of [Ca2+]I were plotted and analyze individually.
With this protocol we found that in the central region like in the lateral region of the pituitary gland, the number of responding cells increases proportionally to doses of TRH, being major at 100 nM. Moreover, the number of cells with [Ca2+]i activity is minor in central region in comparison with the lateral region, but the number of lactotrophs responsive to TRH and DA normalized to total alive cell is mayor in the central region (36.26% and 17% respectively). Finally, we found that the lactotrophs [Ca2+]I response produced by TRH present higher amplitude in cells in the central region of hyphophysis. These results suggest possible patterns of functional lactotrophs regionalization.

Nothing to Disclose: LMR, AH, TF

P2-308

Fish and Chicks: C-Type Natriuretic Peptide and the Development of the Pituitary Gland in *Gallus Gallus* and *Danio Rerio*.

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Mammalian pituitary gland development is well established on a molecular and morphological level. In developmental biology the use of chick and zebrafish models is common but despite chicken pituitary gland development being characterised in 1952 these models have only recently been regularly employed in pituitary studies. The third member of the natriuretic peptide family, C-type natriuretic peptide (CNP), has been investigated in rodent embryos but little is known about its role in chick and zebrafish development. In our study we have used *Gallus gallus* and *Danio rerio* embryos to investigate the role of CNP in pituitary gland development. RNA in situ hybridisation probes were generated to detect expression of chick CNP3 gene and zebrafish cnp3 and Nppc-like (nppcl) genes. The chick CNP3 probe was synthesised from a CNP3 EST (Univ. Manchester Chick EST Database). Primers were designed against zebrafish cnp3 and nppcl. RT-PCR was carried out on whole mixed-age zebrafish cDNA and the PCR products purified and cloned using pGEM-T Easy vector. All plasmids were linearised, reverse transcribed and labelled with digoxigenin for detection with anti-digoxigenin antibody during in situ hybridisation. We have detected expression of CNP3 at days 4, 5 and 6 of *Gallus gallus* development (Hamburger Hamilton stages 24-29), which was especially marked at day 4 in the developing chick pituitary gland. In *Danio rerio* cnp3 expression was detected from 48 hours post fertilisation (hpf) in the brain and presumptive pituitary and hypothalamus, where pomc and prl expression were used as markers for the presumptive hypothalamus and pituitary. Expression of nppcl was widespread in zebrafish from 24hpf but became more specific along the midline of the head up to 72hpf, where marked expression was observed in the presumptive pineal gland. Our findings suggest CNP may be important in the crucial stages before and after Rathke's pouch formation in the chick. In zebrafish CNP appears to be important after internalisation of the pituitary and during the later stages of pituitary patterning.

Sources of Research Support: Project Grant from the BBSRC, and PhD studentships from the Royal Veterinary College and the Anatomical Society of Great Britain & Ireland.

Nothing to Disclose: ANC, SEA, IMM, RCF

P2-309

The Role of the Forkhead Transcription Factor, FOXO1, in Pituitary Gland Development.

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Congenital hormone deficiencies are common, occurring in approximately one in 4,000 live births. Pituitary hormone deficiency can consist of loss of a single hormone (isolated hormone deficiency) or several hormones (combined pituitary hormone deficiency). Absence of anterior pituitary hormones does not interfere with fetal viability, but are required for survival after birth, gonadal differentiation, and maturation of the fetal thyroid. Lesions in the transcription factors PITX1, PITX2, HESX1, LHX3, LHX4, TPIT, PROP1 and PIT1 lead to combined pituitary hormone deficiency in mice and humans. However mutations in these transcription factors account for only a fraction of congenital hormone deficiencies in humans. To identify additional factors that contribute to human congenital hormone deficiencies, we are investigating the forkhead transcription factor, FOXO1, which is important for the normal development of several organs. We find that FOXO1 is present in nuclei of non-dividing pituitary cells, suggesting that it may inhibit cell proliferation. We also find that FOXO1 is present in a fraction of differentiated anterior pituitary cells. FOXO1 is expressed in approximately 1) four-fifth of somatotrope cells 2) three-fourth of gonadotrope cells 3) two-fifth of thyrotrope cells in e18.5 and adult mice pituitary. FOXO1 is expressed in one-third of corticotrope cells at e18.5 and half of adult corticotrope cells. These data suggest that FOXO1 is not specifically required for differentiation of any one cell type, but rather is important for regulating cell cycle progression in pituitary cells regardless of cell type. We are currently investigating the phenotype of the developing mouse pituitary in the absence of FOXO1. Mouse knockout models for Foxo1 result in embryonic lethality at embryonic day (e)10.5 due to vascular defects. To circumvent this problem, we have eliminated FOXO1 specifically in the pituitary gland. Conditional knockout will allow investigate role of Foxo1 in the pituitary without confounding effects of loss of Foxo1 in hypothalamus or surrounding mesenchyme. These studies are critical to determine how loss of FOXO1 affects pituitary gland development. In summary the expression patterns of FOXO1 suggest that it may play a role in preventing cell proliferation of pituitary cells during development.

Sources of Research Support: SIU-SOM-Start Up; SIU-Central Research Committee Faculty Seed Grant.

Nothing to Disclose: SM, CLF, DOJ, BSE

P2-310

Differentiation Capacity of Folliculo-Stellate Cells: A Candidate of Stem Cells in the Anterior Pituitary Gland.

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Folliculo-stellate (FS) cell is a non-endocrine cell producing S100b protein in the anterior pituitary gland. Several functions have been proposed for FS cell. FS cells have lysosomes in their cytoplasm and act as a scavenger cell. In addition, FS cells are known to secrete some cytokines and support neighboring endocrine cells through those cytokines. Intercellular communication through their gap junctions is also noted. However, lots of the biological characteristics of FS cells are still under discussion. For development of new approach to understand the FS cells, we generated a transgenic rat (S100bTg rat) expressing GFP under S100b gene promoter specifically. Using the transgenic rat, we showed the wide differentiation capacity of FS cells.
Firstly, we confirmed a classical differentiation capacity of FS cells, which was that FS cells might differentiate into skeletal muscle cells. It had not been proved with cultured FS cells from the pituitary gland because the possibility was found by the observation of grafted pituitary which had skeletal muscle unexpectedly and by the induction of FS cell-like cell line. In this study, we succeeded to induce skeletal muscle cells from cultured GFP positive FS cells from S100bTg rats, whereas GFP negative cells did not show the differentiation. It suggests the FS cell showed trans-differentiation which is considered the characteristics of organ specific stem cells. Moreover, the capacity to differentiate into another cell was limited to FS cells in the anterior pituitary gland.
Thus we assumed that FS cells could be a stem cell providing endocrine cells and FS cells in the anterior pituitary gland. To validate this hypothesis, we cultured anterior pituitary cells including GFP positive FS cells in the medium containing retinoic acid (RA). RA is known a factor inducing endocrine cells from pituitary progenitor cells. Finally, we found GFP positive FS cells divided asymmetrically and one of daughter cells was Pit1 (pituitary endocrine cell specific transcription factor) protein or/and hormones positive cells. Our present finding suggests that some population of FS cells may be a pituitary specific stem cell.

Nothing to Disclose: MO, YS, KI, YK

P2-311

Thyroid Hormone Regulates Pituitary Development in the Zebrafish Embryo.

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Thyroid hormone (TH) acts as a potent morphogen during metamorphosis in frogs and fish, regulating cell differentiation and cell proliferation. Similar roles for TH during embryonic development have recently been suggested in mammals, but how and where TH acts in the embryo is poorly understood. The zebrafish provides a powerful system for the study of embryonic roles for TH, as hormone levels and function can be easily manipulated in the absence of a blood-placental barrier. Since essential components of the thyroid axis have largely been conserved across vertebrates, studies in zebrafish promise to shed light on basic mechanisms of TH action in the embryo that may also be involved in human congenital diseases.

TH is present in the yolk sack of fish embryos from the beginning of embryogenesis, well before endogenous production from the thyroid gland. Given this abundant source of TH, regulation of TH signaling in target tissues is likely to occur via expression of Diodinase 2 (Dio2), the primary enzyme needed to convert T4 into the more potent form, T3. We found that Dio2 is expressed in a small subset of cells very early in the developing pituitary gland, starting at 31-32 hours post fertilization (hpf), a time when endocrine cells are differentiating and the pituitary is becoming functionally patterned. This population of Dio2 expressing cells in the placode approximately triples by 70-76 hpf. This regional expression suggests a role for TH signaling in very early pituitary differentiation and/or function. To begin to test this we treated embryos with L-thyroxin (T4). We found a dose dependent decrease in the expression of Thyroid Stimulating Hormone (TSH) in thyrotropes with increasing levels of T4. TSH cell numbers remain normal, indicating TH signaling may specifically regulate production of TSH in the early embryo. Dio2 expression was also reduced, consistent with the known negative regulation of this gene by TH.

Our results indicate that T4 acts a negative regulator of TSH expression prior to the onset of TH production in the thyroid and well before metamorphosis in zebrafish. This is the earliest report of negative feedback regulation in the pituitary- thyroid axis. Since functional immaturity of the thyroid axis is a common problem in human infants, especially those born prematurely, these data promise to shed light on the mechanisms of negative feedback system maturation within the developing thyroid axis.

Nothing to Disclose: KNT, ROK

P2-312

HES1 as a Possible Digenic Cause of Hypopituitarism in Two Siblings Harboring a Heterozygous C.301,302AG Deletion in *PROP1*.

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Mutations in *PROP1* (Prophet of *PIT1*) are the most common genetic cause of combined pituitary hormone deficiency (CPHD). This is a recessive disorder and patients are homozygous or compound heterozygous for inactivating mutations. We screened 53 patients with CPHD for *PROP1* mutations: One patient was homozygous for p.F88S, nine patients were homozygous for c.301,302delAG and two siblings heterozygous for c.301,302delAG. In these two latter cases, we also ruled out the presence of mutations in the three highly conserved regions located at the proximal promoter, in the first intron and at the C terminal region of *PROP1*, important for transcription. *Prop1* and *Hes1* (Hairy and enhancer of split 1), a direct target of Notch signaling pathway, are both required for mice pituitary development and have overlapping functions. In addition, double mutant of *Hes1* and *Prop1* mice (*Hes1*$^{-/-}$ and *Prop1* $^{df/+}$) presented eutopic posterior lobe besides small pituitary and a hormonal pattern similar to *Prop1* deficient mice. In front of this, we hypothesized if *HES1* mutation could explain the phenotype of these siblings. **Objective**: To screened *Hes1* gene in CPHD patients heterozygous for *PROP1* mutation. **Methods**: Genomic DNA was extracted from peripheral blood and amplified by PCR using *HES1* primers designed to cover exons 1 to 4. **Results**: No mutations in *HES1* were found. **Conclusion**: *HES1* in not involved as a digenic cause of CPHD in two siblings heterozygous for a *PROP1* mutation.

Nothing to Disclose: APO, LR, MYN, IJPA, BBM, LRC

P2-313

FOXM1 Is Expressed in Actively Proliferating Cells during Pituitary Gland Development.

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FOXM1 is a member of the forkhead family of transcription factors, which are “winged-helix” proteins. FOXM1 is transcribed into three different isoforms: FOXM1a, FOXM1b, and FOXM1c. FOXM1b and FOXM1c are transcriptionally active, while FOXM1a is inactive, and may serve as a dominant negative form. The chief role of FOXM1 is to regulate the cell cycle and mitosis. FOXM1 acts on 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. FOXM1 is necessary for cell proliferation associated with organ development. The central goal of our studies is to understand the molecular mechanisms that govern pituitary gland development. We have found that FOXM1 co-localizes with proliferation markers such as BrdU in mouse embryos at ages e12.5 through e16.5 as well as a subset of cells that express Ki67 at ages e14.5 and e16.5, but not with the cell cycle inhibitor p57. We have acquired Foxm1b knockout mice in order to address the role of FOXM1 during pituitary gland development. These studies are critical to determine whether loss of FOXM1 causes pituitary hormone deficiency as a result of pituitary hypoplasia.

Sources of Research Support: SIU-SOM start up; SIU Central Research Committee Faculty Seed Grant.

Nothing to Disclose: AGP, BSE

P2-314

Characterisation of a Reference Preparation of Indian Water Buffalo Pituitary Glycoprotein Reproductive Hormones.

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Water buffalo is the target for herd improvement by multiple ovulation and embryo transfer technology (MOET) in India. However the details of its endocrinology and reproductive physiology have not been yet completely described. Pituitary hormones from this species have earlier been purified and characterized in fairly good detail in our laboratory. In the initial phase of MOET application, the importance of using homologous hormones was not realised. In addition, buffalo is notorious for its sluggish response to exogenously administered gonadotropins, thus complicating the practice of MOET even further. The success of this MOET protocol in buffaloes is not impressive unlike in cross breed cows. Suspecting this to be due to defects in thyroid status, we thought of obtaining buffalo pituitary hormones in a single preparation which contains all the three hormones i.e. Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Thyroid Stimulating Hormone (TSH). Such a preparation was obtained by isolating the three hormones from a crude pituitary extract by the use of Concanavalin A (Con-A) sepharose as all of them are known to be glycoproteins. The yield of such a preparation was 20 mg protein/g of pituitary tissue. The preparation was further assessed in bio and immunoassays. The preparation had 114 IU of FSH, 769 IU of LH and 6 IU of TSH activity per mg of protein as assessed by *in-vivo* Steelman-Pohley assay using 23-day old female Holtzman strain rats for FSH activity, *in-vivo* ventral prostate assay using 23-day old male Holtzman rats for LH activity, and *in-vitro* cAMP response in homologous buffalo thyroid tissue for TSH activity. Reference preparations of bovine TSH, buffalo LH, human Chorionic Gonadotropin (hCG) and human Menopausal Gonadotropin (hMG) were used as calibration standards. Thus a single characterized enriched preparation of buffalo pituitary FSH, LH and TSH has been obtained and is reported here for the first time. Till today veterinarians have used PMSG for inducing super ovulation in buffaloes. Our preparation will provide FSH and LH replacing PMSG and in addition provide TSH, thus ensuring euthyroid status. In addition it was observed that buLH was more active in buffalo *in-vitro* than in rats. The prime advantage with our preparation therefore, is that it is a homologous hormone mixture. The molecular features in terms of glycan microheterogeneity and the shelf-life are being investigated and will be reported.

Nothing to Disclose: NV, TA, MK

P2-315
Dynamic Imaging of Endocrine Cell Networks.

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Endocrine glands are composed of tightly packed cells responsible for the coordinated generation of hormone pulses into the blood system. Although evidence has been provided that endocrine cells could be organized in distinct networks [1], few studies have been focused on endocrine cell network plasticity and cellular dynamics in response to physiological changes [2]. Recently, the use of two-photon microscopy, together with the development of genetically encoded fluorescent reporters, has made it possible to examine the behavior of cells within living tissues [3]. This approach, already extensively used to study cellular dynamics in the field of immunology, has only recently been applied to the study of endocrine systems. Some recent imaging studies have allowed the visualization of dynamic processes during morphogenesis [4-5], but little is know about endocrine cell network plasticity in the adult. Here, we describe the advantages of in-situ and intravital 2-photon microscopy in the identification of novel mechanisms involved in endocrine cell network remodeling, using two examples with contrasting etiologies: (1) somatotroph plasticity in the pituitary gland following perturbation of steroid hormone balance in adult GH-GFP transgenic mice, and (2) pancreatic islet degeneration following the induction of type I diabetes by multiple low dose streptozotocin treatment.

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Nothing to Disclose: MS, ACM, CL, EG, DC, PLT, IR, PM

P2-316

Rosai-Dorfman Disease Involving the Neurohypophysis and Thyroid.

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Background: Rosai-Dorfman disease is a rare, histiocytic proliferative disorder of unknown etiology commonly affecting lymph nodes. Morphologically, it is characterized by proliferation of foamy histiocytes, lymphocytes and emperipolesis.
Clinical case: A 63 year-old woman presented with 6 weeks history of ataxia, diarrhea, weight loss and severe abdominal pain. Laparotomy revealed a thickening of the intestinal mucosa. Histology demonstrated histiocytic proliferation in the submucosa. Laboratory examination revealed hypoalbuminemia, elevated ALP, GGT, and urea. TSH: 5.37 (0.40-5.50mU/L), Cortisol: 441 (193-690 nmol/L). No evidence of diabetes insipidus.
Because MRI documented dural thickening and enhancement, dural biopsy was performed. Light microscopy revealed massive cellular infiltration adjacent to and involving the dura. Histiocytic proliferation was extensive, accompanied by lymphocytes and plasma cells. CD-3 immunopositive T-lymphocytes were more numerous than CD-20 immunopositive B-lymphocytes. Immunohistochemistry demonstrated many CD-68 immunopositive histiocytes with foamy cytoplasm containing lymphocytes in their cytoplasm, a process known as emperipolesis. Diagnosis was consistent with Rosai-Dorfman disease. The patient's condition deteriorated and she died. Autopsy showed extensive histiocytic-lymphocytic proliferation and emperipolesis in the lungs, spleen, thoracic lymph nodes, thyroid, abdominal and retroperitoneal fat and bowel wall, the latter considered as a source of sepsis causing death.
Histologic examination of the pituitary revealed several large, well demarcated areas in the neurohypophysis replacing the normal structure of the posterior lobe spreading to the pars tuberalis, the lower portion of the pituitary stalk and the adjacent dura. In the adenohypophysis, histiocytic-lymphocytic proliferation was absent indicating that the Rosai-Dorfman disease did not involve the adenohypophysis. PRL immunopositive cells were numerous (52%; normal autopsy pituitary: 10-30%). Immunopositivity was localized mainly in the perinuclearly located conspicuous Golgi zone indicating active secretion. Compression of the pituitary stalk by the proliferating histiocytes and lymphocytes, the so called “stalk section effect” may explain the development of PRL cell hyperplasia.
Conclusion: This is a rare case demonstrating involvement of the neurohypophysis and thyroid by Rosai-Dorfman disease associated with adenohypophysial PRL cell hyperplasia.

Nothing to Disclose: FR, DGM, RGH, BG, NK, MB, KK

P2-317

First Description of Carney Complex (CNC Type 1) with ACTH-Dependent Cushing´s Syndrome (CS) Due to a Pituitary Adenoma.

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Background: CNC Type 1 is an autosomal dominantly inherited, multiple neoplasia syndrome characterized by the existence of multiple endocrine tumors, cardiac myoxomas and typical skin manifestations, first described by J. Aidan Carney in 1985. Endocrine tumors are frequent and often synchronous in patients with CNC. CS frequently occurs in CNC patients and is due to primary pigmented nodular adrenocortical disease (PPNAD).
Clinical case: A 4-year-old boy underwent removal of the left testicle due to a Leydig cell tumor. Twenty years later a pilonidal sinus was operated. In the following year the patient experienced a syncope when playing soccer. Echocardiography revealed a large atrial myxoma protruding in the aorta which was removed. In the same year severe osteoporosis, and one year later bilateral avascular necrosis of the femoral head were diagnosed. About this time hypogonadotropic hypogonadism was first noted and treated with i.m. testosterone. Two years later (at the age of 26) multiple nevi were removed without malignancy. With 32 years he was admitted to a hospital to further investigate a bilateral protrusion of his eye balls. During this stay CS was diagnosed and Carney complex suspected. Neither MRI of the pituitary nor adrenal CT showed an adenoma. For further workup he was transferred to our department. Basal ACTH was elevated (64 pg/ml, normal < 46 pg/ml) and cortisol could neither be suppressed nor stimulated in the Liddle test (4 x 0.5 mg Dexamethasone for 2 day, then 4 x 2 mg for other 2 days). Repeat MRI showed a 6 x 4 mm pituitary adenoma. Petrosal venous sinus catheterisation confirmed the central origin of ACTH and showed stimulation of ACTH during CRF testing. Following removal of the adenoma (immune-histologically expressing ACTH) the patient was cured from his Cushings disease. Molecular genetic analysis confirmed the presence of Carney complex Typ 1 (CNC1) with a stop mutation in the PRKAR1A gene.
Conclusion: This is the first report of Cushings disease in a patient with Carney complex. Patients with this syndrome usually harbour PPNAD as cause of Cushings syndrome, and GH- and PRL- (but not ACTH-) producing pituitary adenomas.

Nothing to Disclose: AG, MK, AL, YW

P2-318

Lack of Mutations in the Gene Coding for the Glucocorticoid Receptor (NR3C1) in a Pediatric Patient with an ACTH-Secreting Pituitary Adenoma, Absence of Stigmata of Cushing Syndrome, and Unusual Histologic Features.

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Background:
Loss of heterozygosity at the glucocorticoid receptor (hGRα) gene (NR3C1) locus has been seen in pituitary adenomas of patients with Cushing disease (CD); rare cases of NR3C1 mutations have also been described in the gemline or somatic state in CD. We describe a pediatric patient with CD, clinical evidence of glucocorticoid resistance (GR) and absence of stigmata of Cushing syndrome (CS), who, however, did not have any evidence of mutations in the hGRα gene (NR3C1).
Clinical Case:
A 14-year-old boy with stunted growth and hypertension but no other signs of CS (i.e. no weight gain, no striae, no facial plethora) was admitted for evaluation of CD. Pituitaty MRI revealed a 3 x 4 millmeter hypoenhancing lesion in the right side of the pituitary gland anteriorly (microadenoma). Urinary free cortisol levels (UFCs) were extremely elevated (100-129 mcg/24 hours, normal range 4 - 56 mcg/24-hours). In addition, diurnal variation of ACTH and cortisol levels was absent. A graded dexamethasone suppression test indicated that the patient had partial GR. Histology confirmed an ACTH-producing pituitary adenoma, characterized by proliferation of anterior lobe cells of similar morphology with reticulin breakdown and strong diffuse ACTH staining. We hypothesized that a NR3C1 mutation was present in the patient's peripheral DNA. However, sequencing studies of the entire coding region of the gene produced normal results. Unfortunately, no pituitary tissue was available for genetic studies; currently efforts focus on the study of paraffin-embedded tissue.
Conclusion:
We conclude with the presentation of a case of a pediatric patient with little evidence of CS, despite the unequivocal presence of an aggressive ACTH-producing tumor and very high UFCs. This patient did not have peripheral DNA NR3C1 coding sequence mutations; possible explanations for this phenotype include somatic mosaicism for NR3C1 mutations or a mutation in another molecule that participated in hGRα-signaling.

Nothing to Disclose: GB, AH, PC, MBL, PX, MA, MK, EL, MQ, NP, CAS

P2-319

An Unusual Presentation of Cushing's Disease: A Recurrent ACTH-Producing Adenoma Located in the Posterior Pituitary Lobe.

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Cushing's syndrome is rare in childhood and adolescence, and represents a diagnostic and therapeutic challenge. The most frequent cause of CS in childhood is Cushing disease (CD), caused by ACTH-producing pituitary tumors or corticotropinomas. Rarely, these tumors can be located in the posterior lobe of the pituitary gland (1). We report one of the first such cases in the pediatric population: a 15-year-old girl with a recurrent corticotropinoma. The tumor was not completely excised in the first surgery because it was located proximal to the posterior lobe of the pituitary gland: at the age of 11, she first presented with classic symptoms of CS. The biochemical investigation was compatible with CD but extensive pituitary imaging was negative. At transsphenoidal surgery, a 2-3 mm lesion was identified in the middle of the gland and within the posterior lobe. Histopathological analysis confirmed an ACTH-secreting pituitary microadenoma in that location. The patient went into remission but 4 years later she presented with recurrence of hypercortisolism; at this time pituitary imaging showed a hypoenhancing microadenoma that was located in the posterior lobe of the gland. The tumor was resected and now, one year later, the patient remains in remission with partial pituitary deficiencies. We conclude that posterior lobe-located corticotropinomas can complicate pediatric CD, just like in adult cases of CS. Surgeons and endocrinologists should bear in mind that pituitary posterior lobe microadenomas can be hard to visualize and completely excise.

Weil RJ et al. J Clin Endocrinol Metab 2006: 91 (7): 2656-2664.

Nothing to Disclose: MFA, PX, NP, EL, MFK, CAS

P2-320

Diagnostic and Therapeutic Challenges in Patients with Cyclical Cushing's Syndrome: A Case Series.

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Background
Cyclical Cushing's syndrome is a very rare condition most commonly related to a pituitary adenoma episodically producing adrenocorticotropic hormones(ACTH). Due to periodic hormone production, it often poses a particularly difficult diagnostic challenge.
Case-1. A 49-year old lady with long standing hypertension presented with Cushingoid features. Low and high dose dexamethasone suppression tests (LDDS and HDDS) failed to adequately suppress serum cortisol(nadir- 637&305 mmol/L) and ACTH concentrations(nadir-14pg/ml &5.6pg/ml). 24-hour urinary free cortisol concentration(24UFCC) was variable. Magnetic resonance imaging (MRI) scan of pituitary was normal. Inferior petrosal sinus sampling showed a significant gradient between petrosal and peripheral samples.Histology from transphenoidal surgery(TSS) confirmed ACTH staining.
Case-2. A 26-year old lady presented with cushingoid features. Serum cortisol was not suppressed by LDDS and HDDS (nadir-346 and 96 mmol/L respectively).24UFCC fluctuated as did her clinical picture with weight variations. Repeat LDDS showed adequate cortisol suppression (<50mmol/L) contrary to initial results.Initial MRI pituitary and adrenal computed tomography(CT)were normal.
Cushingoid features persisted and repeated MRI pituitary revealed a small discrete lesion in the pituitary which led to TSS and histology confirmed ACTH staining.
Case 3. 26-year old lady with history of hypertension during pregnancy presented with uncontrolled hypertension and cushingoid features after delivery.Cortisol(nadir- 363nmol/L)was unsuppressed with LDDS but suppressed adequately with HDDS(cortisol nadir-53nmol/L).24UFCC was variable. CT pituitary showed pituitary macroadenoma.She had TSS and histology confirmed ACTH staining.

24-Hour Urinary Free Cortisol (mmol/24hour)(Reference 33 to 290 mmol/L)

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Case 1	220	146	182	425	76	437	
Case 2	1712	230	1456	646	480		
Case 3	366	120	790	840	180	820	126

Conclusion
All patients demonstrated cyclical pattern of urinary cortisol excretion and abnormal responses to dexamethasone suppression tests. These cases serve to highlight the fact that the diagnosis requires a careful clinical evaluation and high index of suspicion, particularly when the clinical probability is high and some endocrinology test results are normal.

Nothing to Disclose: AM, GS, SS, DS, JOV

P2-321

Cyclic Cushing's Disease in a Patient with an ACTH-PRL Secreting Adenoma.

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Background: Hyperprolactinemia associated with Cushing's disease has been described. Double pituitary adenomas are rare, the incidence in autopsy being 0.4-1.3%, and the majority are GH and PRL secreting. Plurihormonal adenomas are also recognized, most commonly co-secreting GH and PRL. Notwithstanding, the same pituitary adenoma producing PRL and ACTH is exceedingly rare, with few cases reported in literature.
Clinical case: A 16-year-old woman with amenorrhea and galactorrhea was clinically diagnosed with a cystic macroprolactinoma. Other anterior pituitary hormones were normal. The patient was treated with bromocriptine, but after one year, she developed loss of peripheral vision due to optic chiasm compression. She underwent transcranial surgery elsewhere; however, analysis of the surgical specimen revealed only normal anterior pituitary gland. When the patient presented at our institution, she still had signs and symptoms of hyperprolactinemia, hypogonadism, GH deficiency and partial adrenal failure, and a MRI showing a cystic macroadenoma. She was then started on cabergoline with PRL normalization and tumor shrinkage. After three years, she presented with clinical and laboratorial features of hypercortisolism and increase in the size of the sellar mass. She was submitted to transsphenoidal surgery with sub-total tumor resection. Histology and immunohistochemistry depicted a mixed ACTH-PRL adenoma, although ultrastructure analysis displayed corticotroph differentiation of the tumor cells. After surgery, mild asymptomatic hyperprolactinemia persisted and a clinical and laboratorial picture of cyclic Cushing's disease was established. The hypercortisolism is currently in remission.
Conclusion: We described a patient with clinical evidence of concomitant ACTH-PRL secreting pituitary adenoma. This type of tumor is rare and the few cases reported in literature do not have histopathologic confirmation. In our patient, the immunohistochemical profile was demonstrated for both ACTH and PRL. Nevertheless, the ultrastructural findings were most consistent with corticotroph cell differentiation. To our knowledge this is the first documented case of macroadenoma secreting ACTH-PRL associated with cyclic Cushing's disease.

Nothing to Disclose: LCSL, BBA, DBP, AG, MBL, MDB

P2-322

Cushing's Disease Presenting as Pituitary Apoplexy with Co-Secretion of ACTH and Growth Hormone.

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Pituitary apoplexy in patients with Cushing's disease is an uncommon event. We describe a patient with co-secretion of ACTH and growth hormone (GH) who presented with pituitary apoplexy.
This 38-year-old woman with a 5 year history of type 2 diabetes mellitus, hypertension and obesity presented to the ER with 2 days of severe, incapacitating headache, and abrupt bitemporal hemianopsia on the morning of admission. She exhibited classic features of Cushing's syndrome with round facies, dorsocervical fat pad, centripetal distribution of fat and red-purple striae. MRI of the brain revealed a heterogeneously enhancing mass within the sella with suprassellar extension and compression of the optic chiasm, findings most consistent with a pituitary macroadenoma with features suggesting pituitary apoplexy. The patient was given IV corticosteroids and taken to the operating room for emergent transphenoidal adenomectomy to decompress her optic apparatus. A gross total removal of her hemorrhagic pituitary tumor was performed. On histopathologic examination, the tumor demonstrated hemorrhagic necrosis of a basophilic pituitary adenoma with positive ACTH staining on immunocytochemistry. Preoperative laboratory data demonstrated a GH level of 6.9 ng/ml and IGF-1 level of 505 ng/ml despite the lack of clinical features of acromegaly. Immunohistochemical stain of the tumor for GH was negative. Post operatively, the patient had complete restoration of her vision and a postoperative serum cortisol level of 2 mcg/dl suggestive of biochemical remission. At 3 months after surgery, the patient exhibited a 40 lb weight loss and improvement of her diabetes and hypertension.
Plurihormonal pituitary adenomas are not common, although ~25% of GH-producing adenomas concomitantly produce prolactin. We have found only 5 cases of concurrent secretion of GH and ACTH from pituitary tumors; none had clinical Cushing's syndrome and none presented with pituitary apoplexy.
Summary: To our knowledge, this is the first case of a patient with clinical Cushing's disease with concomitant production of GH that presented with pituitary apoplexy. Although we have few preoperative laboratory studies because of the emergent presentation, the secretion of GH from the adenoma is supported by the elevation of IGF-I. It is likely that the GH did not retain antigenicity on immunohistochemical staining, related to the hemorrhagic necrosis of the tumor.

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2. Oki K, Yamane K, Oda Y, et al. Combined acromegaly and subclinical Cushing disease related to high-molecular-weight adrenocorticotropic hormone. J. Neurosurg. 2009;110:369

Nothing to Disclose: EAN, NHE, MR, LRS, JKL

P2-323

Remission of Hypercortisolism: Presumed Asymptomatic Apoplexy in ACTH-Producing Pituitary Macroadenoma.

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Background: Cushing´s disease (CD) is caused by excessive secretion of ACTH usually by a pituitary corticotroph microadenoma, 4-10% of cases being due to ACTH-secreting macroadenomas. We report a patient with 2 years evolution of CD harboring a solid-cystic ACTH-macroadenoma who presented spontaneous remission of hypercortisolism due to presumed asymptomatic tumor apoplexy. **Case:** A 36-year woman showed typical symptoms of Cushing's syndrome (CS). Initial tests were consistent with ACTH-dependent CS: elevated 24h urinary free cortisol (1200, 1000, 1180 µg/24h; n: 10-90 µg/24h), abnormal 1 mg dexamethasone overnight test (cortisol 2.5 µg/dL; n: <1.8 µg/dL), elevated midnight salivary cortisol (460 ng/dL; n: <130 ng/dL) and ACTH of 89.5 pg/mL (n: <60 pg/mL). Pituitary MRI showed a 13 mm sellar mass with supra-sellar extension leading to chiasmatic compression, with hyperintense T2 weighted imaging suggesting a cystic component. She had no visual impairment. After 4 months, while waiting for pituitary surgery, she presented with spontaneous resolution of CS (lost of 26.4 pounds, return of regular menses, improve of hypertension and abdominal obesity). Tests were consistent with remission of hypercortisolism: normal 24h total urinary cortisol (54, 47, 49 µg/24h; n: 30-310 µg/24h), normal midnight salivary cortisol (<100 ng/dL) and ACTH of 24 pg/mL. Pituitary MRI disclosed tumor shrinkage to 9 mm without chiasmatic compression. She has been currently without CS or hypopituitarism for six months. Hormonal and image data suggested that silent infarction of pituitary tumor probably took place, leading to spontaneous remission of CS. **Conclusion:** Pituitary apoplexy occurs as a result of infarction, hemorrhage, or both in a pituitary tumor. It could be symptomatic leading to neurological and/or visual complaints or clinically asymptomatic. Although there are previous reports of CD remission following apoplexy induced by radiation therapy or by corticotrophin-releasing hormone test, to our knowledge no CD remission after spontaneous asymptomatic pituitary apoplexy was reported. A careful long-term follow-up is required for patients with CD whose remission was due to pituitary apoplexy concerning the risk of hypercortisolism recurrence or hypopituitarism.

Nothing to Disclose: PSG, MCM, DJPCR, MDB, MCBVF

P2-324

Case Report: Benign Intracranial Hypertension (BIH) during Treatment of Cushing's Disease (CD) with Ketoconazole in Children.

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Background: Benign intracranial hypertension (BIH) is a syndrome characterized by headache, visual disturbances and papilloedema due to high intracranial pressure with normal brain imaging and composition of cerebrospinal fluid (CSF). Scattered cases of BIH have been described after Cushing's Disease (CD) control by pituitary surgery or mitotane. We report a case of BIH in a child patient after Cushing's syndrome control with ketoconazole. Its association to ketoconazole or normalization of cortisol levels with this treatment has not yet been described. Furthermore, CD is known to be uncommon during childhood. Case report: We present a case of a 12-year-old girl with CD confirmed by elevated urinary free cortisol, cortisol after 1mg dexamethasone suppression of 13.2 µg/dL (n: <1.8), ACTH of 58 pg/mL (n: 10-52), normal pituitary MRI and bilateral simultaneous inferior petrosal sinus sampling with a significant ACTH response to CRH from 26.6 to 250 pg/mL (positive to DC: elevation bigger than 3 times the baseline). Ketoconazole therapy was implemented to control the disease in order to obtain aeration of the sphenoid sinus and then be able to underwent transsphenoidal surgery. Ketoconazole doses were gradually incresead based on clinical and laboratory parameters of Cushing's syndrome. At the dose of 800mg/day, the patient recovered growth rate, lost 10% of weight and normalized twenty-four hours urinary free cortisol (Table 1). After a few weeks of farmacological control, the patient complained of frontal headache and vomiting, which was treated as sinusitis with azithromycin for 6 days. No symptomatic relief was observed and the patient reported a black spot on the right temporal visual field. Immediate ophthalmologic evaluation identified significant bilateral papilloedema. Brain MRI remained normal and lumbar puncture confirmed elevated CSF pressure and normal liquor composition. The patient was treated with acetazolamide with resolution of the symptoms, including the visual impairment. Conclusion: This case emphasizes the association between BIH and ketoconazole therapy in CD.

Table 1

	Reference	Baseline	Kt 200mg	Kt 400mg	Kt 600 mg	Kt 800mg
24h UFC ug/dl	37-136	303	282	110	54.6	25.0
ACTH pg/ml	10-52	25	132	177	149	186
Cortisol ug/dl	6.2-19.4	9.1	22.7	18	17.3	
SDHEA ug/dl	33.9-280	64.3	86.2	137	86.2	58

kt: ketoconazole

Nothing to Disclose: MAC, FC, NF, DA, TS, TGDC, VB, PBDL

P2-325

Intracranial Hypertension Following Treatment of Cushing's Disease.

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Background: While withdrawal from exogenous steroid treatment has been associated with pseudotumor cerebri, only a few reports have suggested such an association shortly following treatment of Cushing's disease. In this case study, we report a patient who presented with pseudotumor cerebri 11 months after correction of hypercortisolism, and review the literature and the putative mechanisms for this complication.
Clinical Case: A 33-year-old woman with symptoms and signs of hypercortisolism was diagnosed with hypercortisolism based on elevated urinary free cortisol (163 mcg/24hr) and an unsuppressed cortisol (21.6 mcg/dL) the morning after 1 mg dexamethasone administration. A pituitary MRI revealed a 4 mm microadenoma. The patient underwent an uneventful endoscopic transsphenoidal resection of an ACTH-secreting tumor. Postoperative serum cortisol reached a nadir of 1.2 mcg/dL and hydrocortisone replacement therapy was begun. The patient had complete resolution of her symptoms and signs associated with the Cushing's disease. She presented 11 months following surgery with complaints of nausea, vomiting, headaches, blurred vision and inability to take her oral medications. Brain CT and MRI studies were unremarkable and her random cortisol on admission was undetectable. A fundoscopic examination revealed asymmetric signs of early papilledema and a lumbar puncture documented an opening pressure of 360 mm H_2O. A diagnosis of idiopathic intracranial hypertension was made. Within 5 days of treatment with dexamethasone and acetazolamide the patient's symptoms improved. Literature review revealed 7 previous cases in which pseudotumor cerebri developed following treatment of Cushing's disease either by resection of the pituitary lesion, bilateral adrenalectomy or pharmacologically induced adrenal blockade. Lessons from animal models have suggested an important role for glucocorticoids in affecting rates of cerebrospinal fluid production and absorption. In all previous cases, symptoms had developed within a few weeks after correction of hypercortisolism. To the best of our knowledge this is the first report of increased intracranial pressure presenting almost a year following resection of an ACTH secreting pituitary adenoma.
Clinical Lesson: Cessation of steroid exposure either from exogenous or endogenous source may result in intracranial hypertension. This condition should be suspected even several months following corticosteroid withdrawal.

Nothing to Disclose: AT, GZ, UBK, ERL, WWW

P2-326

Mifepristone (RU-486) in the Treatment of Refractory Cushing's Disease.

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Cushing's Disease (CD) carries a five-year mortality of up to 50%. When surgical resection is not possible, pharmacologic suppression using dopamine agonists (cabergoline) and 17α hydroxylase inhibitors (ketoconazole) offer variable success. We describe a patient with refractory CD and use of a novel agent, Mifepristone.
The patient presented with weight loss, fatigue, worsening diabetic control and exertional dyspnea. Physical exam showed facial plethora, centripetal obesity, skin lesions, muscle weakness, spontaneous bruising, hypertension, and preserved visual fields. A 24-hour urine cortisol and ACTH were elevated. Culture of the skin lesions grew Mucor species. Brain MRI revealed a large enhancing macroadenoma in the sella with extension into the sphenoid sinus and third ventricle displacing the optic chiasm.
Two months later, she was hospitalized with volume overload and respiratory failure. Evaluations were negative for primary pulmonary, infectious or cardiac causes. Ketoconazole was started until the patient was stable to tolerate surgical resection. Her mental status then deteriorated, and visual field deficits developed indicating tumor expansion. Surgical resection was only able to remove 70% of the mass. Cabergoline was started postoperatively, however, cortisol and ACTH levels continued to increase. Six weeks following resection, the patient developed lower extremity weakness and new composite fractures of T6-8 with cord compression. Having lost her ability to walk, sustained several infections and developed end-organ damage, experimental therapy was sought.
Mifepristone, a glucocorticoid receptor antagonist, was added on compassionate use protocol. Subsequently, the patient's insulin and hypertensive medication requirements dropped dramatically. Potential side effects, including hyperkalemia and elevated liver enzymes did not develop. However, the patient grew despondent, hypotensive, and hypoglycemic, requiring short-term treatment with dexamethasone. Adrenal insufficiency, a known potential complication of Mifepristone therapy, was likely exacerbated by the P450-inhibiting properties of ketoconazole. The patient subsequently underwent a successful bilateral adrenalectomy.

Disclosures: MJM: Principal Investigator, Corcept, Novartis Pharmaceuticals.
Nothing to Disclose: EED, BGCL

P2-327

Treatment of a Postsurgical Residual Silent Corticotroph Macroadenoma.

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Background: In a recent study by Tateno et al, silent corticotroph adenomas (SCAs) were shown to have mRNA expression of somatostatin receptor SSRT1 and SSRT2 (1). There are no clinical trials done to date examining octreotide, an inhibitor of SSTR2, as a potential therapeutic agent to be used as a bridge to repeat surgery in reducing residual tumor size in SCAs.
Clinical Case: A 76-year old woman with 3 months of headache and blurry vision found to have a pituitary macroadenoma on CT showed no clinical signs of Cushing's disease or other endocrine abnormality. MRI showed a 5.1 x 3.4 x 4.5cm sellar mass with mass effect on the optic chiasm, encasing the right cavernous artery, and touching the left, and displacing the ACAs and third ventricle. Laboratory tests showed low IGF-1 (<25 ng/mL, n 57-172 ng/mL), LH (0.4 mIU/mL, n 7.7-58.5 mIU/mL), and FSH (2.2 mIU/L, n 25.8- 134.8 mIU/L), and a slight elevation in ACTH (66 pg/mL, n 6-58 pg/mL) and prolactin (106 ng/mL, 4.8-23.3 ng/mL) consistent with stalk effect. Cortisol and TSH were within normal limits. She underwent a partial transsphenoidal resection of the pituitary tumor given a firm capsule and the location. Post-operatively she became hypotensive requiring treatment with pressors and stress dose steroids. She was stable off pressors within 24 hours and after two days of stress dose steroids began steroid taper to physiologic doses.
Post-operative MRI showed a 3.1 x 4.1 x 3.1cm mass with continued mass effect on the optic chiasm and third ventricles. The final pathologic diagnosis is silent corticotroph adenoma. One week after discharge, labs showed ACTH within normal limits (25 pg/mL, n 6-58 pg/mL) consistent with SCA. Hydrocortisone tapered to 15mg AM and 5mg PM. Neurosurgery scheduled repeat MRI for three months after surgery to evaluate for possible tumor descent that would allow repeat surgery via transsphenoidal approach. She will be started on octreotide for medical management of the SCA in the interim.
Conclusion: Given the patient's large residual tumor, mass effect on the optic chiasm and prior case series indicating the aggressive nature and rate of recurrence of SCAs (2), she would likely benefit from medical management to prevent further tumor growth. The demonstration of SSRT1 and SSRT2 gene expression in SCA by Tateno et al (1) provides the rationale for considering treatment with octreotide, which is SSTR2 selective, for medical management for residual SCAs prior to repeat surgery.

1. Tateno T, Kato M, Tani Y, Oyama K, Yamada S, Hirata Y. Differential Expression of Somatostatin and Dopamine Receptor Subtype Genes in Adrenocorticotropin (ACTH)-secreting Pituitary Tumors and Silent Corticotroph Adenomas. Endocrine Journal 2009; 56(4): 579-584.
2. Scheithauer BW, Jaap AJ, Horvath E, Kovacs K, Lloyd RV, Meyer FB, Laws ER, Young WF. Clinically Silent Corticotroph Tumors of the Pituitary Gland. Neurosurgery 2000; 47(3): 723-730.

Nothing to Disclose: NL, DP, SS

P2-328

ACTH-Secreting Pituitary Tumor: Evolution of an Adenoma to Carcinoma.

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BACKGROUND:
Pituitary gland is an uncommon site of primary carcinoma. Of 800 cases of pituitary tumors seen at our institution since 1975 only 2 pts. had primary pituitary carcinoma. Many aspects of natural history of pituitary cancer have remained unexplored. Gathering clinical data in all such cases is crucial to improve the diagnosis & management. We have previously reported 2 cases of ACTH-secreting pituitary carcinoma consisting of metastases to the liver in one case & cervical lymph nodes in the other (1). In our previous publication we had suggested the emergence of pituitary carcinoma may involve a proliferative continuum from a pre-existing pituitary adenoma to an invasive carcinoma. We now present an additional case of an invasive ACTH-secreting adenoma that transformed from an adenoma to carcinoma & metastasized to lumbar vertebra over a follow-up period of 5 yrs.
CLINICAL CASE:
A 50-yrs. old lady had recurrent ACTH-secreting (ACTH 239 ng/l RR: <=46, AM serum cortisol: 1138 nmol/l RR: 171-536) invasive pituitary macroadenoma (2.9x2.2x1.6 cm) involving suprasellar & bilateral parasellar extension w/- invasion of cavernous sinuses. She underwent repeated x 4 direct endonasal transsphenoidal resection. She had right 3^{rd} & 6^{th} cranial nerve palsy & decreased vision in rt. eye (20/100).Whole body PET scan done 5 years after initial presentation revealed intensely hypermatabolic (SUV 16) sellar/supresellar tumor w/- metastatic lesions in right 4^{th} rib & L4 vertebra. Histopathology of pituitary tumor: malignant epithelial tumor. Immuo stains positive for: ACTH, Synaptophysin, Chrmogranin, AE1/AE3, CAM 5.5, CK19, Vimentin, CEA, CD 15. P53 expression had increased sequentially from 0% (2003) to, 1% (2006) & finally to 100% (2007). Proliferative index (KI-67) had progressively increased from <3% initially (2003) to10% (2006), & finally to >80% (2007). CT-guided Bx of L4 vertebral pedicle showed metastatic carcinoma compatible w/- pituitary origin.
CONCLUSION:
The findings in this case indicate transformation of pituitary adenoma to carcinoma involves a proliferative continuum eventuating into metastatic lesions. Adjunctive events such as XRT given to this & other previously reported patients may predispose to the evolution of carcinoma in genetically susceptible individuals. Because XRT is an effective Rx for pituitary adenoma; its use will continue. It is important to be aware of the potential adverse effect of XRT for invasive pituitary tumors.

Mohammed Ahmed, Imaduddin Kanaan, Abdullah Alarifi, et al: ACTH-Producing pituitary cancer: Experience at the King Faisal Specialist Hospital & research Center. Pituitary 2000;3:105-112.

Nothing to Disclose: MA, HA-H, IK

P2-329

Temozolomide-Induced Regression of Hepatic Metastases in a Pituitary Corticotroph Carcinoma with Low O6-Methylguanine-DNA Methyltransferase (MGMT) Expression.

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Background: Pituitary carcinoma occurs in ~0•2% of resected pituitary tumours, and carries a poor prognosis (mean survival <4 years), with standard chemotherapeutic regimens showing limited efficacy. However, recent evidence suggests that temozolomide, an orally active alkylating agent used principally in the management of glioblastoma, may also be effective in controlling aggressive/invasive pituitary adenomas/carcinomas. Low levels of expression of the DNA-repair enzyme MGMT, as assessed by immunohistochemistry, predicts temozolomide responsiveness. Here, we report a case of a pituitary corticotroph carcinoma with hepatic metastases, which responded clinically, biochemically and radiologically to temozolomide therapy.
Case report: A 65-year-old man presented as an emergency with frontal headache and evolving bilateral 3rd nerve palsies. Imaging showed a sellar-based mass with parasellar and suprasellar extension. At transphenoidal surgery a necrotic ACTH-staining pituitary adenoma was resected. There were no features of Cushing's syndrome clinically or biochemically, and hydrocortisone replacement was required post-operatively. No residual tumour was identified on post-operative MRI and the patient elected for surveillance follow-up.
However, two years later he developed clinical and biochemical evidence of ACTH-dependent Cushing's syndrome. Pituitary MRI showed no evidence of tumour regrowth and, although a peripheral CRH test suggested a corticotroph origin, IPSS did not demonstrate a central:peripheral gradient. Further imaging with CT and FDG-PET revealed multiple hepatic lesions, and subsequent biopsy confirmed metastatic ACTH-staining pituitary carcinoma. MGMT expression was very low in both the primary pituitary tumour and hepatic metastases.
Temozolomide therapy was commenced (200mg/m2 daily for five consecutive days every 28 days) and has been well tolerated. ACTH levels have fallen from a peak of 5685 to 2318 ng/L after 5 cycles, with CT demonstrating partial regression of the hepatic lesions. Metyrapone and ketoconazole were also used to help control hypercortisolism and the patient has undergone pituitary radiotherapy to mitigate against the potential risk of local tumour recurrence.

Nothing to Disclose: AKA, NK, HB, DJH, KK, AD, NA, HKC, SJ, VKKC, HLS, RWK, JDP, NB, MG

P2-330
Kaposi's Sarcoma in the Setting of Cushing's Disease.

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Background: Kaposi's sarcoma (KS)is commonly associated with immunosuppression, particularly in HIV patients and in transplant patients on immunosuppressive therapy. To our knowledge, there have been no reports of KS associated with Cushing's disease (CD).
Clinical Case: A 54-year-old Hispanic male with a past medical history of hypertension and DM Type 2 presented with typical symptoms of CD with easy bruisibility, fatigue, muscle wasting of the proximal muscles and fat deposition around the belly. He had an atypical presentation of 40lbs weight loss over 3 months and hypokalemia. In addition, he exhibited numerous non-blanching and raised purplish plaques extending throughout the lower extremities. They ranged in size from 0.5 cm to 2 cm in diameter. The left leg had increased redness and warmth with 2+ pitting edema up to the mid thigh when compared to the right leg. Initial tests were consistent with CD: elevated 24 hour urine free cortisol (1503 µg/24hr), abnormal 1mg dexamethasone suppression test (cortisol: 33.3 µg/dL and ACTH: 116 pg/mL). MRI showed a 1.6 x 0.4 cm nonenhancing right sided sellar lesion. Inferior petrosal vein sampling confirmed pituitary CD with central ACTH to peripheral ratio of 5.72 (n > 2.0 for pituitary CD). Punch biopsy of the skin from the left calf showed atypical vascular proliferation with immunohistochemical stain positive for Human Herpes Virus-8 (HHV-8), all consistent with the diagnosis of KS. HIV test was negative by ELISA. The patient had two failed transphenoidal surgeries followed by a bilateral adrenalectomy. Upon recovery from his surgery, he was started on systemic chemotherapy for the treatment of KS with a combination of Doxil, Taxol, and Bleomycin. This resulted in improvement of his leg edema, flattening of the KS lesions along with decreased pain of the lower extremities.
KS is caused by the HHV-8 in the setting of immunosuppression. In non-HIV patients, KS has been reported in patients on immune-suppressants after having undergone organ transplantation. It has also been associated with corticosteroid use. We have not found any published reports of KS in the setting of Cushing's disease or syndrome.
Conclusion: As far as we know, this is the first case citing an occurrence of KS in the setting of a non-HIV patient with either Cushing's disease or syndrome. Combination of treatment with chemotherapy and treatment of Cushing's are effective therapies for KS in this setting.

Nothing to Disclose: LJ, AR, MDR

P2-331

Predictors of Bone Mineral Density (BMD) Response to Growth Hormone (GH) Replacement in Adults with GH Deficiency (GHD) – A KIMS (Pfizer International Metabolic Database) Analysis.

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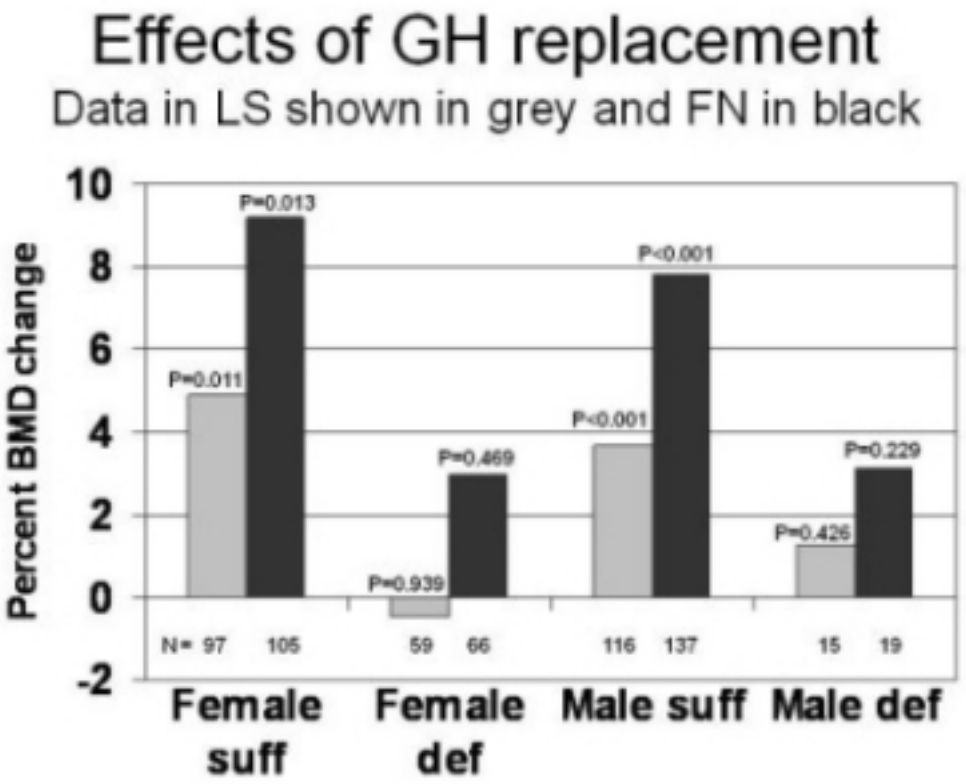
GH replacement may lead to increase in BMD in adults with GHD. To investigate factors associated with BMD response to GH replacement, the KIMS database was searched. The search identified 371 adults (see Table) matching our criteria which were as follows: stringently defined GHD, GH-naive at study entry, baseline BMD measurement at femoral neck (FN) and/or posterior-anterior lumbar spine (PA LS) as well as at the same site after 2 years on GH replacement, and BMD measurements on the same densitometer (Hologic, Lunar/GE or Norland).
GH therapy for 2 years led to increase in BMD in the LS by 3.1% ($P < 0.001$) and FN by 7.0% ($P < 0.001$). In both genders there was significant increase in BMD (LS and FN) in patients who were sex-steroid sufficient or replaced (“suff” in the Figure represents both those groups combined).
The predictors of 2-year BMD response to GH (multivariate regression analyses) were as follows: 1. LS: lower baseline BMD ($P < 0.001$) was associated with greater BMD increase; 2. FN: lower delta IGF-1 SDS (change from baseline; $P < 0.001$), and higher GH dose ($P < 0.001$) were associated with greater BMD increase; lower baseline BMD ($P = 0.002$) was associated with greater percent BMD increase.

Clinical characteristics of the study population

Variable	Mean ± SD or percent
Age at KIMS entry (yr)	48.4 ± 12.7
Gender (% F/M)	52.3 / 47.7
IGF-1 SDS (baseline)	-1.8 ± 1.5
Baseline BMD Z-scores	Female LS (-0.4 ± 1.5); Female FN (-0.2 ± 1.2); Male LS (-0.3 ± 1.6); Male FN (-0.3 ± 1.1)
Sex steroid deficient / sufficient or substituted (%)	26.7 / 73.3
Number of additional pituitary hormone deficiencies (%)	none (7); one (17.8); two (18.3); three (40.2); four (16.7)
GH dose (mg/d)	0.20 ± 0.12



Conclusions: Normal sex steroid levels, either endogenous or replaced, appeared to be essential for achieving an increase in BMD after 2 years of GH replacement. The interaction between GH and sex steroid replacement requires further study. Patients with lower baseline BMD showed greater LS BMD improvement. Higher GH dose and lower delta IGF-1 SDS led to greater FN BMD improvement.

Disclosures: SLG: Investigator, Merck & Co., Lilly USA, LLC, Sanofi-Aventis; Consultant, Amgen, Merck & Co. DK: Employee, Pfizer, Inc. PJJ: Employee, Pfizer, Inc. MPW: Employee, Pfizer, Inc. MK-H: Employee, Pfizer, Inc.
Nothing to Disclose: NAT, AHH, DMC, BMKB

P2-332

The Effect of Recombinant Human Growth Hormone on Linear Growth in Children with Inflammatory Bowel Disease: Results of a Randomised Controlled Trial.

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Background:Despite optimal gastrointestinal management, some children with inflammatory bowel disease(IBD) may have growth retardation. The role of rhGH in these children is unclear.
Design:Randomised controlled trial of rhGH(0.067mg/kg/day) over a 6 month period. Primary outcome measure: height velocity (HV).
Subjects:22 children with IBD and HtSDS<-2 or HtSDS<-1 and HVSDS<-1. Eleven(9M) were in the control group (C) and eleven(9M) in the treatment group(Rx). All children had Crohn's Disease (CD) except 2 in the C group who had ulcerative colitis (UC).
Methods:HtSDS, HV, HVSDS were compared between in Rx and C at baseline(T0) and 6 months(T6). HVSDS was adjusted for Tanner stage(TS) for girls≥11yrs and boys≥12yrs. Disease severity including CRP, ESR, albumin(Alb), haemoglobin(Hb) and cumulative dose of Prednisolone were collected at T0 and T6. Glucose homeostasis was assessed by fasting glucose, insulin, c-peptide and HBA1C at T0 and T6. All data are expressed as median(10th,90th).
Results:CA at T0 was 14.7yrs(9.3,16.2) & 13.7(9.1,15.5);median CA-BA at T0 was 1.7yrs(-0.3,3.6) & 1.7yrs(-0.7,4.1) for Rx and C. TS at T0, in Rx were TS1-2; TS2-5; TS3-2; TS4-2. In C, they were, TS1-3;TS2-6;TS3-1;TS4-1. By T6, pubertal progress was noted in 5/11 & 3/11 of Rx and C groups. HtSDS at T0 was in Rx and C: -2.8(-4.1,-1.5) & -1.8(-2.7,-1.3,(p=0.001). Change in HtSDS at T6 in Rx and C was significantly different: 0.3(0.1,0.8) & -0.1(-0.3,0.3),p<0.0001. HV at T0 was similar in Rx and C: 5.0cm/yr(0.8,8.8) & 3.8cm/yr(1.6,6.5), and so was HVSDS: -3.1(-6.0,4.4) & -2.4(-6.2,1.8). HV at T6 in Rx and C was 10.8cm/yr(6.1,14.3) & 3.5cm/yr(2.0,9.3),p<0.0001.HVSDS at T6 in Rx and C was 3.2(-0.4,16.4) & -2.0(-6.3,4.9),p=0.0001. CRP, ESR, Alb, Hb and cumulative prednisolone dose were similar between the Rx and C group at T0 and T6. 2 children in each group had clinical relapse during the study period. ΔBA/ΔCA was similar in the two groups at T6. At T6, in Rx and C, fasting insulin, was 7.0mU/L(2.1,15.7) & 3.8mU/L(2.1,6.6),p=0.04; C-peptide was 0.7nmol/L(0.4,1.2) & 0.3nmol/L(0.2,0.8),p=0.002,and HOMA index was 1.5(0.3,3.7) & 0.3(0.2,0.8),p=0.05. Fasting glucose and HBA1C, were similar in both groups at T6.
Conclusion:rhGH at a dose of 0.067mg/kg/day in children with IBD and growth retardation can increase HV by over 100% without excessive skeletal maturation. Therapy is associated with a reduction in insulin sensitivity but no overt abnormality of glucose tolerance.

Nothing to Disclose: SCW, PK, DHC, AMD, JCB, MD, KH, PM, SFA

P2-333

Diabetes Mellitus Incidence in Growth Hormone Treated Patients: Experience from the Global GeNeSIS Observational Program.

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[1]Eli Lilly and Co Windlesham, UK ; [2]Eli Lilly and Co Indianapolis, IN and [3]Eli Lilly and Co Bad Homburg, Germany.

Because of the action of growth hormone (GH) as an insulin antagonist, it has been suggested that GH treatment (tx) may alter glucose metabolism. Cutfield *et al.* (1) reported a 6-fold increase in risk for type 2 diabetes (T2DM) in GH-treated patients (pts) compared to general population data, but no increase for type 1 diabetes (T1DM). We assessed T1 and T2DM incidence rates from the GeNeSIS global observational program vs. recent rates of DM in the general pediatric population (2).
Data from GH-treated pts with ≥ 1 follow-up visit available (N=11,136) were screened for potential DM cases. At GH tx onset, median [Q1-Q3] age was 10.2 [6.5-12.7] y and GH dose was 0.26 [0.19-0.33] mg/kg/wk. There were more males (60 %); the main diagnostic groups were growth hormone deficiency (GHD, 65%), idiopathic short stature (ISS, 14%) and Turner syndrome (TS, 10%). Questionnaires were issued to verify diagnoses and pre-tx risk factors regarding 66 pts. Cases were assigned by standard diagnostic criteria (3), using questionnaire responses, existing study data and serious adverse event reports. Standardized incidence ratios (SIR) and 95% confidence intervals (CI) were calculated for US cases alone and all cases globally, using US general population DM rates stratified for age and ethnicity (2).
During GH tx (duration of 2.5 [1.3-4.4] y), 28 tx-emergent DM cases were identified: 14 T1 (GHD N=8, ISS 3, TS 2, other 1), 12 T2 (GHD N=7, TS 2, other 3) and 2 of unspecified DM type. Table 1 indicates T1 and T2DM SIR and 95% CI for T1 and T2DM cases.

Table 1: DM cases and calculated SIRs

	DM Cases	US Cases SIR (95% CI)	All Cases SIR (95% CI)
T1DM	14 (6 US, 8 non-US)	1.4 (0.5-3.1)	1.6 (0.9-2.6)
T2DM	12 (5 US, 7 non-US)	8.5 (2.8-19.9)	7.1 (3.7-12.4)

We observed no significant increased risk for T1DM but a 7.1-8.5 fold increase for T2DM. Pre-tx risk factors for T2DM were present in 75% of cases, including: Asian ethnicity, comorbidity (sideroblastic anemia, obesity), pre-existing abnormal glucose metabolism (insulin resistance, impaired glucose tolerance), history of leukemia/irradiation and corticosteroid use.
This analysis and previous data (1) must be interpreted in light of limitations of comparing observational study event data vs. general population rates, potential surveillance bias and change in DM diagnostic criteria over time. Nevertheless, it is recommended that pts treated with GH should be monitored for alterations of glucose tolerance and development of T2DM.

(1) Cutfield W et al.,Lancet 2000; 355:610-613
(2) The Writing Group for the SEARCH for Diabetes in Youth Study Group, JAMA 2007; 297:2716-2724
(3) American Diabetes Association, Diabetes Care 2008; 31:S55-S60

Sources of Research Support: Eli Lilly and Company.

Disclosures: CJC: Employee, Lilly USA, LLC. RSS: Employee, Lilly USA, LLC. AGZ: Employee, Lilly USA, LLC. WFB: Employee, Lilly USA, LLC.

P2-334

Interaction between Testosterone and Growth Hormone on Whole Body Protein Anabolism Is Mediated through the Liver.

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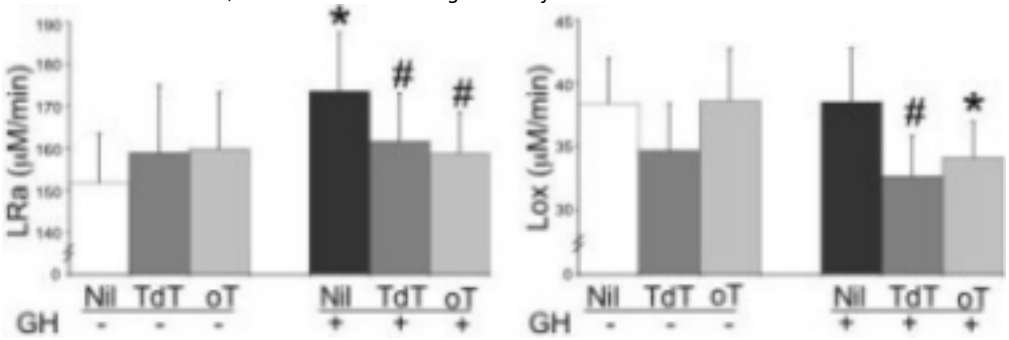
Growth hormone (GH) and testosterone (T) both exert protein anabolic effect and may act synergistically. Liver and muscle are major sites where protein metabolism is regulated. We aimed to determine whether the site of GH and T interaction is primarily hepatic or extrahepatic. We therefore compared the impact on whole body protein metabolism of T administered via oral route (solely hepatic T exposure) with transdermal T replacement (systemic T exposure) in the presence or absence of GH.

Fifteen hypopituitary men with GH and T deficiencies participated in a randomised open-label crossover study of 2-wk treatments with transdermal T (tdT 10 mg) and oral T (oT 40 mg), with or without GH replacement (0.6 mg/day) for 3 months. The dose of T administered orally achieves physiological portal T concentrations without spill-over into the systemic circulation (Clin Endocrinol 2009; 71:715-21). Whole body protein turnover was measured, from which protein breakdown (LRa) and protein oxidation (Lox), as an inverse measure of anabolism, was estimated at the end of each treatment period.

In the absence of GH, neither TdT nor oT affected LRa (left panel). GH therapy significantly increased LRa, which was reduced by T treatment with the effect not different between TdT and oT administration. In the absence of GH, neither TdT nor oT affected Lox (right panel). Treatment with GH did not significantly affect Lox. However addition of T treatment reduced Lox, with the effect not significantly different between TdT and oT.



$p<0.05$ * vs no treatment, # vs GH only

In summary, in the absence of GH, neither systemic nor hepatic T exposure significantly influenced protein metabolism. In the presence of GH, T administration to achieve either systemic or solely hepatic T exposure reduced protein turnover and oxidation to a similar extent.

We conclude, in the doses used, T alone did not affect protein metabolism. In the presence of GH, T stimulates anabolism by reducing protein breakdown and oxidation. Because there was no difference between systemic and solely hepatic effects of T, the primary site of the GH and T interaction on protein metabolism is most likely the liver.

Sources of Research Support: NHMRC of Australia; Eli Lilly provided Humatrope® and Maynepharma provided Androderm® patches.

Nothing to Disclose: VB, UJM, DJH, KKYH

P2-335

The Metabolic and Cardiovascular Consequences of Long-Term Growth Hormone Replacement in Hypopituitarism.

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Background: In hypopituitary adults growth hormone replacement (GHR) has positive effects on body composition, lipid levels and bone mineral density (BMD). However important questions regarding the long-term effects of growth hormone (GH) on fracture rates and cardiovascular risk in these patients remain unanswered.
Objective: To investigate the benefits ≥10 years GHR on body composition, BMD and cardiovascular risk factors in hypopituitary adults.
Design: We studied 46 adult patients (20 female, 26 male) with established treated hypopituitarism and severe GH-deficiency. 25 individuals (60.2±10.3, years, mean±SD) had been receiving continuous GHR for over 10 years whilst the remaining 21 (50.7±12.7, years) had replacement of pituitary hormone deficiencies with the exception of GH (over a similar duration). We assessed and compared the effect of GHR on body composition, lipid & glucose levels, BMD and blood pressure.
Results: GH replacement was associated with sustained increases in IGF-1, LBM and glucose concentration (IGF-1, 29.2±2.9 v 12.8±1.7, nmol/L, $p<0.003$; LBM, 51.7±2.5 v 46.7±2.7, kg, $p<0.02$; glucose; 5.7±0.3 v 4.5±0.1, mmol/L, $p<0.02$, all variables 10 years v baseline) and reduction in diastolic blood pressure (DBP, 71.6±1.5 v 81.9±2.9, mmHg, $p<0.02$, 10 year v baseline).
Following 10 years GH replacement, IGF-I and BMD were higher than compared with the GH naïve patients (IGF-I, 29.2±2.9 v 11.7±1.5, nmol/L, mean±SE, $p<0.001$; Z-score lumbar spine, -0.09±0.4 v -1.5±0.5, $p<0.02$; 10 year GHR v GH naïve). Diastolic BP, waist hip ratio and LDL cholesterol were lower following GHR (DBP, 71.6±1.5 v 78±3.3, mmHG, $p<0.05$; WHR, 0.94±0.02 v 1.01±0.03, $p<0.04$; LDL-cholesterol 3.1±0.2 v 3.9±0.3, mmol/L, $p<0.05$; 10 year GHR v GH naïve). Glucose concentrations were higher in the GHR group compared with GH naïve subjects (glucose, 5.7±0.3 v 4.8±0.2, mmol/L, $p<0.05$).
Conclusion: The effects of GHR on LBM, WHR, BMD, lipid levels & BP observed in short-term studies were maintained up to 10 years after initiating treatment in these hypopituitary adults. Long-term GH was therefore associated with improvement in markers of cardiovascular risk, however higher glucose levels were seen in those who had received GH during this period.

Nothing to Disclose: TM, LAB, JKP, SMT, BMM, PVC

P2-336

Analysis of Height Outcomes by Gender and Pubertal Status in Response to Treatment with Growth Hormone Therapy in Children with Short Stature and Different Diagnoses: Data from the ANSWER Program®.

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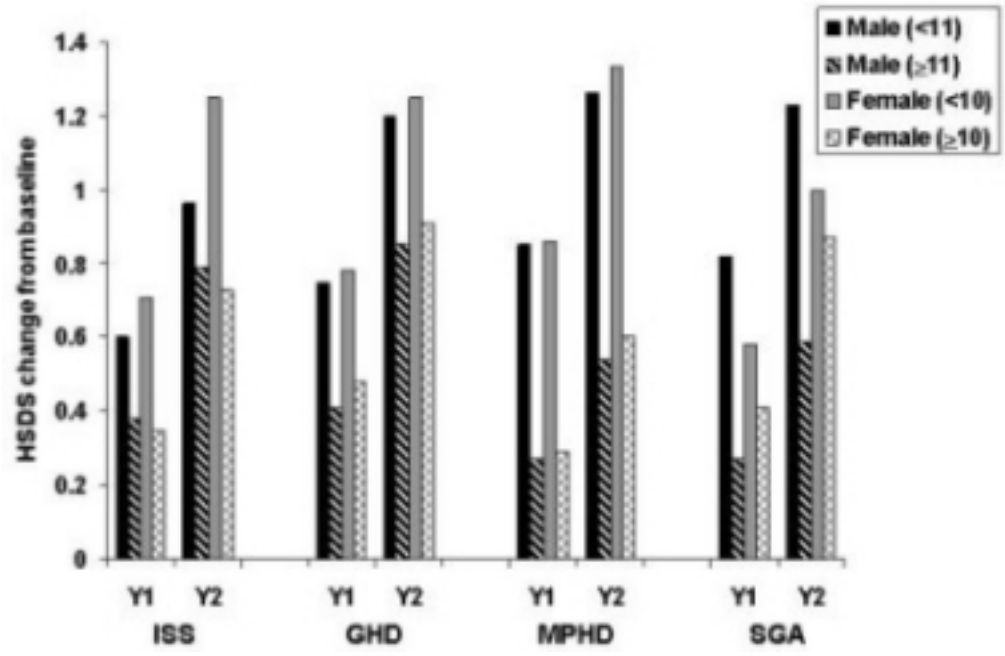
The American Norditropin Studies: Web-enabled Research (ANSWER) Program®, a US-based registry, has collected long term safety and efficacy information on patients treated with Norditropin® (somatropin rDNA origin) at the discretion of participating physicians. By November 30, 2009, data were collected on 7,197 treatment naïve pediatric patients with different diagnostic conditions, including isolated/idiopathic growth hormone deficiency (GHD), multiple pituitary hormone deficiency (MPHD), small for gestational age (SGA), and idiopathic short stature (ISS). Mean age at GH treatment start was generally younger in patients with MPHD (7.5±5.45 yrs) and SGA (8.4±3.74 yrs) than in other diagnostic categories (ISS, 11.2±3.06; GHD, 10.8±3.54). The lowest mean peak GH levels at baseline were observed in patients with GHD (5.3±2.77 ng/mL) and MPHD (3.1±3.04 ng/mL), compared to the other diagnostic categories (ISS, 17.1±20.09; SGA, 14.4±13.69). Limited gender differences in HSDS change from baseline (D HSDS) were found across diagnostic categories with a significant effect observed in SGA patients at year 1 (male > female, p=0.016). When examined by pubertal status for patients with GHD, the best D HSDS was observed in prepubertal patients (Table 1). When stratified by age at treatment start, similar results were observed in all diagnostic categories for both sexes (Figure 1). In conclusion, a better growth response was apparent in younger and in prepubertal children, emphasizing the importance of starting growth hormone treatment at a younger age.

Table 1. HSDS change from baseline by pubertal status for patients with GHD

	Year 1		Year 2	
	N	Mean±SD	N	Mean±SD
Pre-pubertal[a]	1128	0.64±0.53	610	1.15±0.73
Pre-pubertal/ Pubertal	572	0.50±0.41	638	0.94±0.53
Pubertal	772	0.48±0.36	474	0.95±0.56

[a]Pubertal status assigned by the physician; pre-pubertal were Tanner I throughout, pre-pubertal/pubertal patients transitioned into Tanner II or more, pubertal patients were Tanner II or more at baseline.

Figure 1: HSDS change from baseline stratified by age at treatment start



Disclosures: JR: Consultant, Novo Nordisk. PL: Consultant, Novo Nordisk; Researcher, Novo Nordisk. RG: Employee, Novo Nordisk. JG: Employee, Novo Nordisk.

P2-337

The Effect of Gonadotropin-Releasing Hormone Agonists with or without Growth Hormone on Predicted Adult Height in Korean Boys with Central Precocious Puberty and Early Puberty.

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Objective: Gonadotropin-releasing hormone agonists (GnRHa) are the drug of choice for treatment of central precocious puberty (CPP). However, because CPP is less common in boys than in girls, little data is available on the long-term efficacy and safety of GnRHa treatment in boys with CPP. We studied the effect of GnRHa combined with or without growth hormone on predicted adult height in Korean boys with CPP and early puberty after one year treatment.
Patients: This study included 18 boys with CPP and 63 boys with early puberty who were treated with depot leuprolide acetate from 2003 to 2009 in the Ajou university hospital, Korea. Among them, 4 boys with CPP and 28 with early puberty were treated with GnRHa and growth hormone. Anthropometry, bone age, sexual maturity rating and predicted adult height (PAH) were assessed at baseline, after 6 months, and after one year.
Results: In boys, PAH (SDS) of GnRHa group of early puberty (n=35) was similar to their pretreatment PAH (SDS) [175.40.92 cm (0.360.16) vs 176.91.04 cm (0.620.18)] (P=0.1907). In GnRHa combined GH group of early puberty (n=28). PAH was significantly increased [169.81.38 cm (-0.640.25) vs 173.91.40 cm (0.080.25)] (P□0.001). PAH (SDS) of GnRHa group of CPP (n=14) was significantly increased [172.62.1 cm (-0.150.38) vs 175.12.11 cm (0.290.38)] (P=0.0014) and that of combination group of CPP (n=4) was increased, but not significant [171.12.42 cm (-0.410.43) vs 174.12.67 cm (0.130.47)] (P<0.1542). In multiple logistic regression, pretreatment bone age SDS [OR 2.71(S.E. 1.58) (P=0.048)] was significant influencing factor on changes of predicted adult height.
Conclusion: Our data indicate that GnRHa treatment significantly improves growth potential in boys with CPP. However, GnRHa treatment dose not affect growth prognosis and GnRHa combined with growth hormone improves growth prognosis in boys with early puberty. But, longer follow up of larger group of patients is necessary to conclude the efficacy and safety of GnRHa with or without Growth hormone treatment.

Nothing to Disclose: HSL, HSL, JSH

P2-338

Difficult Transition of Patients with Growth Hormone Deficiency from Pediatric to Adult Care.

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Objectives: Adult patients with childhood-onset growth hormone deficiency (COGHD) and persisting growth hormone deficiency (GHD) were questioned on their status of medical attendance.
Patients: 52 patients (17 females) at the age of 17.56 ± 1.7 years were diagnosed as persistent GHD due to transition criteria with a maximal GH level during insulin induced hypoglycemia of less than 6 ng/ml. There were 37 patients with an isolated GHD (iGHD) and 15 patients with multiple pituitary hormone deficiencies (MPHD). Patients were contacted by letter and telephone at a mean age of 23.64 ± 2.3 years. No contact was possible in 7 (13%) patients, including 5 patients with iGHD and 2 with MPHD.
Results: Overall 15 out of remaining 45 patients were still in endocrine medical care, 9 patients showing MPHD and 6 iGHD. Ten patients were at least once in endocrine care, but not presently and 20 patients never attended adult endocrine care. Therefore, 31% of patients with MPHD and 81% of patients with iGHD had no current endocrine medical attendance. Based on a questionnaire these patients do not suffer from subjective health problems and state to have a good quality of life.
Conclusions: It seems to be difficult to convince patients with iGHD to continue GH therapy and therefore endocrine care. Even in patients with MPHD nearly one third is not followed by an adult endocrinologist. New strategies are necessary to ensure long-term adult endocrine medical care especially in those with MPHD.

Nothing to Disclose: SMB, VM, WB, CW, HS, HPS

P2-339

Relationship of Estrogen and IGF-1 during Menopause and Geripause.

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We have indicated the importance of comparing hormonal changes with aging in the menopausal transitions of women (Endocrinology,'09). Correlation of the signs and symptoms of menopause with hormone modifications through serum estradiol levels (SEL) has been defined. SEL forms a bell-shaped curve from birth through ages 40-49, followed by a rapid decline during ages 50-65 (geripause), with a milder decline thereafter. Postmenopausal women from ages 65 and above show only a slight but detectable decrease of estrogen in the serum. The present study evaluated women in these age groups,, comparing SEL, serum FSH and IGF-1. The results show distinct relationships between all these reproductive-related hormones. It is possible to predict some symptoms and transitions caused by estrogen loss. However, these relationships are distinct for premenopausal (up to age 51) and late geripause (age 85+). Thus, our current study reflects dramatic changes in women, showing decreases in estradiol after menopause with subsequent rise in FSH. Additionally, a significant inverse relationship of estrogen is seen with the serum levels of IGF-1. Using the MRS Questionnaire for our study, we have evaluated menopausal signs and symptoms according to laboratory data. These conclusions may define more clearly the basic cause for several of the featured symptoms of the late menopause and geripause

Nothing to Disclose: BAE, JT, MVW, OCM, GDA, DF, CS

P2-340

Pharmacological and Toxicological Evaluation of AEZS-130, a Novel, Oral Synthetic Growth Hormone Secretagogue for the Diagnosis of Growth Hormone Deficiency.

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AEZS-130 is a novel growth hormone secretagogue, which exerts its effect by binding to the growth hormone secretagogue receptor GHS-R1A, which is the receptor for the physiological peptide ligand ghrelin. This mechanism of action was determined previously by binding studies assessing the affinity for the GHS-R1A by using membrane preparations from human pituitary gland, as well as transiently expressing LLC-PK1 cells (Ref1).
Here we further confirm the high affinity binding of AEZS-130 to GHS-R1A and demonstrate its functional ghrelin agonistic activity in various cell based assay systems covering ghrelin receptors of different species. Binding to the human GHS-R1A was determined with an EC_{50} value of 15.0 nM and analysis of AEZS-130 in two independent functional cell-based assays revealed even improved potency with EC_{50} values of 7.9 nM and 0.5 nM based on CRE-dependent reporter gene activation and calcium release as respective read-outs. Comparable potency was seen against the rat GHS-R1A, i.e. with EC_{50} values of 5.8 and 3.1 in the CRE reporter gene assay and calcium release, respectively.
Previous pharmacological studies in an infant rat model following single subcutaneous administration at a dose of 300 µg/kg AEZS-130 have demonstrated an increase in growth hormone release comparable to the effect exerted by the positive control Hexarelin (Ref1). Here we show that also single oral treatment of fed rats with 24 mg/kg AEZS-130 is capable of inducing significant growth hormone release in a time dependent manner, which is paralleled by AEZS-130 plasma levels.
The ability of AEZS-130 to increase growth hormone levels after repeated oral treatment of five days was demonstrated in beagle dogs, showing a 4-10 fold increase in the AUC of growth hormone levels.
Safety of AEZS-130 was shown in various early safety and toxicity assays *in vitro*, as well as in preclinical safety and toxicity studies in rats and dogs.
In summary, AEZS-130 is a potent and safe oral synthetic GH releasing compound with potential utility as a diagnostic for growth hormone deficiencies as well as a therapeutic for a range of diseases including treatment of cancer induced cachexia. AEZS-130 is currently undergoing late phase clinical evaluation for development as a diagnostic for adult growth hormone deficiency.

(1) Guerlavais V et al. (2003). J. Med. Chem. 46, 1191-1203.

Disclosures: BA: Employee, Aeterna Zentaris GmbH. PS: Employee, Aeterna Zentaris GmbH. EB: Researcher, Aeterna Zentaris GmbH. VL: Collaborator, Aeterna Zentaris GmbH. DP: Employee, Aeterna Zentaris GmbH. MT: Employee, Aeterna Zentaris GmbH.

P2-341

Patient Acceptability of a New Injection Device System To Administer Human Growth Hormone: Results from a Multinational Handling and Questionnaire Survey.

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Objective: To measure patient usability of a new injection system, Norditropin FlexPro and FlexPro PenMate (FlexPro/PenMate) for administration of human growth hormone (hGH) in children and adolescents with growth hormone deficiency (GHD).
Study Design and Methods: In this multinational, open-label, uncontrolled study, patients (n=50) using Norditropin NordiFlex (NordiFlex) without NordiFlex PenMate were trained to use the FlexPro/PenMate and NordiFlex/PenMate systems to deliver test medium into a cushion. Participants' opinions were recorded by questionnaire during a face-to-face interview following the training session. Handling of the device systems were assessed using a 4-point rating scale.
Results: Patient characteristics were similar between countries (58% male; ages 4-10yrs, 38%; 11-14yrs, 38%; 15-17yrs, 11%; not stated, 1%; duration of GH therapy, ≤1yr, 36%; >3yrs, 38%; right-handed injection, 80%; self-injection, 50%).

Patient preferences shown as n (%)

	US	Germany	Sweden	Netherlands	Japan
No. of participants	10	10	10	10	10
Preference for using injecting system					
FlexPro/PenMate	9(90)	7(70)	8(80)	9(90)	7(70)
NordiFlex/PenMate	1(10)	1(10)	2(20)	1(10)	1(10)
Neither	0(0)	2(20)	0(0)	0(0)	2(20)
Confidence with self-injecting in the future with each pen system					
No. of self-injectors	6	6	5	8	0
FlexPro/PenMate					
Confident/very confident	6(100)	3(50)	4(80)	8(100)	0(0)
NordiFlex/PenMate					
Confident/very confident	6(100)	2(34)	3(60)	8(100)	0(0)
Ease of learning to use					
FlexPro/PenMate	10(100)	9(90)	10(100)	9(90)	10(100)
NordiFlex/PenMate	10(100)	6(60)	9(90)	8(80)	7(70)

Overall, 80% preferred the FlexPro/PenMate system over the NordiFlex/PenMate system and 84% reported that they would be confident or very confident about self-injecting using the new system. 96% found it 'very easy' or 'easy' to learn how to use the FlexPro/PenMate system.
Conclusions: The majority of participants preferred the FlexPro/Penmate system over the NordiFlex/PenMate system and stated that they would be confident using it for self-injection. The improved features of the new device system suggest that it is user-friendly, which may facilitate greater treatment adherence thereby enhancing treatment outcome.

Disclosures: AMK: Employee, Novo Nordisk. SM: Employee, Novo Nordisk. CB: Employee, Novo Nordisk. GSF: Employee, Novo Nordisk.

P2-342

Muscle Strength in Elderly Growth Hormone (GH) Deficient Adults after Ten Years of Replacement.

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Context: Only few studies have investigated the effects of GH replacement on muscle strength in elderly GH deficient (GHD) patients.
Objective, Design, and Patients: In this prospective open-labeled study, the effects of 10 yr of GH replacement on muscle strength and neuromuscular function were followed in 24 elderly GHD adults (mean age 65.2 yr; range 61-74 yr).
Results: The mean initial GH dose of 0.72 mg/d was lowered to 0.37 mg/d. The mean IGF-I SD score increased from -1.10 at baseline to 1.17 at study end. GH replacement induced a sustained increase in lean body mass and a transient increase in isometric knee flexor strength (60°). Other measures of upper leg and handgrip strength remained unchanged except for isometric knee extensor strength (60°), which was reduced after 10 years. However, after correction for age and gender, using observed/predicted value ratios, there were sustained and even progressive increases in most variables reflecting muscle strength. At study end, knee flexor strength had increased to 108-113% of predicted, knee extensor strength to 95-118%, and handgrip strength to 87-93%. Measurements of neuromuscular function showed unchanged voluntary motor unit activation after 10 years.
Conclusions: Ten years of GH replacement therapy in elderly GHD adults did not increase absolute levels of muscle strength but provided protection from most of the normal age-related decline in muscle performance and neuromuscular function resulting in nearly normalized muscle strength.

Nothing to Disclose: GNG, ME, KS-S, B-AB, GJ, JS

P2-343

Gender, but not Sex-Steroid Milieu, Determines GH Response to Triple Secretagogues.

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Growth hormone responses to individual and dual secretagogues often exhibit significant variability due to confounding factors such as age, BMI, gender, and sex-steroid milieu. We tested the hypothesis that simultaneous stimulation with three distinct pathway-selective secretagogues would drive maximal GH secretion independently of BMI, gender and sex-steroid milieu, thereby offering a more general test of GH reserve. The cohort comprised 26 healthy older adults (ages 50-74 yr, BMI 21-30 kg/m^2, gender 14 F 12 M), in whom the sex-steroid milieus were clamped by leuprolide + placebo/E_2 or leuprolide + placebo/T replacement. Subjects each received L-arginine (to restrain somatostatin outflow), GHRP-2 and GHRH as a triple-secretagogue combination. By ANOVA, nadir GH concentrations were maximal in women receiving E_2 supplementation ($P < 0.001$): **Figure 1**. Mean triply stimulated GH concentrations were 1.8-fold higher in hypogonadal women than men without sex-steroid supplementation: **Figure 2**. Peak triply stimulated GH concentrations were highest and equal in women with and without E_2 supplements (97 ± 20 and 97 ± 17 ug/L), compared with men given leuprolide plus placebo (32 ± 17, $P = 0.006$). Peak GH was intermediate in men given leuprolide plus T (47 ± 2.2 ug/L). BMI was a strong negative correlate of stimulated GH peaks ($R^2 = 0.42$, $P = 0.002$). Deconvolution analysis corroborated the rank order of descending mass of triply stimulated GH secreted as: women + E_2 = women - E_2 > men + T ≥ men - T ($P < 0.01$), and further disclosed that GH secretory-burst maxima occurred significantly earlier in women (± E_2) than men ($P < 0.01$). These data refute the hypothesis that a triple secretagogue combination acts independently of gender and BMI, while affirming independence from the short-term sex-steroid milieu.

Figure 1.

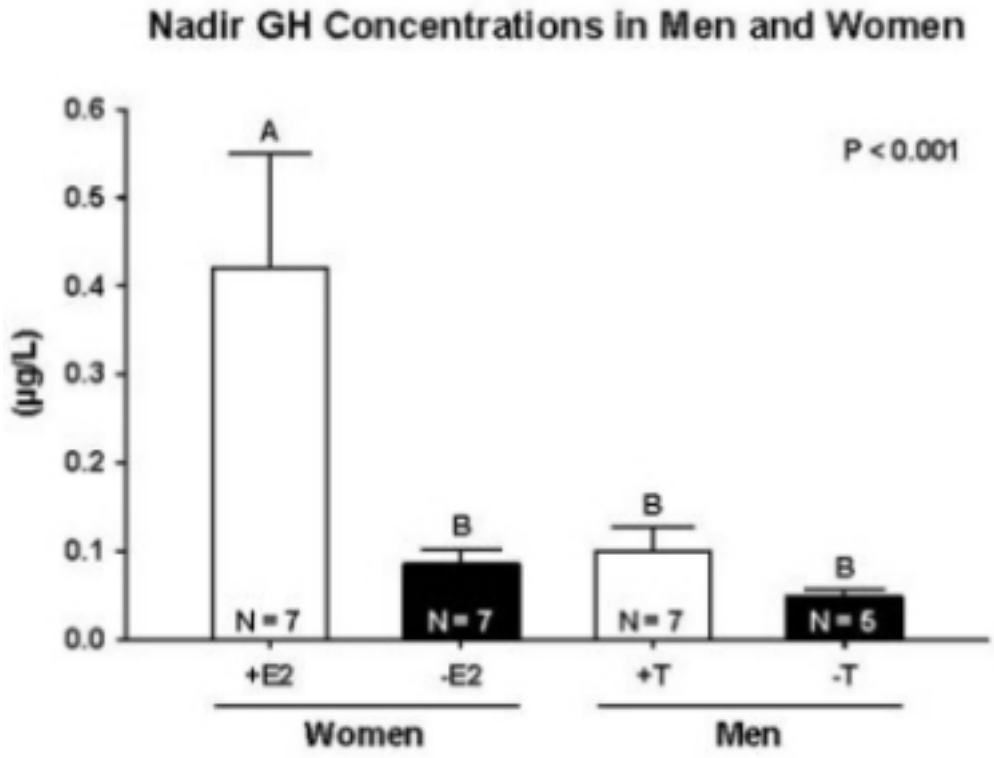
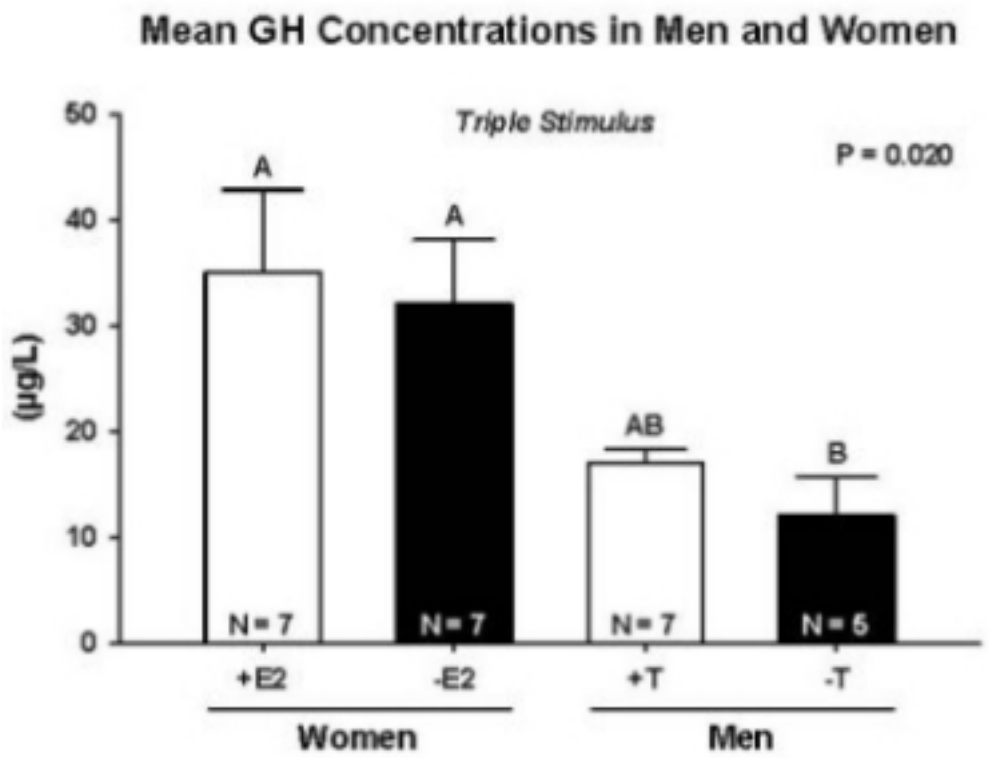


Figure 2.



Sources of Research Support: AG019695 and AG029362 from the National Institutes of Health (Bethesda, MD).

Nothing to Disclose: JDV, NLR, DE, JMM, CYB

P2-344

Usability Testing of a New Pen Injector and a Combination of the New Pen Injector and a New Penmate System To Administer Human Growth Hormone in Three Open-Label User Surveys.

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Objective: To measure patient acceptability of a new injection system, Norditropin FlexPro and FlexPro PenMate for human growth hormone (hGH) administration in children and adolescents with growth hormone deficiency (GHD) currently on GH therapy.
Study Design and Methods: Study UT03 (n=70; 10-18yrs) conducted in the USA, evaluated the safety and performance of Norditropin FlexPro. UT14 (n=74; 6-18yrs) conducted in Japan investigated the overall ease of handling of Norditropin FlexPro. UT13 [n=50 (10 patients/country); 4-17yrs], (USA, Japan, Germany, Sweden, The Netherlands), preference for the combined Norditropin FlexPro/FlexPro PenMate system was evaluated and compared to the Norditropin NordiFlex/NordiFlex PenMate system. Patients were trained to use both injection systems in random order and to inject test medium into a foam cushion. Participants' opinions on the device systems were recorded by questionnaire during a face-to-face interview. Handling of the device systems were assessed using a 4-point rating scale.
Results: The proportion of self-injecting patients varied between countries (USA 100%; Japan 0-24%; The Netherlands 80%; Germany 60%; Sweden 50%). In UT03 and UT14 most patients, 64 and 81%, respectively, preferred Norditropin FlexPro over their current device. In UT03, 100% of patients found it easy to learn how to use Norditropin FlexPro. 99% of patients found it easy to push the dose button and 97% felt confident the correct dose was delivered. In UT13, 80% of participants preferred the Norditropin FlexPro/FlexPro PenMate system over the Norditropin NordiFlex/NordiFlex PenMate system. When questioned 64% of patients in study UT13 said they would be very confident and 20% said they would be confident of using the new device in the future. In contrast 44 and 32% said they would be very confident or confident of using NordiFlex/NordiFlex PenMate in the future. Ease of handling the new device was rated as 'very easy' or 'quite easy' by 99% of patients in UT14.

Patient preferences n (%)

	Norditropin FlexPro	Current	Not sure
UT03	45(64)	11(16)	14(20)
UT14	60(81)	9(12)	5(7)
UT13	40(80)	6(12)	4(8)

Conclusions: The majority of participants in all three studies reported a preference for the new device, Norditropin FlexPro. In the multinational study (UT13), most patients expressed a preference to continue use of the new device. Compared to other countries there were fewer self-injectors in Japan than in other countries.

Disclosures: AMK: Employee, Novo Nordisk. SM: Employee, Novo Nordisk. CB: Employee, Novo Nordisk. TKK: Employee, Novo Nordisk. GSF: Employee, Novo Nordisk.

P2-345

Serum IGF-1 in Patients with Traumatic Brain Injury as a Predictor of Growth Hormone Secretory Response to Glucagon Stimulation Testing.

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Background: The diagnosis of Adult Growth Hormone Deficiency (AGHD) is established through growth hormone stimulation testing (GHST), which is often complex, expensive, time consuming, and may be associated with significant side effects for the patient[1-4]. The decision to perform GH provocative testing is influenced by clinical findings, medical history, and biochemical evidence. Serum IGF-1 can provide important information regarding GH secretion and may yield clues to the likely response of an individual in whom GH stimulation testing is being contemplated [4,5] .
Methods: One hundred thirty eight adult subjects (98 male/40 female) with a history of moderate to severe traumatic brain injury (TBI) were assessed for AGHD using the Glucagon Stimulation Test [6] (GST) and serum IGF-1 levels. Age and body mass index (BMI) were also recorded. All subjects were tested at least one year post TBI. IGF-1 values were compared to peak GH values obtained following GST. A ROC curve analysis[7] was performed to determine the ability of various IGF-1 cutoffs to predict diagnosis of AGHD at several potential diagnostic cutoff points (<3 ng/ml, <5 ng/ml, and <10 ng/ml).
Results: An IGF-1 cutoff value of 175 ng/ml minimized the misclassification of AGHD patients and GH sufficient patients and provided a sensitivity of 83% and specificity of 40%, considering a criterion for peak GH response of 3ng/ml or less. An IGF-1 cutoff value of 175 ng/ml minimized the misclassification of AGHD patients and GH sufficient patients and provided a sensitivity of 74% and specificity of 39%, considering a criterion for peak GH response of 5ng/ml or less. An IGF-1 cutoff value of 200 ng/ml minimized the misclassification of AGHD patients and GH sufficient patients and provided a sensitivity of 84% and specificity of 35%, considering a criterion for peak GH response of 10ng/ml or less.

GST Cutoffs	IGF-1 Cutpoint	Sensitivity	Specificity	ROC AUC
GST < 3 ng/ml	175 ng/ml	83%	40%	0.69
GST < 5 ng/ml	175 ng/ml	74%	39%	0.65
GST < 10 ng/ml	200 ng/ml	84%	35%	0.66

Conclusion: Our current findings provide useful clinical data that may help guide a clinician's decision in the implementation of GHST to detect growth hormone deficiency. Further, our data appear to be consistent with previous work assessing IGF-1 levels in the prediction of GH response using ITT[8].

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Nothing to Disclose: SG, DJZ, JJG, CRG, BEM, RJU

P2-346

Validation of Multi-Frequency Bioelectrical Impedance Analysis (MFBIA) for Measurements of Body Composition: A Study of Growth Hormone and Testosterone Administration in Healthy Adults.

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MFBIA is a convenient, non-invasive and inexpensive method for estimating body composition. It provides an indirect estimate based on the compartmentation of extracellular water (ECW) and intracellular water, from which fat free mass (FFM) and fat mass (FM) are derived. There is a paucity of data comparing MFBIA to established techniques. For the measurement of body composition, dual-energy X-ray absorptiometry (DXA) is a widely accepted method for estimating FM and FFM. FFM comprises body cell mass and ECW, which can be quantified by bromide dilution.
The aim was to compare estimates by MFBIA (Impedimed SFB7TM, Brisbane, Australia) of ECW, FM, and FFM against bromide dilution and DXA. Seventy-one healthy recreational athletes (43 men, 28 women; aged 18-40 years; BMI 24 ± 0.4 kg/m2) were studied in a double-blinded, randomized, placebo-controlled design before and after treatment with growth hormone and testosterone (1). Data were analyzed using linear regression and the Bland-Altman method.
At baseline, there was a significant correlation between MFBIA and bromide dilution for ECW ($r2 = 0.84$, $p < 0.001$) and DXA for FM and FFM ($r2 = 0.79$ and 0.91, respectively; $p < 0.001$). ECW was 3.5 ± 1.1 % lower, FM was 22.4 ± 3.7 % lower and FFM was 13.7 ± 1.0 % higher with MFBIA compared to the established techniques ($p<0.01$). During the treatment, GH increased ECW and FFM and reduced FM; these changes correlated significantly between the methods ($r2$ for ECW, = 0.35; FM = 0.21; FFM = 0.61; $p < 0.001$). The change in ECW and FFM was not significantly different between the methods, however FM was 29.0 ± 8.8 % lower with MFBIA ($p<0.01$).
In summary, good correlation was observed between MFBIA and the established techniques for measuring ECW, FM, and FFM. At baseline, MFBIA estimates of ECW was slightly and FM substantially lower, while FFM higher compared to bromide dilution and DXA. During the treatment, only FM estimated by MFBIA was lower compared to DXA. We conclude that MFBIA is an acceptable tool for measuring ECW, however it substantially under-estimates FM compared to DXA.

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Nothing to Disclose: CHK, VB, UM, AEN, KKYH

P2-347

Cephalometric Features in Adults with Isolated and Untreated GH Deficiency.

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Craniofacial growth (CFG) has never been accurately studied in adult individuals with untreated isolated GH deficiency(IGHD). We assessed CFG in nine adult GH-naive IGHD individuals with a mutation in the GHRH receptor gene from Itabaininha, Brazil. We performed a cephalometric study, including nine linear and five angular measurements. In addition, posterior facial height (PFH)/anterior facial height (AFH) and lower-anterior facial height(LAFH)/AFH ratios were calculated. Cephalometric measurements were compared to an atlas of normal Brazilian population, while stature and cephalic perimeter (CP) to normal Itabaininha controls.Stature and CP were reduced in IGHD subjects, both in absolute values and standard deviation scores (SDS). In absolute values, all linear cephalometric measurements were reduced, with AFH, total mandibular length, and PFH being the most markedly reduced. PFH/AFH and LAFH/AFH ratios were not different from those of the reference group. Angular measurements were similar between IGHD and the reference groups, with the exception of the gonial angle (the angle between the ramus and the corpus of the mandible) which was significantly greater in IGHD subjects. Looking at SDS' values, total maxillary length was the most reduced parameter, followed by a cluster of 5 measurements, while the less affected ones were the lower-anterior facial height and the mandibular ramus height.

Table 2: Standard Deviation Score (SDS) of the variables

	Mean ± SD
Height	-6.5 ±1.2
Cephalic Perimeter	-3.7 ±1.0
Linear Measurements	
Total Maxillar length	-6.5 ± 1.7
Posterior Cranial Base Length	-4.9 ± 1.1
Total Mandibular length	-4.4 ± 0.7
Total Posterior Facial Height	-4.4 ± 1.1
Total Anterior Facial Height	-4.3 ± 0.9
Mandibular Corpus length	-4.2± 0.8
Anterior Cranial Base Length	-4.1 ± 1.7
Lower-anterior	-2.7 ± 0.7
Mandibular Ramus height	-2.5 ± 1.5
Angular Measurements	
Mandible-Cranial base relation	-1.0 ± 2.4
Maxilla-Cranial base relation	-1.0 ± 1.6
Maxilla-Mandible relation	-0.2 ± 1.5
Mandibular Plane Angle	-0.0 ± 1.8
Gonial Angle	2.5 ± 1.1

In conclusion, congenital, lifetime untreated IGHD causes reduction in all linear measurements of CFG, particularly in total maxillary length, which is probably the most characteristic craniofacial feature of this syndrome. Angular measurements and facial height ratios are less affected, suggesting that lack of GH causes proportional blunting of CFG.

Disclosures: RS: Advisory Group Member, Novo Nordisk; Researcher, Novo Nordisk.
Nothing to Disclose: LAO-N, MFBM, AAF, AHAO, AHOS, EHOV, MBG-J, MHA-O

P2-348

Assessment by Short Form-36 of Quality of Life in the Adult Patients with Severe Growth Hormone Deficiency from the Data of 6-Month Growth Hormone Treatment in Post-Marketing Surveillance for Humatrope® in Japan.

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Impaired Quality of Life (QOL) is recognized as one of the important clinical feature of patients with growth hormone deficiency in adults (AGHD). Beneficial effects of growth hormone (GH) therapy on QOL improvement have been topics of reports in Europe and the US. In addition, the degree of QOL impairment is mild in the patients with childhood-onset type AGHD (CO-AGHD) compared to those with adult-onset type AGHD (AO-AGHD). The similar trends have been reported in small-sized preliminary Japanese clinical trials. To further assess the effect of GH treatment on QOL in Japanese patients with AGHD, we studied time-dependent Short Form (SF)-36 scores in 402 patients participating in post-marketing surveillance for Humatrope® in Japan. At present, QOL data were collected 142 cases (AO-AGHD: 97 cases, CO-AGHD: 45 cases) at the enrollment and from 105 cases (AO-AGHD: 70cases, CO-AGHD: 35cases) after 6-month GH treatment. The mean sub-domain scores of AGHD patients at the enrollment were all lower than those of Japanese general populations, except for 'Bodily Pain' sub-domain score. In 105 cases treated with growth hormone during 6-month treatment, the mean scores of QOL sub-domains in Physical Functioning ($p=0.002$, paired t-test), Vitality ($p=0.002$), Social Functioning ($p=0.002$) and Role Emotional ($p=0.017$) were significantly improved as compared with those at enrollment. In AO-AGHD, sub-domains in Vitality ($p=0.015$), Social Functioning ($p=0.011$), and Role Emotional ($p=0.032$) were significantly improved, whereas in CO-AGHD only sub-domain in Physical Functioning ($p=0.007$) was significantly improved. Although SF-36 is not a specific questionnaire for AGHD so as being rather insensitive to assess the QOL of AGHD patients, the present study revealed that impaired QOL of Japanese AGHD patients was improved with 6-month GH treatment at least in some sub-categories of SF-36.

Sources of Research Support: Eli Lilly and Company.

Disclosures: AS: Advisory Group Member, Lilly USA, LLC. ST: Clinical Researcher, Lilly USA, LLC. TT: Consultant, Lilly USA, LLC. KF: Advisory Group Member, Lilly USA, LLC. AT: Consultant, Lilly USA, LLC. KC: Advisory Group Member, Lilly USA, LLC.

P2-349

Dutch National Registry of Growth Hormone Treatment in Adults – Diagnostic Procedures.

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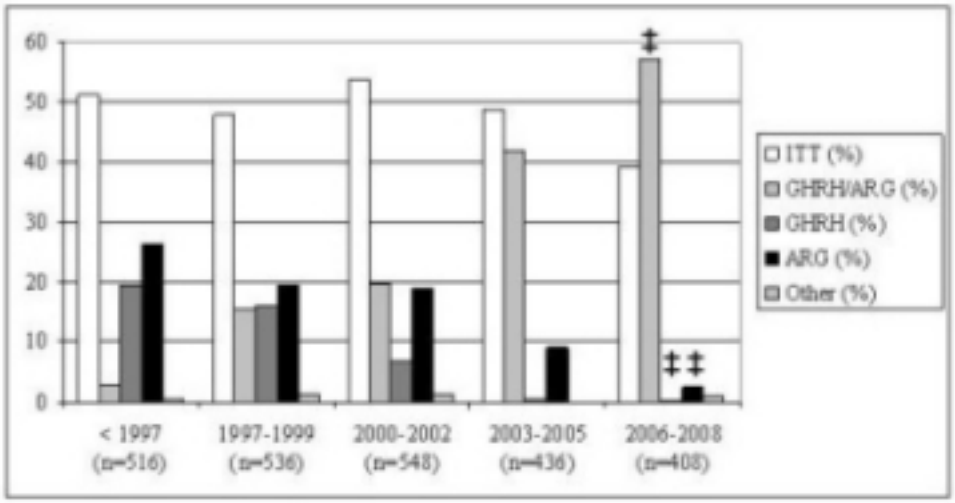
Summary

Objective: The Dutch National Registry of Growth Hormone Treatment in Adults was established in 1998 on the advice of the former minister of health. Main goals were to gain more insight into long-term efficacy, safety and costs of growth hormone therapy (GHT) in growth hormone deficient (GHD) adults in the Netherlands.

Methods: Diagnostic test procedures of all registered patients were evaluated and divided into three categories: insulin-like growth factor-I (IGF-I) level ≤ - 2 SD combined with peak GH response below the threshold for the specific stimulation test used, IGF-I level below -2 SD and two or more additional pituitary hormone deficits, or continuation of GHT without retest in children with multiple pituitary deficits and evident hypothalamic-pituitary disease.

Results: Until January 2009, 2890 patients, 1474 men, were registered. Each year, 219 ± 35 patients entered the registry. Mean age at time of (re)evaluation was 43.1 ± 16.2 years. In 85% of the patients, a GH stimulation test was performed, in the majority an ITT (49%) or combined GHRH-arginine test (25%). In 12% of the patients, IGF-I levels were ≤ -2 SD combined with two or more additional pituitary hormone deficits, and 2% of the patients were children continuing GHT. Over the years, the test of first choice shifted from the ITT towards the GHRH-arginine test.

Figure 1. Provocative GH tests used for diagnosing severe GHD in adults per 3-year period.



‡ Significant difference over time when compared to percentage ITT performed, $p \leq 0.01$.

Conclusion: 2890 patients were included in the registry until January 2009. In 85% of these patients, the diagnosis GHD was established by provocative testing, particularly an ITT or a combined GHRH-arginine test.

Nothing to Disclose: ICvN, CCvB, LIA, AAMF, HPFK, AJvdL, JWRT, MB, MLD

P2-350

Growth Hormone Treatment in Adults with Prader-Willi Syndrome Increases Bone Formative Markers, but Not Bone Resorption and Bone Mass: Results from a 12 Months Placebo-Controlled Study.

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Background: Bone mineral density (BMD) in adult Prader-Willi syndrome (PWS) is reduced, maybe due to high bone turnover. In this study BMD and biochemical markers of bone formation and resorption were assessed before and after 12 months of growth hormone (GH) treatment.
Design: Forty-three adults (22 women), mean age of 28.3 (+/-6.8) yr with genetically verified PWS were randomized to GH or placebo treatment for 12 months. The first 4 weeks doses were 0.3 mg/day respectively 0.4 mg/day if body weight was below or above 100 kg, followed by 0.6 mg/day (0.8 mg/day). BMD was assessed by dual x-ray absorptiometry at baseline and after 12 months of treatment as were serum markers of bone formation (PINP, osteocalcin) and resorption (NTx), together with insulin-like growth factor 1 (IGF-1).
Results: IGF-1 increased from mean (+/-SD) 15.4 (6.5) to 23.8 (9.6) nmol/l with GH treatment, $p< 0.01$. Compared to age matched controls BMD at baseline was (z-score and 95% confidence interval) in lumbar spine L1-L4 (LS) -1.43 (-1.03- -1.83), total femur -1.52 (-1.28- -1.78), femoral neck -1.54 (-1.29- -1.80) and total body(TB) -0.97 (-0.60- -1.34). Men had lower z-score BMD than women in LS mean (+/-SD) -1.9 (1.2) versus -1.0 (1.0) and in TB -1.5 (1.1) versus -0.5 (1.0), both $p< 0.05$. No difference in BMD was observed with or without sex hormone replacement (testosterone in 8 men, oestrogen in 6 women). At 12 months there was a change in LS BMD of -2.1% (3.4) on GH treatment compared to placebo +3.1 (5.4), $p< 0.05$, mainly do to a decrease in women by -3.1% (2.9) on GH versus an increase of +4.1% (2.4) on placebo, $p< 0.05$. Other significant changes in BMD were not observed.
Increase with GH were observed for PINP from 66.4 (30.8) to 118.6 (60.2) µg/l, $p< 0.001$ (expected values female 19-83, male 22- 87) osteocalcin from 4.4 (3.2) to 10.1 (8.6) ng/l, $p< 0.05$, (expected values 5- 25). NTx did not change, baseline 19.9 (6.7) nM, (expected values female 7.7- 19.3, male 8.1- 24.8).
Conclusions: This cohort of PWS adults, and in particular the men, had low BMD that did not improve with 12 months of GH treatment, despite an increase in bone formation markers. To obtain an increase in BMD, longer GH treatment seems necessary.

Disclosures: RS-C: Clinical Researcher, Novo Nordisk. TS: Clinical Researcher, Novo Nordisk. KFR: Clinical Researcher, Novo Nordisk. SF: Clinical Researcher, Novo Nordisk. JSC: Investigator, Novo Nordisk; Speaker Bureau Member, Novo Nordisk; Advisory Group Member, Novo Nordisk. JB: Investigator, Novo Nordisk.
Nothing to Disclose: APJ, TU, CH

P2-351

Application of a Pharmacokinetic-Pharmacodynamic Model for a Pegylated, Long-Acting Human Growth Hormone Developed for Once-Weekly Administration.

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Recombinant human Growth Hormone (rhGH) is usually administered as daily s.c. injections, which poses a challenge for both children and adults treated with rhGH for several years and has prompted research into alternative approaches of GH administration. NNC126-0083 is a pegylated rhGH developed to prolong the exposure of the product in the blood circulation, allowing for a once weekly treatment. The objective of this analysis was to develop a population pharmacokinetic/pharmacodynamic (PK/PD) model for NNC 126-0083 and its effects on circulating insulin-like growth factor-I (IGF-I) levels in adults with growth hormone deficiency (AGHD) and to use the model in the dose design of a long-term trial.
Methods/Subjects: The model was developed using the software NONMEM and was based on data from 30 AGHD patients (26-63 years; 54-103 kg). Subjects were dosed in four cohorts of 7-8, once weekly for three weeks, with s.c. injections of NNC126-0083 (n=6) or placebo (n=2). Doses escalated between cohorts (10, 20, 40, and 80 mg protein/kg). Individual PK/PD profiles were sampled for 168 and 240 hours after the first and the third dose, respectively.
Results: In the PK model, drug is absorbed into a central compartment by two serial first-order processes. Elimination takes place by a first-order and a saturable pathway. The simulation showed the predicted weekly mean IGF-I levels to be centred at the target IGF-I mean value of 1 SDS, after fixing of the dose by the end of Week 8. The dose titration target is to reach a mean IGF-I SDS value during steady state after fixing the dose, reflecting the "average" IGF-I exposure during the week.
Conclusion: A PK/PD model for NNC 126-0083 in AGHD patients has been developed to be used during dose titration in a long-term trial in AGHD patients.

Disclosures: TK: Employee, Novo Nordisk. JM: Employee, Novo Nordisk. BSH: Employee, Novo Nordisk. MHR: Employee, Novo Nordisk.

P2-352

Twelve Months Efficacy and Safety of a Novel Once-a-Week Sustained Release rhGH (LB03002): A Phase III Study in Adults with GHD.

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LB03002 is a novel sustained-release rhGH formulation in sodium hyaluronate microparticles, administered once-a-week (wk) by subcutaneous injection. The long term efficacy and safety of LB03002 administered to adults with GHD were evaluated in a phase III study for 26+26 wks. Adults with proven GHD (N=152) of either adult- or childhood-onset from Europe and the US were treated in a ratio of 2:1 (LB03002:Placebo [PL]) for 26 wks (as reported at ENDO 09; P2-746); after which, subjects were offered the opportunity to participate in a 26 wk open-label extension (rollover) study in which 136 subjects were treated with LB03002, reported herein. Doses were adjusted according to IGF-I and safety profiles. At wks 26 and 52, most patients who had been randomized to LB03002 during the first part of the study remained at the target range of IGF-I SDS within -0.5 and +1.5. Patients rolling over from PL to LB03002 had the expected low IGF-I SDS at wk 26, but reached target IGF-I SDS at wk 52. Treatment with LB03002 for either 26 or 52 wks produced a consistent and sustained reduction of fat mass as assessed by DXA.

Mean Fat Mass Change(kg)

	LB03002(26wks)→LB03002(26wks)	PL(26wks)→LB03002(26wks)
N	93	50
Baseline(SD)	27.83(8.88)	26.72(10.07)
N	93	50
Change from baseline at Wk 26(95%CI)*	-1.07(-1.543,-0.596)	0.57(-0.205,1.345)
N	88	39
Change from baseline at Wk 52(95%CI)*	-1.06(-1.733,-0.396)	-1.12(-1.749,-0.482)

*p<0.001

LB03002 showed a good safety profile at 26 and 52 wks, with a low incidence of SAEs or AEs leading to discontinuation/ modification of study drug. There were no clinically relevant abnormal laboratory parameters. There was a lower incidence of injection site reactions during the rollover study than during the first 26 wks. The majority of patients assessed the discomfort at injection site, including pain, warmth, erythema and swelling, as none or mild at most visits. **Conclusion:** The dose adjustment algorithm was effective in maintaining IGF-I SDS within the target range. Treatment with LB03002 for 26 or 52 wks in adults with GHD demonstrated a sustained reduction of fat mass and other body composition parameters with a good safety profile and acceptable tolerability. These additional 26 wk open-label data extend the initial positive report from the PL-controlled phase, demonstrating continued efficacy and safety in a large group of patients taking LB03002.

Disclosures: BMKB: Study Investigator, Biopartners; Consultant, Novo Nordisk, Pfizer, Inc.; Researcher, Lilly USA, LLC. CJS: Advisory Group Member, Lilly USA, LLC, Pfizer, Inc.; Study Investigator, Biopartners; Researcher, Ipsen, Novartis Pharmaceuticals; Consultant, Merck & Co., Novo Nordisk.
Nothing to Disclose: H-JJ, SL, CS, VP, MC, JR, MP, DMC

P2-353

A Novel Human Growth Hormone XTEN Construct (VRS-317) for Monthly Administration: Long Half-Life and Sustained In Vivo Potency.

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The extended half-life (XTEN) recombinant human growth hormone product, VRS-317, may provide comparable efficacy and safety to daily rhGH with a monthly dosing interval. VRS-317 consists of rhGH with XTEN amino acid sequences genetically fused to the N terminus and the C terminus, and is produced efficiently as a soluble protein in E. coli without the need for refolding. The XTEN domain of VRS-317 is a non-immunogenic and biodegradable amino acid sequence. In rats, VRS-317 had a terminal half-life after intravenous dosing of 15 hr. VRS-317 administered every other day showed equal potency to daily injections of hGH as measured by body weight gain in hypophysectomized rats, even when administered at a three-fold lower molar dose (Fig 1). In monkeys, VRS-317 administered at 1.5 mg/kg IM or SC had very rapid absorption, >80% absolute bioavailability, and a 90-110 hr terminal half-life (Fig 2). A dose dependent increase in IGF-I levels was noted in monkeys administered 0.3, 1.5, or 7.5 mg/kg VRS-317 with increases above baseline of 71%, 204% and 335% , respectively at 216 hr post-dose. Predictions from allometric scaling suggest that the half-life of VRS-317 in humans will be greater than 200 hr. Based on preclinical efficacy and pharmacokinetics, a likely monthly dose of no more than 1.5 mg/kg in pediatric patients is predicted for VRS-317. No significant lipoatrophy was observed in a pig model. VRS-317 may be formulated to enable room temperature stability. Repeat dose immunogenicity and monthly maintenance of IGF-I responses are being assessed in juvenile monkeys. VRS-317 may provide a significant improvement in convenience, safety and efficacy for growth hormone deficient patients. Further development of this promising, long-acting rhGH product is ongoing with initial clinical development projected for late 2010.

Figure 1: Change in body weight of hypophysectomized rats (n=6/group) after SC administration of hGH or VRS-317.

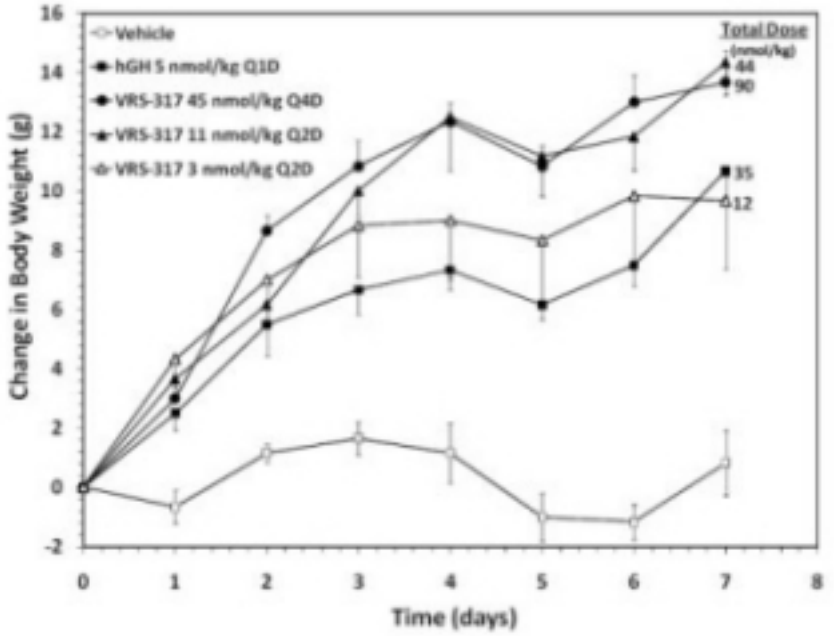
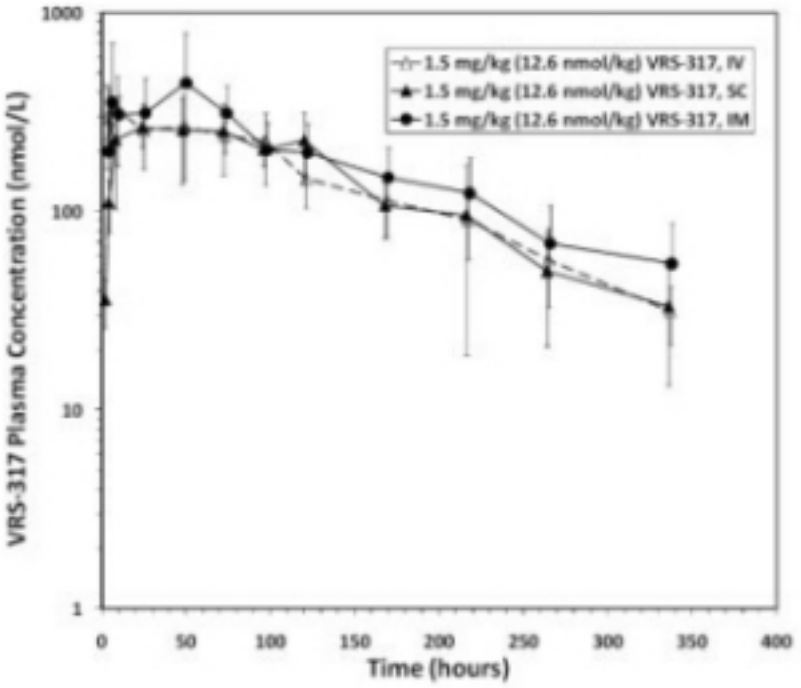


Figure 2: VRS-317 Pharmacokinetics in Cynomolgus Monkeys (n=4/group) dosed with 1.5 mg/kg IV, SC or IM.



Nothing to Disclose: JLC, NG, BS, LL, SM, YY, JS

P2-354

Dose-Ascending, Safety, Tolerability, Pharmacokinetics and Pharmacodynamics Study of a Novel Long-Acting Recombinant Growth Hormone in Healthy Volunteers.

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Background
ACP-001 has been developed with the aim of alleviating the need for daily growth hormone injections in growth hormone deficient patients. ACP-001 has been developed as a long acting prodrug suitable for once-a-week dosing. ACP-001 is designed as a PEGylated prodrug that releases unmodified hGH with a known mode of action and original volume of distribution while maximizing the stimulating effect in adipose and bone tissue. Due to the inactive nature of the prodrug complex, the risk of injection site lipoathrophy is minimized as the growth hormone activity at the injection site is greatly reduced. The prodrug is absorbed into the blood compartment, where it acts as a circulating hGH reservoir, releasing the majority of hGH payload.
Objectives
The objectives of this study were to investigate 1) safety and tolerability of ACP-001, a once-a-week human Growth Hormone, in comparison to Placebo and 2) pharmacokinetics and pharmacodynamics of ACP-001, in comparison to daily hGH injections.
Method
44 healthy, male volunteers in the age range of 20-45 and BMI of 18.5 - 29.9 kg/m2 were randomized to one of four dose levels of ACP-001 (equivalent to 0.04; 0.08; 0.16 and 0.24 mg/kg/week liberated growth hormone) given once, or one of two dose levels of daily hGH (0.08 and 0.16 mg/kg/week), given as 7 daily injections, or placebo. Pharmacokinetic and pharmacodynamic (IGF-1, IGFBP-3) samples were taken up to 240 hours, followed by an additional follow-up of 32 days for binding and neutralizing antibodies.
Results
Four different doses of ACP-001 and two different doses of daily hGH were administered. All doses were well tolerated, with no observations of concerning injection site tolerability issues. No SAE occurred. Preliminary data showed pharmacokinetic dose proportionality of different dose levels of ACP-001. Dose-dependent IGF-1 elevation was observed for all doses with IGF-1 levels remaining elevated throughout the week.
Conclusion
ACP-001 was safe and well tolerated in this Phase 1 study. Injection site reactions were rare, transient and mild in nature. No antibodies were detected up to 42 days. Pharmacokinetic results are in agreement with a once-a-week dosing. ACP-001 demonstrated a consistent IGF-1 elevation over one week, indicating it is at least as potent as daily hGH with regards to IGF-1 elevation based on protein equivalents.

Disclosures: MB: Clinical Researcher, Ascendis Pharma. PG: Employee, Biovail Contract Research. ZT: Employee, Biovail Contract Research. DG: Employee, Ascendis Pharma A/S. KS: Employee, Ascendis Pharma A/S. TSN: Employee, Ascendis Pharma Inc.

P2-355

Protocol for Early Introduction of Growth Hormone Therapy in PWS Yields Good Clinical Results.

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Background: Growth Hormone therapy (GH) in children with Prader-Willi Syndrome (PWS) has been shown to be beneficial in decreasing body fat percentage, improving lean body mass, and increasing linear growth and resting energy expenditure. GH is often initiated after several years. Very early initiation of growth hormone therapy could have direct benefits on psychomotor development and muscle tone; however, studies have not been conducted in infants. We therefore report a protocol for early initiation of growth hormone therapy in infants with PWS.
Approach: Our multidisciplinary PWS clinic has been successfully using a protocol for early initiation (ages 3-4 months) of growth hormone therapy with good clinical results. All patients undergo polysomnography assessments to evaluate for central apnea and sleep disordered breathing prior to the initiation of growth hormone. Low-dose growth hormone is started as early as 3 months and gradually increased with careful attention to subsequent sleep studies and side effect profiles with close follow-up.
We compared our patients with early GH start to a cohort of PWS patients whom did not receive growth hormone therapy. The early intervention group demonstrated earlier achievement of developmental milestones (sitting up, talking, transfer of objects, ect), younger age of g-tube removal, and improved body composition profiles.
Conclusion: Introduction of growth hormone therapy as early as 3-4 months of age in the Prader-Willi population can be safe and effective when closely monitoring for obstructive/central apnea with repeated polysomnography assessments and side effect evaluations. Growth hormone is beneficial for improving body composition and motor development which impacts cognitive development and should be considered in early infancy.

Disclosures: DSH: Researcher, Pfizer, Inc., Ipsen.
Nothing to Disclose: ASD, JFA, KA

P2-356

Age- and Muscle Mass-Independent Effect of Body Fat on GH Release after an Overnight Fast.

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Endogenous release of GH in men has been variably reported to be determined individually or jointly by age, fat mass, muscle mass, androgenic state, and physical activity. These factors were considered in the present study of 32 healthy men with diverse levels of physical activity and fitness, and respective age and BMI spectrum of 19-78 yrs, and 21-38 Kg/m^2. Study protocol was conducted in each subject after an overnight fast, starting between the hours of 0800-0900 and continuing for a total period of 6.5 hours. Serum concentrations of GH were measured in all blood samples collected at 10-min intervals over the study period. IGF1 and IGFBP-3 were assayed in a single fasting blood specimen. DXA was used to compute total body fat mass (FM), trunk fat mass (TFM), and appendicular muscle mass (AMM). Appendicular muscle mass index (AMMI), fat mass index (FMI), and trunk fat mass index (TFMI) were calculated by dividing AMM, FM, and TFM in Kg by square of height in meter. Visceral fat area (VFA) was determined by CT scan. GH secretory properties were assessed by decovolution analysis. Regression analysis did not identify age or muscle mass (AMMI) as a significant correlate of either measures of GH concentration or secretion, except for age having a positive correlation with the number of GH pulses during the 6.5 hr period (R: 0.45; P:0.01). On the other hand, VFA was negatively and significantly correlated with the 6.5 hr sum mass (R: -0.62; P: 0.0001), and mean (R: -0.62; P: 0.0001) GH concentrations. While not true for pulse frequency, VFA was a negative determinant of basal (R: -0.36; P: 0.048) and pulsatile (R: -0.59; P: 0.0003) GH release, as well as mass of GH secreted per burst (R: -0.46; P: 0.008). DXA-derived measures of body fat correlation with GH secretory parameters and GH concentrations were identical to VFA. Alternatively, age proved to be a negative predictor of IGF-1 (R: -0.67; P: <0.0001) with no effect of visceral fat, when adjusted for age. Neither age nor VFA were correlated with IGFBP-3. In summary, body fat (being general or local) independent of age has significant impact on circulating GH concentration, which appears to be due to compromised basal and to a much higher extent pulsatile mode of GH release. Correlation of age rather than body fat with IGF-1 is in contrast to GH results, and requires further investigation.

Sources of Research Support: Salem V.A. Medcial Center Research Institute.

Nothing to Disclose: DL, BD, JDV, AI

P2-357

The GH Monitor Registry: Trends in Serum IGF-I in Saizen®-Treated Patients.

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Many of the actions of growth hormone (GH) are mediated by insulin-like growth factor-I (IGF-I). Measurements of serum IGF have been used to assess adherence and effectiveness during GH treatment, and as a guide to dosing. The GH Monitor[SM] was a longitudinal, electronic registry for collecting information related to recombinant human GH (Saizen®) therapy in pediatric patients (0–20 y). In addition to demographic, efficacy, and safety data, the GH Monitor also allowed for entry of IGF-I levels. Other GH databases contain scant IGF-I data. Herein, we present the trends in IGF-I data from the GH Monitor. A total of 2446 patients were enrolled in the database, 1955 of whom were naive to GH treatment. IGF-I data were available for 1603 (82.0%) of the GH-naive patients. Although Saizen is indicated only for GHD, the registry also allowed patients to be entered under other diagnoses, the two most common of which were idiopathic GHD (IGHD) and "idiopathic short stature" (ISS). IGF-I data were available for 877 IGHD patients (829 baseline values, 1729 interim values, and 38 exit values) and 337 ISS patients (309 baseline values, 478 interim values, and 5 exit values). IGF-I data for patients in these two subgroups with a pre-treatment value and at least one follow-up value are presented in the Table. Regardless of diagnosis, IGF-I levels started at and rose to similar levels.

IGF-I Data for IGHD and ISS Patients

	Baseline	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
IGHD						
Median mo. from baseline	0.0	9.8	19.2	26.5	31.8	37.3
No. of patients	688	389	246	151	78	43
Mean IGF-I (ng/mL)∗†	143.3	360.4	391.0	394.2	419.6	457.0
Mean IGF-I SDS∗	-1.85	0.04	0.27	2.74	0.65	0.67
Mean height SDS∗†	-2.17	-1.77	-1.44	-1.25	-1.22	-1.11
Mean change in height SDS∗†		0.47	0.81	1.07	1.22	1.27
ISS						
Median mo. from baseline	0.0	9.1	19.5	30.6	33.9	36.0
No. of patients	277	141	81	47	29	15
Mean IGF-I (ng/mL)∗†	169.4	349.3	405.3	422.4	473.1	366.8
Mean IGF-I SDS	1.08	0.20	0.56	0.46	1.09	0.60
Mean height SDS∗†	-2.32	-2.08	-1.71	-1.46	-1.38	-1.30
Mean change in height SDS∗†		0.46	0.73	0.92	1.08	1.23

∗Increases over time: baseline to visit 2 (P<0.005), †Increases over time: visit 2 to later visits (P<0.005)

In conclusion, IGF-I concentrations increased during Saizen treatment and may be a useful early indicator of treatment efficacy.

Sources of Research Support: EMD Serono.

Disclosures: RR: Speaker, Serono, Genentech, Inc., Novo Nordisk, Pfizer, Inc.; Principal Investigator, Lilly USA, LLC. CB: Medical Advisory Board Member, Serono. SF: Medical Advisory Board Member, Serono. MG: Principal Investigator, Lilly USA, LLC, Genentech, Inc., Novo Nordisk; Pfizer, Inc., Tercica; Medical Advisory Board Member, Serono, Endo Pharmaceuticals, Pfizer, Inc., Tercica; Speaker Bureau Member, Endo Pharmaceuticals, Genentech, Inc., Novo Nordisk, Tercica; Grant Review Panel, Genentech, Inc. PP: Medical Advisory Board Member, Serono; Speaker Bureau Member, Serono. JS: Medical Advisory Board Member, Serono. SD: Employee, Serono. BH: Employee, Serono. RB: Employee, Serono. AA-S: Employee, Serono.

P2-358

The GH Monitor Registry: Efficacy of the Delivery of Saizen Via the cool.click® Needle-Free Device Versus Needle-Based Devices.

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Saizen® is a recombinant human growth hormone (GH) therapy indicated for the treatment of GH deficiency (GHD). The GH Monitor[SM] was a longitudinal, electronic registry established to gather data on pediatric patients treated with Saizen. The majority of patients in the registry used the cool.click® needle-free device (CCNFD) for administration of Saizen with the remainder using various needle-based devices (NBD). Demographic, efficacy, and safety (presented elsewhere) data were collected over a 6-yr period, with analysis herein confined to only GH-naive patients. Efficacy was evaluated through changes in height standard deviation score (SDS) and mean annualized height velocity (AHV). Although originally designed to capture data from children treated for GHD, the registry also allowed patients to be entered with other diagnoses. The two most common diagnoses were idiopathic GHD (IGHD) and idiopathic short stature (ISS) (Table). Generally, demographics in these two populations were similar; however, CCNFD patients had a significantly higher mean height SDS at study entry compared with NBD patients in both the IGHD (0.27 SDS higher, *P*<0.01) and ISS (0.34 SDS higher; *P*<0.01) groups. Efficacy of Saizen delivered via CCNFD appeared comparable to NBD devices (Table), although, in IGHD patients, CCNFD users had a significantly greater mean 1st-yr AHV compared with NFD users (0.68 cm/yr greater, *P*<0.01). In conclusion, registry patients receiving Saizen using CCNFD had similar overall growth as those who received Saizen via NBD.

Demographics and Efficacy Results for the Two Most Common Diagnoses

	IGHD		ISS	
	CCNFD (n=712)	NBD (n=274)	CCNFD (n=233)	NBD (n=113)
Age at screening, y	10.6 (3.5)	10.4 (4.2)	11.1 (2.9)	10.8 (3.6)
Male, n (%)	519 (73.7)	202 (74.0)	158 (68.4)	74 (65.5)
Height SDS at entry	-2.0 (0.9)∗	-2.3 (0.9)	-2.2 (0.7)∗	-2.6 (0.8)
Saizen dose at entry, mg/kg/wk	0.30 (0.1)	0.28 (0.1)	0.33 (0.1)	0.31 (0.1)
Change in height SDS from study entry to end (all pts)	0.86 (0.8)	0.74 (0.7)	0.83 (0.8)	0.67 (0.6)
Change in height SDS from study entry to end (pts with ≥3 y follow-up	1.34 (0.8), n=168	1.41 (0.8), n=48	1.5 (0.7), n=42	1.33 (0.6), n=17
1st-y AHV, cm/y	9.05 (2.4)∗	8.37 (2.7)	8.56 (2.9)	8.61 (2.1)

Data are presented as mean (SD) unless otherwise noted, ∗P<0.01 compared with NBD.

Sources of Research Support: EMD Serono.

Disclosures: MG: Principal Investigator, Lilly USA, LLC, Genentech, Inc., Novo Nordisk, Pfizer, Inc., Tercica; Medical Advisory Board Member, Serono, Endo Pharmaceuticals, Pfizer, Inc., Tercica; Speaker Bureau Member, Endo Pharmaceuticals, Genentech, Inc., Novo Nordisk, Tercica; Grant Review Panel, Genentech, Inc. CB: Medical Advisory Board Member, Serono. SF: Medical Advisory Board Member, Serono. PP: Medical Advisory Board Member, Serono; Speaker Bureau Member, Serono. RR: Speaker, Serono; Principal Investigator, Lilly USA, LLC; Speaker, Genentech, Inc.; Speaker, Novo Nordisk; Speaker, Pfizer, Inc. JS: Medical Advisory Board Member, Serono. SD: Employee, Serono. BH: Employee, Serono. RB: Employee, Serono. AA-S: Employee, Serono.

P2-359

Growth Hormone Treatment in Australia: Historical Trends and Demographics (OZGROW).

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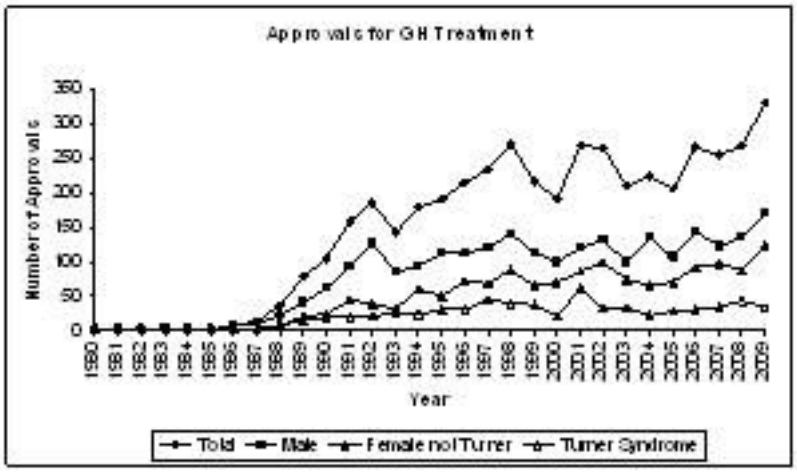
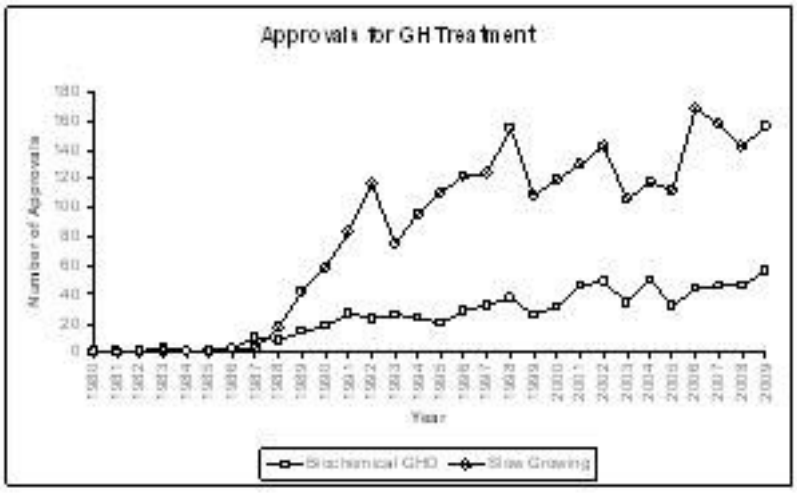
Background: Since 1989 all children receiving growth hormone (GH) as part of the Australian Government's Pharmaceutical Benefit Scheme (PBS) have been recorded and their treatment monitored. Basic demographic and clinical information has been collected on each patient at each visit and recorded in a national database (OZGROW). Here we investigate the trends in diagnosis and demographics of the GH treated cohort in Australia since 1980.
Methods: Analyses were based on the whole cohort, by gender, and by indication. There are 7 clinical indications defined by the Government for which GH is subsidised by the PBS: Biochemical GH Deficiency (GHD), Slow Growing (SG), Turner Syndrome (TS), Chronic Renal Insufficiency (CRI), Cranial Lesion/Irradiation (CLI), Hypoglycaemia (Hg), and Prader-Willi Syndrome (PWS, first included in 2009).
Results: Fig. 1 shows the total number of Approvals in each year from 1980 to 2009 with Male, Female-(not TS), and TS shown separately. Fig 2 shows the Approvals of the two major indications SG and GHD. Total Approvals for each Indication and gender are GHD (Male:456, Female:283), SG (1548, 918), TS (5,656), CRI (153,74), CLI(212,132), Hg (18,17), and PWS (25,28). Significantly ($P<10^{-5}$) more males than females were approved for all indications except Hg, PWS, and TS. Median age at Approval was 104.2mths (Male:102.3, Female106.1, P=0.11), GHD(82.5), SG(107.0), TS(99.9), CRI(99.1), CLI(129.2), Hg(6.0), PWS(67.0). Median period Approval to Ceased, 53.4mths (Male:57.4, Female:49.5, P=0.002), GHD(62.0), SG(50.3), TS(66.8), CRI(43.2), CLI (51), Hg(170.3) but this is highly influenced by age at Approval. Median age at Approval of Active GH recipients: 87.6mths (Male:91.8, Female 82.7 P=0.001, Female –TS 85.2, P=0.003).
Conclusions: Total, (mostly SG), Approvals increased rapidly from 1989 to 1998 before again increasing from 2005. There have consistently been more boys than girls Approved for GH treatment for most indications. Duration of treatment is longer for males although, of Active recipients, Females are Approved earlier.





Sources of Research Support: We would like to thank the participants and their carers for consenting to provide the

clinical data used in this work. We also thank the treating paediatric endocrinologists and nurses for collection of this data. We acknowledge the Pharmaceutical Benefits Division of the Department of Health and Ageing for their support of OZGROW and in the in the collection of GH data.

Nothing to Disclose: IPH, CC, AC, MH

P2-360

Socio-Economic Parameters in Adults with Childhood Onset Growth Hormone Deficiency.

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Introduction
A high number of primary diseases may result in growth hormone deficiency (GHD). Questionnaire studies in adults with childhood onset GHD have identified various affected socio-economic parameters, among others a significantly reduced income and number of partners. The persons investigated were typically only adults treated with GH in childhood.
Materials and methods
Using the unique Danish registries, we compared socio-economic parameters in a nationwide identified cohort of adults with childhood onset GHD with a cohort of controls with the same gender, all individually matched on age.
Four-hundred-and-three GHD persons, whereof 246 were men, were compared with 38,558 controls, whereof 23,653 were men. The parameters were marital status, income, children, education, unemployment, and retirement. Statistical analyses applied were conditional logistic regression and Cox regression, where each GHD person and his or her controls were one stratum. Furthermore, we calculated mortality ratios adjusted for age and calendar-time, and further adjusted for marital status and education. Subgroups of GHD persons with malignant tumors, craniopharyngioma, idiopathic GHD, and others were investigated separately.
Results
Severe consequences were identified on all socio-economic parameters. Total mortality was significantly increased, however when adjusting for socio-economic factors the GHD men seemed to reduce their mortality ratio markedly.
Conclusion
Socio-economic parameters in adults with childhood onset GHD are severely affected; only in the subgroup of persons with idiopathic GHD some parameters were comparable with the background population.

Sources of Research Support: Kirstine Stochholm was supported by a research grant from Central Denmark Region; Kirstine Stochholm was supported by a research grant from the Danish Ministry of Science, Technology and Innovation.

Nothing to Disclose: KS, CHG

P2-361
Role of Bcl-G in Male Germ Cell Apoptosis.

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Introduction: We have previously demonstrated that germ cell apoptosis is an important mechanism to suppress spermatogenesis after testicular hyperthermia or hormonal deprivation. We demonstrated by gene microarray experiment that Bcl-gonad (Bcl-G) was upregulated by 2.4-fold in human testes induced by intratesticular hormonal deprivation. Bcl-G is a novel proapoptotic member of the Bcl-2 family. Bcl-G has 3 isoforms in which Bcl-G median form (Bcl-Gm) and Bcl-G short form (Bcl-Gs) are shown to be testis specific. Thus, we hypothesize the testicular specific isoforms of Bcl-G are involved in germ cell apoptosis.
Objectives: To determine in rat testes 1) the expression and localization of Bcl-G; 2) the effect of either testicular hormonal deprivation or testicular hyperthermia on Bcl-G expression.
Methods: 1) Groups of 4 rats were treated with a single s.c. injection of GnRH-Antagonist (30mg/kg BW) or a water vehicle as control for 5 days. All rats were sacrificed 6 days after treatment. 2) Groups of 4 rats received testicular hyperthermia at 43C for 15min under light anesthesia of isoflurane. The animals were sacrificed at 30min, 2hrs, 4hrs, and 6hrs after heat treatment. Testicular sections were used for in situ detection of apoptosis by TUNEL assay and protein localization by immunohistochemistry. Western blots were performed to quantify the protein expression.
Results: We found testicular hormonal deprivation induced germ cell apoptosis mainly occurred at middle stages, and testicular hyperthermia induced germ cell apoptosis at early and late stages of spermatogenesis. In control rats, Bcl-Gm and Bcl-Gs were expressed in the testis and localized in spermatocytes and Leydig cells. Bcl-G isoforms are accumulated in apoptotic germ cells. Western Blots showed no changes in Bcl-Gs expression, Bcl-Gm was increased starting at 30min, and maintained elevation thereafter after heat treatment as compared with controls. Bcl-Gs was up-regulated after testicular hormonal deprivation without changes in Bcl-Gm expression.
Conclusions: Bcl-Gm and Bcl-Gs are present in rat testes. Testicular hyperthermia induced germ cell apoptosis is associated with an increased Bcl-Gm expression in apoptotic germ cells. In contrast, intratesticular hormone deprivation induced germ cell apoptosis is associated with an increased Bcl-Gs expression. The testicular specificity of Bcl-Gm and Bcl-Gs makes it a potential target for male infertility intervention and contraceptive development.

Disclosures: RS: Consultant, Clarus.
Nothing to Disclose: JCV, YHL, CW

P2-362

Leydig Cell Specific Inactivation of Glucocorticoid Receptor Causes Delayed Maturation of the Testis and Resistance to Restraint Stress.

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Leydig cells are the main site of testosterone biosynthesis in males. The levels of testosterone (T) in circulation are set by the steroidogenic capacity of individual Leydig cells and the total number of Leydig cells per testis. Previous studies have shown that glucocorticoid directly inhibits testosterone biosynthesis via the glucocorticoid receptor (GR). However, whether this action specifically involved GR in Leydig cells, or GR in supporting cells, was unknown. Transgenic mice carrying an insertion of cre-recombinase downstream of a 4.5 kb Cyp17 promoter (Cyp17cre) were cross-bred with mice carrying GR with loxP sites flanking exon 3 (L/L). F1 mice (L/+, Cyp17cre) were backcrossed to L/L mice. Female F2 L/L-Cyp17cre were backcrossed to L/L males to generate L/L-Cyp17cre males, with Leydig cell specific inactivated GR, and L/L littermate controls for this study. Blood and testes were collected from 90 day-old males. We found that L/L-Cyp17cre males had levels of testosterone that were 50% that of L/L controls, but that Leydig cell numbers were not affected. Expressions of proteins that are key to the steroidogenic pathway were examined by real-time PCR, Western blot analysis, and by enzyme assays. L/L-Cyp17cre males showed decreased expression levels for luteinizing hormone receptor, steroidogenic acute regulatory protein, 3β-hydroxysteroid dehydrogenase 6, 17α-hydroxylase and 17β-hydroxysteroid dehydrogenase 3. Leydig cells of L/L-Cyp17cre adult males had lower gene expression of prostaglandin D synthase and insulin-like growth factor 3, and could thus be considered immature relative to L/L controls. We hypothesized that without GR in Leydig cells, mice would be resistant to stress-induced suppression of testosterone production. After 3 hrs of restraint stress serum T levels were lower in L/L males compared to L/L-Cyp17 cre, which supports a conclusion that stress induces suppression of testosterone production via Leydig cell GR. In summary, the GR in Leydig cell supports maturation of the steroidogenic pathway, although compensatory mechanisms enable fertility in mice with Leydig cell inactivated GR.

Sources of Research Support: RO1 HD050570 from the National Institutes of Health (RSG). Natural Science Funding of China grant 30871434 (RSG); the New Century Talent Program of Chinese Ministry of Education 2008 (YDH).

Nothing to Disclose: YDH, BBC, JHZ, DOH, DSG, QQL, RSG

P2-363

Humanin Prevents GnRH-Antagonist Induced Male Germ Cell Apoptosis Via Inhibiting p38 MAPK and Reactivating STAT3 Pathway in Rats.

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Objective: Humanin (HN) has been reported as a potential peptide for neuroprotective therapy against Alzheimei's disease, and it also has been previously shown by our group to inhibit GnRH-antagonist (GnRH-A) induced male germ cell apoptosis (a model for hormonal male contraception) (1). We have demonstrated that p38 MAPK signaling is the upstream pathway of the male germ cell apoptosis cascade induced by intratesticular testosterone deprivation (2,3). In this study, we tried to determine the possible signaling pathway(s) of HN for its anti-apoptotic effect in male germ cells. We hypothesized that HN can inhibit GnRH-A induced p38 MAPK activity and reactivate the STAT3 pathway in male germ cells.
Study Design: Groups of 4 adult SD rats were treated with GnRH-A (sc, 30mg/Kg body weight) with or without HN (intratesticular injection, 50mcg daily for 5 days). Another group of 4 rats was injected with placebo as control. Animals were sacrificed at day 6 and testes were collected and frozen for protein extraction, fractionation and Western Blotting analysis.
Results: After GnRH-A treatment, p38 MAPK activity increased about 4.4 folds compared with the control group. HN inhibited the activation of p38 MAPK induced by GnRH-A but not to the control level. We found no change in ERK expression which is consist with our previous finding that p38 MAPK is the key pathway in male germ cell apoptosis. In this study, we did not find significant changes in STAT3 protein level, but we showed that Ser727 and Tyr705 phosphorylated STAT3 were decreased after GnRH-A treatment and restored with HN administration.
Conclusions: p38 MAPK activation and STAT3 phosphorylation may play important roles in the anti-apoptotic actions of HN in GnRH-A induced male rat germ cell apoptosis. More experiments are needed to explore whether there is crosstalk between the p38 MAPK pathway and STAT3 pathway in this animal model with HN treatment. Understanding the role of the cytoprotective HN peptide in testis physiology and pathology may help us to find a new target for male infertility treatment and contraceptive development.

(1)Lue YH et al., Endocrinology 2010; 151:350
(2)Jia Y et al., Biology of Reproduction 2009; 80:771
(3)Vera Y et al., Mol Endocrinol. 2006; 20:1597

Disclosures: RSS: Consultant, Clarus. CW: Consultant, Clarus.
Nothing to Disclose: YJ, YHL, KWL, LJC, PC

P2-364
Humanin Is a Potent Germ Cell Survival Factor.

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Background: Humanin (HN), an endogenous 24-amino acid peptide, is known for its ability to suppress neuronal cell death as well as many other cell types. A derivative of HN (HNG) with substitution of Glycine for Serine14, enhances its neuroprotective activity by about 1000-fold compared to HN. We have previously shown that intratesticular administration of HN inhibits germ cell apoptosis (GC-A) induced by gonadotropin releasing hormone antagonist in rats. In this study, we investigated the effect of HNG, a potent HN agonist on GC-A in an ex vivo culture system.
Study design: Immediately after removal of one testis of an adult male rat under the anesthesia with Isoflurane; the seminiferous tubules (ST) were dissected to one mm length of dark (middle stage) or light (late or early stages) segments. 10-12 pieces of ST were cultured in each well with serum free culture medium with the addition of 0.1% bovine serum albumin for the control group and HNG (CPC Scientific) at a concentration of 250ng/ml for treatment group. The culture plate was incubated at 34° C for 4 and 24 hours. The pieces of ST were: 1) fixed in formalin on a slide for TUNEL assay to detect cell apoptosis or 2) dispersed as single cell suspension with collagenase (0.2%) and filtered through cell strainer to separate the germ cells from Sertoli cells. After equalization of germ cell concentration between the groups, we used an ELISA to quantify cellular apoptosis.
Results: In the ex-vivo ST culture, spontaneous germ cell apoptosis occurred in serum free culture medium. The rate of GC-A was significantly suppressed (P <0.001) at 4h (apoptosis index in control; 14.6±2.52 vs. HNG; 5.86±0.58) and 24h (apoptosis index in control; 9.83±1.05 vs. HNG; 2.51±0.66) after HNG treatment. The potent inhibitory action of HNG on GC-A was further quantified by ELISA, which showed significant decrease in GC-A after 4h HNG treatment by 41 % in middle stages of the spermatogenesis cycle and by 39% in early and late stages of spermatogenesis in comparison with controls.
Conclusion: We demonstrated significant protective effect of HNG to prevent germ cell death in cultured seminiferous tubules. We have established an ex vivo model to rapidly, conveniently and quantitatively evaluate the effects of different proteins and peptides on germ cell death. This ex vivo model can facilitate future studies for development of male contraceptive or infertility treatment.

Nothing to Disclose: NI, YHL, JK, RSS, PC, KWL, LJC, CW

P2-365

Regulation of Spermatogonial Differentiation to Spermatocytes in *Utp14b* Mutant (*jsd*) Mice.

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The differentiation of spermatogonia to spermatocytes is blocked in adult *Utp14b* mutant (*jsd*) mice, due to a defect in the spermatogonia but this block can be reversed *in vivo* by suppression of testosterone (T) or elevation of testicular temperature[1,2]. To elucidate mechanisms of these *in vivo* effects, we utilized 9-day tissue culture of pieces of testes. Culture of *jsd* testes at approximate scrotal temperatures (32.5ºC) failed to induce the differentiation of type A spermatogonia to spermatocyte stage. When the incubation temperature was increased, differentiation of spermatogonia was induced reaching a maximum at 37°C, resulting in production of ~24 spermatocytes per 100 Sertoli cells. To determine whether T has a direct role in regulating differentiation of spermatogonia, we modulated T levels and action during culture of *jsd* testes. Without any addition of T, T levels could rise up to between 60 and 600 ng/ml at the ends of the 3-day intervals before medium changes. Addition of hydroxyflutamide (HOF) to inhibit the action of T failed to induce the differentiation of spermatogonia in tissue cultured at 32.5°C. Conversely, addition of T to 1000 ng/ml to stabilize the levels of T in culture, also failed to inhibit differentiation of spermatogonia in *jsd* testis tissue cultured at 37°C. We futher demonstrated that HOF and T used in the culture medium exhibited their biological activities, by analyzing the mRNA levels of the AR regulated gene, *Rhox5*, by real-time RT-PCR. Thus changes in the levels or action of T *in vitro*, under conditions in which it was biologically active, failed to alter spermatogonial differentiation. These data support our hypothesis that the direct mechanism of overcoming the differentiation block is the elevation of temperature and that T suppression is acting indirectly *in vivo* by elevating the testicular temperature. To address the question of whether the stimulation at 37°C was due to increased cell kinetics, we cultured testes from 6-7 day-old *jsd* heterozygotes and found that there was actually greater differentiation to the spermatocyte stage at 32.5°C than at 37°C. Equivalent differentiation was observed in tissue from 6-7 day-old *jsd*-mutants as in heterozygotes, indicating that the differentiation observed in juvenile *jsd* mice is not simply be due to the elevated testicular temperature prior to testicular descent but may also be due to a different phenotype of the cells in the first wave of spermatogenesis.

(1)Shetty G et al., Endocrinology 2001; 142: 2789
(2)Shetty G and Weng CC., Endocrinology 2004; 145:126

Sources of Research Support: NIH Grant HD-40397.

Nothing to Disclose: GS, WZ, KLP, SHS, CCW, MLM

P2-366

Ghrelin Diminishes Cisplatin-Induced Testicular Germ Cell Apoptosis and Restores the Normal Expression Patterns of p53 and NFkB in the Testis.

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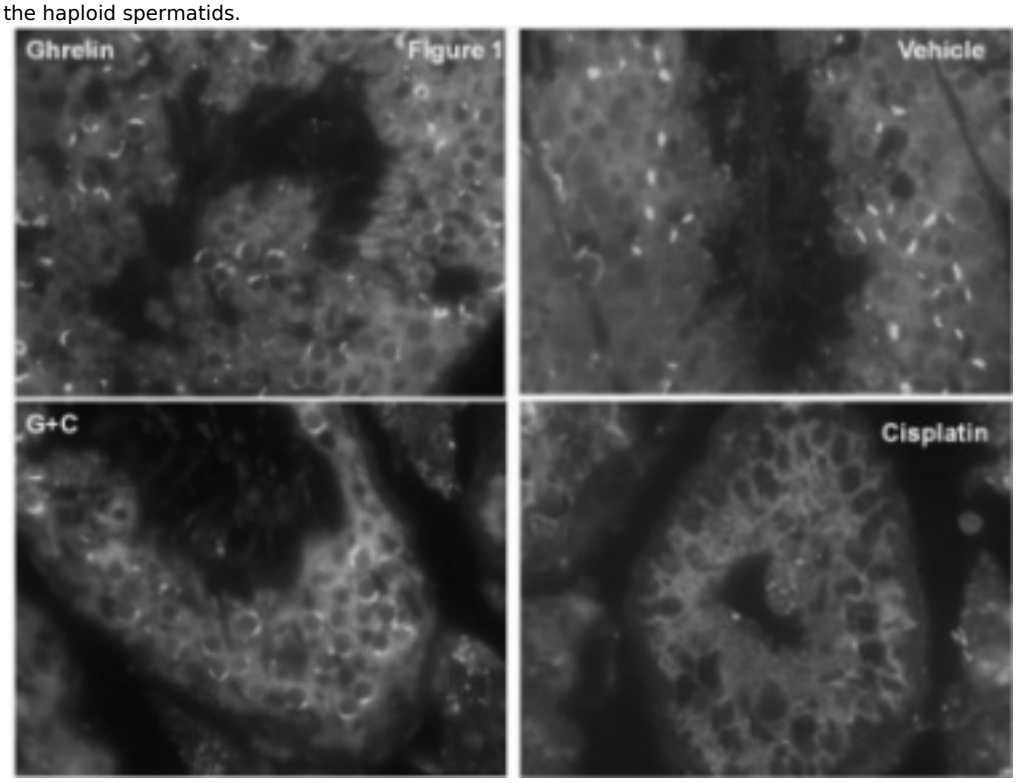
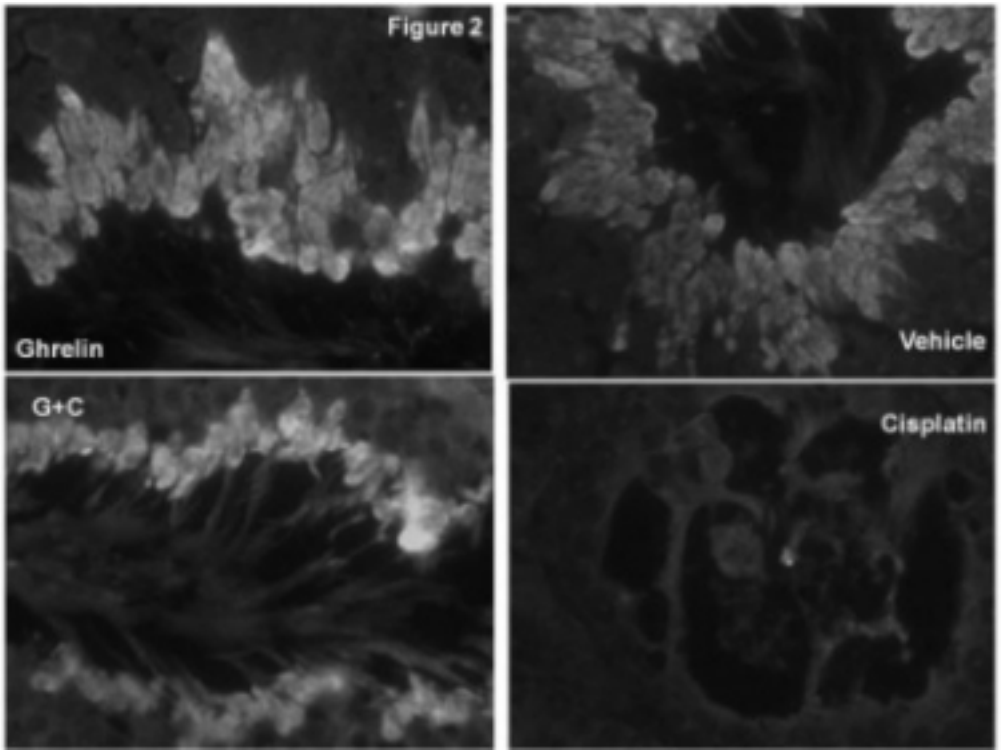
Cisplatin, a chemotherapeutic agent commonly used for the treatment of cancer, causes testicular damage and germ cell apoptosis. Although >50% of male cancer patients receiving cisplatin suffer from long−term infertility, medical treatment for or prevention of chemotherapy−induced infertility is not available. We showed that ghrelin, a hormone with anti-apoptotic properties, diminishes cisplatin−induced germ cell apoptosis in the testis. We tested the hypothesis that NFkB and p53-dependent pathways, associated with testicular apoptosis, may be altered by ghrelin to prevent testicular cell death following exposure to cisplatin.
Objectives: To determine the effects of cisplatin and ghrelin on testicular p53 and NFkB-dependent pro-apoptotic pathways.
Methods: Young adult C57bl/6J mice expressing GFP under control of a promoter that contains NFkB consensus binding sites were treated with vehicle (V), cisplatin (C, 2.5 mg/kg/day), ghrelin (G, 0.8 mg/kg/twice a day) or ghrelin+cisplatin (GC). NFkB and p53 activation were assessed by immunostaining.
Results: Cisplatin administration increased germ cell apoptosis and this was partially prevented by ghrelin coadministration. NFkB activity and p53 were present in round and elongated spermatids of V, G and GC animals. Total testicular NFkB and p53 were decreased in testis from cisplatin-treated animals predominantly resulting from the loss of the haploid spermatids.





GC partially prevented this loss.
Conclusions: NFkB and p53 are activated during normal testicular apoptosis. Cisplatin-induced testicular damage results in a decrease in total testicular p53 and NFkB activation because of the loss of the haploid spermatids. Ghrelin diminished cisplatin−induced testicular germ cell apoptosis and restored the normal expression patterns of p53 and NFkB.

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Nothing to Disclose: JMG, SDW, VP, TH, RGS, LD, DJL

P2-367

Mutations in NR5A1 Encoding Steroidogenic Factor 1 Are Associated with Spermatogenic Failure.

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One in seven couples worldwide are infertile and male factor infertility accounts for approximately 30-50 percent of these cases. Chromosomal anomalies and rearrangements or microdeletions on the Y chromosome account for a small fraction of cases of male infertility. Although many genes are known to be essential for gametogenesis there are surprisingly few monogenic mutations that have been conclusively demonstrated to cause spermatogenic failure. The nuclear receptor, NR5A1 (also called steroidogenic factor 1), is a key transcriptional regulator of genes involved in the hypothalamic–pituitary–steroidogenic axis. Mutation of NR5A1 causes various anomalies of human gonadal development and function including 46,XY disorders of sex development and 46,XX primary ovarian insufficiency. Considering the key role that NR5A1 plays in reproductive processes, we hypothesized that mutations in the NR5A1 gene may be associated with spermatogenic failure in otherwise healthy men. To test this hypothesis, we sequenced the NR5A1 open reading frame in 315 men seeking infertility treatment because of unexplained spermatogenic failure. We identified seven men with oligozoospermia or azoospermia, who carried missense mutations in the NR5A1 gene. Functional studies indicated that these mutations impaired NR5A1 transcriptional activity. We did not observe these mutations in more than 2000 control individuals including 346 normospermic men and 270 men of known fertility. In the latter two groups the entire NR5A1 coding region was sequenced. Our data indicate that NR5A1 mutations are associated with infertility in approximately 4% of otherwise healthy men with severe spermatogenic failure. This broadens the spectrum of phenotypes associated with mutations in the NR5A1 gene and suggests that mutations in this gene may be the most common single gene defect associated with male infertility to date.

Sources of Research Support: Grants from the Agence Nationale de la Recherche-GIS Institut des Maladies Rares (to Dr. McElreavey); by a research grant (1-FY07-490) from the March of Dimes Foundation (to Dr. McElreavey); by a research grant from the EuroDSD in the European Community's Seventh Framework Programme FP7/2007–2013 under grant agreement n° 201444 (to Dr McElreavey and Dr Bashamboo), by a research grant from the Portuguese Foundation for Science and Technology (to D. Lourenço), by the National Institute for Health Research (to UCL Institute of Child Health Biomedical Research Centre); and a Wellcome Trust Senior Research Fellowship in Clinical Science (079666, to Dr. Achermann).

Nothing to Disclose: AB, LL, BFdS, DL, DM, CR, HR, JA, KM

P2-368

The Molecular Basis for the Mismatch Repair Deficiencies in Men with Non-Obstructive Azoospermia (NOA).

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Mismatch repair (MMR) genes are well studied in hereditary non-polyposis colorectal cancer. MMR mutation carriers are also at high risk for several extracolonic malignancies. Interestingly, Pms2, Mlh3 and Mlh1-deficient mice display sterility associated with abnormal chromosome pairing in meiosis. These deficient mice possess not only microsatellite instability but also are predisposed to tumors. Testicular pathologies of these deficient mice were similar to men with non-obstructive azoospermia (NOA). We characterized mismatch repair deficiencies in a cohort of infertile patients with NOA. A group of 96 men with idiopathic non-obstructive azoospermia (with no Y chromosome microdeletions) provided testis biopsies for fibroblast culture. These patients underwent testicular sperm extraction and intracytoplasmic sperm injection (ISCI) with in vitro fertilization. The control group consisted of fibroblast cultures, derived from vasectomy specimens of 63 normal fertile men. Genomic DNA and RNA were extracted for characterization of mutations and gene expression studies of MMR proteins respectively. Cells were analyzed for immunocytochemistry and immunoprecipitated with MLH1, MSH2 and MSH6 antibodies. MLH1 displayed persistent diffuse cytoplasmic staining with a strong perinuclear accentuation and MSH2 showed a distinct punctate nuclear staining. Five patients showed aberrant localization of the MMR proteins (both MLH1 and MSH2) with significant decreased MSH2 staining in one patient. Immunoblots of this patient further confirmed the relative absence of MLH1 and MSH2 proteins. Gene expression studies of the MMR genes exhibit significant down regulation of MLH1, MSH2 and MSH6. DHPLC reveal polymorphisms and genetic aberrations in exons 8 and 9 of MLH1 gene spanning 57559bp, exon 13 of MSH2 gene spanning 80298bp and exon 4 of MSH6 gene spanning 24702bp. Sequencing data further confirmed the variants in these NOA patients. Functional consequences of MMR proteins deficiency were assessed by MNNG toxicity. Fibroblasts from Sertoli Cell Only (SCO) patients with defects in MMR proteins are resistant to MNNG toxicity, similar to cancer cells. Defective MMR results in a “mutator” phenotype with increased rate of spontaneous mutation and microsatellite instability. These characteristics may have health consequences for the NOA patients and present a risk to their children conceived by ICSI.

Sources of Research Support: In part by by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (P01HD36289) to DJL.

Nothing to Disclose: SM, JA, KH, LIL, DJL

P2-369

Proliferative and Steroidogenic Capacity of Leydig Cells in Cyclin-Dependent Kinase Inhibitor 1A and 1B Double Knockout Mice.

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Cyclin-dependent kinase inhibitors (CDKIs) regulate the actions of cyclin-dependent kinases (CDKs). The CDKIs consist of two families, Cip/Kip and INK4. Two Cip/Kip proteins CDKN1A (also know as p21Cip1) and CDKN1B (also know as p27kip1) are expressed in Leydig cells. Previously, we reported that Cdkn1b knockout mice had increased Leydig cell proliferation accompanied by lower steroidogenic capacity. The goal of the present study was to determine whether p21Cip1 had a role in Leydig cell proliferation and steroidogenesis by examining these parameters in Cdkn1a knockout (Cdkn1a–/–) and Cdkn1a and Cdkn1b double knockout (DBKO) mice. Leydig cell number, BrdU incorporation rate, steroidogenesis, steroidogenic enzyme mRNA levels and activities were compared in wildtype, Cdkn1a–/– and DBKO mice. Relative to the wild-type control, Leydig cell number per testis was doubled in DBKO mice and unchanged in Cdkn1a–/– mice. Testicular testosterone concentrations and mRNA levels for luteinizing hormone receptor (Lhcgr), steroidogenic acute regulatory protein (Star), cholesterol side-chain cleavage enzyme (Cyp11a1) and their respective proteins were significantly lower in DBKO mice, while testicular testosterone level was unchanged in Cdkn1a–/– mice. In Cdkn1a–/– mice, Leydig cells were hypertrophic, and 5α-reductase 1 (Srd5a1) mRNA and its activity were lower relative to wild-type controls. We conclude that Cdkn1a–/– mice do not have increased Leydig cell proliferation or adult numbers or impairments in steroidogenic enzymes, indicating that the changes seen in Leydig cells in DBKO mice appear to predominately result from loss of CDKN1B, rather than CDKN1A, although the changes in overall testis growth and sperm production in Cdkn1a–/– mice indicate that CDKN1A does have clear effects on overall testis development and function.

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Nothing to Disclose: RSG, YDH, YHC, HL, QQL, MM, DRH, PSC, BRZ

P2-370

Androgen Receptor Is Required in the Transition from Fetal to Adult-Type Leydig Cells.

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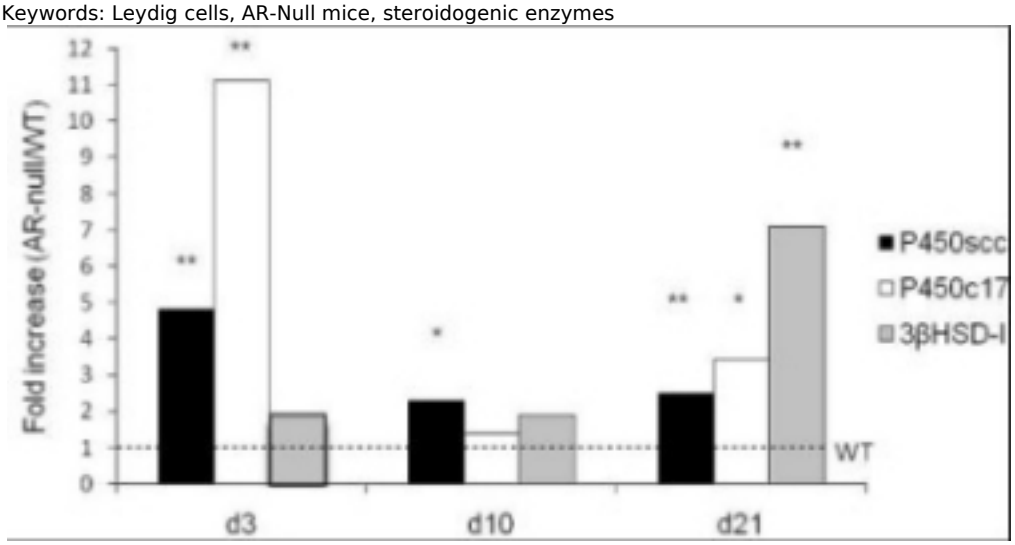
During testis development, Leydig cells (LCs) arise as two discrete populations: fetal LCs that populate the fetal testis and decline dramatically in number at birth and adult-type LCs that differentiate from mesenchymal cells at postnatal days 7-14. To examine the role of the androgen receptor during the transition from fetal to adult-type LCs, the expression of P450scc, P450c17, 3β-HSD-I, and 3β-HSD-VI was examined in AR-null and wild-type (WT) mice at postnatal days 3, 10 and 21 by real time PCR, immunofluorescence, and in situ hybridization. The relative mRNA expression of P450scc, P450c17 and 3β-HSD-I by real-time PCR in testes of AR-null mice was expressed more abundantly than in WT mice (Fig). By immunofluorescence, the intensity of P450scc staining in the testes of AR-null mice is stronger than that of WT mice at d3, d10, and d21, and similarly, the intensity of P450c17 staining in AR-null mice is stronger than WT mice at d3 and d21. The number of interstitial cells staining for P450scc and P450c17 gradually decreases from d3 to d10 in both AR-null and WT mice. At these ages, interstitial cells expressing steroidogenic enzymes are presumed to be fetal LCs. In contrast, by both real time PCR and in situ hybridization, the mRNA expression of 3β-HSD-VI (a marker of adult-type LCs) in the testis of AR-null mice is significantly lower than that of WT littermates at d10, d21 and d24. Collectively, these results indicate that the absence of androgen receptors results in persistence of fetal LCs in the postnatal testis and inhibits differentiation of adult-type LC in the prepubertal testis. These data suggest that the androgen receptor helps mediate the regression of fetal LCs after birth, and may also play a role during the initial differentiation of adult-type LCs from mesenchymal precursors. We speculate that the timely regression of androgen receptor-mediated fetal LCs is necessary for the establishment of a normal prepubertal population of adult-type LCs.

Keywords: Leydig cells, AR-Null mice, steroidogenic enzymes



Nothing to Disclose: XW, XC, NZ, MML

P2-371

New Model for Age-Related Prostatic Disease: Smooth Muscle Cell-Specific Knockout of Androgen Receptor.

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Androgen-driven stromal-epithelial interactions are vital for normal development and function of much of the male reproductive system, including the prostate. They also play a key role in prostatic diseases such as benign prostatic hyperplasia and prostate cancer, which are a significant medical problem in older men; withdrawal of androgens is the current primary therapy for treating prostate cancer. However, the cell-specific role for androgen action in the healthy and diseased adult prostates remains unclear. The aim of this study was to determine the extent to which androgen action in a subset of prostatic stroma, the smooth muscle cells, regulates normal epithelial cell function, identity, and homeostasis using prostates from mice with targeted ablation of androgen receptors (AR) specifically from the smooth muscle cells.
These KO mice have significantly smaller prostates in adulthood despite hyperplasia of the epithelial and stromal compartments. Prostates from KO mice have reduced mRNA expression of epithelial cell identity markers such as p63, Sox 9 and cytokeratin 14 and 18. None of these changes could be explained by altered serum testosterone or estrogen concentrations. We also demonstrate an exaggerated response to exogenous testosterone and estradiol in prostates from these KO mice. In conclusion, these results demonstrate *in vivo* that the smooth muscle cells play a vital role in androgen-driven stromal-epithelial interactions in the prostate, determining epithelial cell identity and function, as well as limiting the prostatic epithelial proliferative response to exogenous hormones. This may have implications for understanding onset and progression of age-related prostatic diseases, highlighting smooth muscle cells as a possible key therapeutic target.

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Nothing to Disclose: MW, LM, AM, PTKS, RMS, LBS

P2-372

The Expression Pattern and Regulation of Testis Specific Serine Protease (TESSP) Genes during Spermatogenesis.

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Spermatogenesis is a precisely controlled process generating spermatozoa. In mature testis, some spermatogonia enter meiosis to differentiate into spermatocytes and subsequently haploid spermatids. The spermatids then undergo morphological modifications to become mature spermatozoa. Many factors are known to be involved in this process but its molecular mechanism is not fully understood. This time we focused on two unsolved issues, the function of proteases and the regulatory mechanism of male germ cell-specific gene expression. We first searched for serine proteases, the largest family of proteases, expressed in the mouse testis by using degenerate primers designed at their active sites. This resulted in the cloning of four novel serine protease genes namely testis-specific serine proteases TESSP1-4. TESSP-1, located on chromosome 17 forming a gene cluster with other proteases, was expressed in spermatogonia, spermatocytes, and Sertoli cells as reported by Takano et al. (1, 2). We investigated expression patterns of the other three TESSP genes by Northern blot and RT-PCR analyses. All three genes were specifically expressed in the testis and their expression began at two weeks after birth with their expression peaks at three weeks. In situ hybridization revealed their mRNAs were localized in spermatocytes at seminiferous epithelial stages IX to XII. The only difference we observed between the three genes was their expression levels. By quantitative RT-PCR, TESSP-2 mRNA was expressed at the highest level, followed by TESSP-3, and -4. These results strongly suggest the relationship of TESSPs with spermatogenesis and may give a hint of the role of proteases in spermatogenesis. We next examined the regulatory mechanism of the four TESSP genes. Because TESSP-2, -3 and -4 genes form a cluster on chromosome 9, it is especially interesting how their expressions are controlled. The in vitro reporter gene assay indicated that the promoter activity was very weak but seemed to reflect the difference of their expression levels. We also assessed hormonal control of the TESSP genes and the result revealed that TESSP-1 gene was up-regulated by the treatment with 5alpha-dihydrotestosterone. These data suggest that the expression of TESSPs is regulated by a complicated mechanism involving androgen and regulatory elements other than the promoter.

(1) N. Takano et al., Mol Reprod Dev. 2005; 70:1-10
(2) N. Takano et al., Zoolog Sci. 2009; 26:294-300

Nothing to Disclose: RY, MK, TT, APK

P2-373

Oocyte Meiotic Resumption Involves LH- and EGFR-Dependent Activation of Multiple Pathways.

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Transactivation of the epidermal growth factor (EGF) network by luteinizing hormone (LH) is essential for oocyte maturation, cumulus expansion and ovulation. These events are impaired in mutant mice with disruption of the EGF signaling network (*Areg*$^{-/-}$ *Egfr*$^{wa2/wa2}$). LH transactivation of EGFR occurs rapidly, preceding the onset of oocyte meiotic resumption. LH also induces MAPK-dependent phosphorylation and closure of connexin 43 (Cx43) gap junctions prior to oocyte meiotic resumption. To gain insight into the role of the EGF network in LH-induced oocyte maturation, we used a genetic approach and the preovulatory follicle (POF) culture model to examine the regulation of Cx43 gap junction phosphorylation and of different known signaling pathways downstream of LH and the EGFR. LH stimulation of POFs from *Areg*$^{-/-}$ *Egfr*$^{wa2/wa2}$ mice produced no oocyte maturation. Here, we show that LH-induced Cx43 phosphorylation on S255 and S262, two serine residues implicated in gap junction closure, is impaired in *Areg*$^{-/-}$ *Egfr*$^{wa2/wa2}$ POFs. The EGFR tyrosine kinase inhibitor AG1478 also completely prevented LH-induced phosphorylation of these two serines. Western blot analyses of LH activation of different signaling pathways in wild type and double mutant POFs revealed that phosphorylation of MAPK and p38MAPK, but not AKT, is dependent in part on EGFR signaling. Under conditions where MAPK activation and Cx43 phosphorylation were both prevented, AREG-induced oocyte maturation still occurred, suggesting that another signal can promote meiotic resumption in the absence of gap junction closure. Because LH fails to induce oocyte maturation in *Areg*$^{-/-}$ *Egfr*$^{wa2/wa2}$ POFs in which gap junction closure is impaired, this signal may be EGFR-dependent. Thus, closure of Cx43 gap junctions appears sufficient but not necessary for oocyte meiotic resumption. An LH-dependent decrease in cGMP in the follicle may be involved in PDE3A activation in the oocyte, resulting in decreased cAMP and resumption of meiosis. Here, LH induced a decrease in cGMP in *Areg*$^{-/-}$ *Egfr*$^{wa2/wa2}$ POFs, but to levels that were significantly higher than in wild type POFs. Whether these levels of cGMP are sufficient to maintain meiotic arrest remains to be determined. These findings indicate that multiple pathways regulated by both LH and EGFR coordinate or act in parallel to promote the development of a mature egg.

Sources of Research Support: NIH grant HD052909.

Nothing to Disclose: MH, MC

P2-374

Growth Differentiation Factor-9 Is Anti-Apoptotic during Follicular Development from Preantral to Early Antral Stage.

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Ovarian follicular atresia represents a selection process that ensures the release of only healthy and viable oocytes during ovulation. The transition from preantral to early antral stage is the “penultimate” stage of development in terms of gonadotropin dependence and follicle destiny (survival/growth versus atresia). We have examined if and how oocyte-derived growth differentiation factor-9 (GDF-9) and follicle-stimulating hormone (FSH) regulate follicular development and atresia during the preantral to early antral transition, by a novel combination of in vitro gene manipulation (i.e. intra-oocyte injection of GDF-9 antisense oligos) and preantral follicle culture. Injection of GDF-9 antisense suppressed basal and FSH-induced preantral follicle growth in vitro, while addition of GDF-9 enhanced basal and FSH-induced follicular development. GDF-9 antisense activated caspase-3 and induced apoptosis in cultured preantral follicles, a response attenuated by exogenous GDF-9. GDF-9 increased phospho-Akt content in granulosa cells of early antral follicles. Although granulosa cell apoptosis induced by ceramide was attenuated by the presence of GDF-9, this protective effect of GDF-9 was prevented by the PI3K inhibitor LY294002 and a dominant negative form of Akt. Injection of GDF-9 antisense decreased FSH receptor mRNA levels in cultured follicles. The data suggest that GDF-9 is anti-apoptotic in preantral follicles, and protects granulosa cells from undergoing apoptosis via activation of the PI3K/Akt pathway. Adequate level of GDF-9 is required for follicular FSH receptor mRNA expression. GDF-9 promotes follicular survival and growth during the preantral to early antral transition by suppressing granulosa cell apoptosis and follicular atresia.

Nothing to Disclose: MO, BKT, FK

P2-375

Bone Morphogenetic Protein-2 (BMP-2) Increases Gene Expression of FSH Receptor and Decreases Gene Expression of LH Receptor and StAR in Human Granulosa Cells.

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Objective: A growing body of evidence indicates that BMP cytokines, members of the TGF-ß superfamily, play a key role in female fertility in mammals The object of this study was to examine the expression and function of BMP-2 in the human ovary.
Material and Method: The experimental procedure was approved by the institutional review board. All specimens were obtained under signed informed consent. Normal human ovaries were obtained from 6 patients who underwent salpingo-oophorectomy for the treatment of uterine cervical cancer. BMP-2 expression in human ovaries was examined by in situ hybridization. Human granulosa cells obtained IVF patients were cultured with recombinant BMP-2 (100 ng/ml) or hCG (10 IU/ml) for 24 hrs. The level of mRNA of granulosa cells were measured by real time RT-PCR.
Result(s): BMP-2 expression was detected in granulosa cells of antral follicles but not of corpus luteum. The *in vitro* study showed that BMP-2 induced FSH receptor expression, while decreasing LH receptor and StAR expression in human granulosa cells. HCG decreased gene expression of BMP-2 and increased BAMBI, an antagonist of BMP-2.
Conclusion(s): Expression and disappearance of BMP-2 might contribute to folliculogenesis and luteinization by regulating gonadotropin receptors expression in human granulosa cells. HCG can modulate BMP-2 function by controlling BMP-2 and BAMBI expression. BMP system and gonadotropin system might cooperate with mutual communication to regulate an ovarian function.

Nothing to Disclose: OY, JS, YO, TY, YT, ON

P2-376

The Role of Pituitary Adenylate Cyclase-Activating Polypeptide as a Local Factor in the Zebrafish Ovary.

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide originally purified from ovine hypothalamus for its potent activity to stimulate cAMP production. However, its presence and action have also been demonstrated in various peripheral tissues including the ovary. In the zebrafish, two isoforms of PACAP (adcyap1a and adcyap1b) and three PACAP receptors (adcyap1r1, vipr1 and vipr2) have all been demonstrated to express in the ovary. Interestingly, while both follicle cells and oocytes express adcyap1b, the expression of adcyap1a was restricted to the oocytes only. Among the three receptors, adcyap1r1 and vipr2 were expressed in the oocytes whereas the expression of vipr1 was exclusively located in the follicle cells. In cultured zebrafish follicle cells, PACAP significantly up-regulated activin beta A (inhbaa and inhbab) and follistatin (fst) expression but down-regulated activin beta B (inhbb) expression in a time- and dose-dependent manner, similar to the effects of gonadotropin (hCG). Since adcyap1b expression in the follicle cells is highly responsive to gonadotropin, PACAP might mediate gonadotropin actions as a local factor to propagate and/or amplify the actions of gonadotropins in a paracrine manner and its functional roles are likely, at least to some extent, related to the ovarian activin-follistatin system. On the other hand, the temporal expression analysis of PACAPs and their receptors during folliculogenesis suggested that PACAP might play differential roles in regulating follicle growth and maturation through different receptors. The two receptors that are expressed in the oocyte (adcyap1r1 and vipr2) showed a significant increase in expression at the transition from the primary growth (PG) stage to pre-vitellogenic (PV) stage, suggesting a direct effect of PACAP on the oocyte to drive follicle activation and recruitment via these receptors. In contrast, vipr1, the receptor expressed in the follicle cells, decreased in expression at the PG-PV transition and afterwards; however, its expression surged dramatically at the full-grown (FG) stage, suggesting its potential involvement in final oocyte maturation. Our data indicate that the PACAP system may also work as part of the putative PACAP/GHRH-GH-IGF mini-axis in the ovary as it significantly stimulated the expression of growth hormone (gh) and two isoforms of growth hormone receptors (ghra and ghrb) in the follicle cells.

Sources of Research Support: Research Grants Council of the Hong Kong Grants CUHK4422/04; CUHK4578/05; CUHK4587/06; CUHK4647/07; CUHK4643/08; and CUHK4644/09 awarded to W. Ge.

Nothing to Disclose: RZ, AHKT, WG

P2-377

Peroxisome Proliferator-Activated Receptor-gamma Coactivator-1 alpha Regulates Progesterone Production in Ovarian Granulosa Cells with Steroidogenic Factor-1 and Liver Receptor Homolog-1.

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Previously, we demonstrated that bone marrow-derived mesenchymal stem cells (MSCs) differentiate into steroidogenic cells such as Leydig and adrenocortical cells by the introduction of steroidogenic factor-1 (SF-1) and treatment with cAMP. In this study, we employed the same approach to differentiate umbilical cord blood-derived MSCs (UCB-MSCs). Even though UCB-MSCs also differentiated into steroidogenic cells, they showed characteristics of granulosa-luteal like cells. We found that peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1α) was expressed and further induced by cAMP-stimulation in UCB-MSCs. Consistent with the results from UCB-MSCs, PGC-1α was also expressed in the ovary, and the expression was detected only in granulosa cells. PGC-1α binds to the NR5A family (SF-1 and liver receptor homolog-1, LRH-1) of proteins and markedly enhances their transcriptional activities. Reporter assays revealed that PGC-1α activated the promoter activities of SF-1 and LRH-1 target genes. Infection of KGN cells (a human cell line derived from granulosa cells) with adenoviruses expressing PGC-1α resulted in the induction of steroidogenesis related genes, and the stimulation of progesterone production. Surprisingly, PGC-1α also induced SF-1 and LRH-1; the latter was induced to a greater extent. Knock down of Pgc-1α in cultured rat granulose cells resulted in attenuation of those gene expression as well as progesterone production. Transactivation of NR5A family by PGC-1α was repressed by Dax-1. PGC-1α binds to activation function 2 (AF-2) domain of NR5A proteins via its consensus LXXLL motif. These results indicate that PGC-1α is involved in the progesterone production in ovarian granulosa cells by potentiating transcriptional activities of the NR5A family proteins.

Nothing to Disclose: TY, AU, KM

P2-378

Mouse Model of Metabolic Syndrome Reveals a Negative Effect on Oocyte Quality and Quantity.

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Metabolic syndrome is becoming increasingly problematic. These patients have diminished fertility. Associated anovulatory cycles and implantation defects are believed to be causative factors. The negative impact to the oocyte is less well established. Using an *in vitro* follicle culture system, we recapitulate follicular development and oocyte maturation, starting with secondary follicles. Our culture system allows us to determine if secondary follicles and resulting oocytes from lean and obese animals are equivalent. We conducted a comparative study of weight-related differences in survival, follicle growth and oocyte quality. Follicles were isolated from CD1 mice on day 100 (n=19 mice) during estrus, diestrus and metestrus. A separate diet induced obesity cohort (n=15) was fed a higher calorie and fat chow since time of weaning. Serum cholesterol, testosterone, AMH, estradiol and intraperitoneal glucose tolerance test assays were performed. Follicles were encapsulated in a 3-D alginate-fibrin hydrogel and cultured for 9 days. Oocytes were then extracted and matured *in vitro*. Follicle survival and growth were measured. Egg quality was determined by examining morphology, oocyte spindle and polar body structures by light and confocal microscopy. Estradiol, cholesterol and glucose levels were significantly higher in the diet induced obese group. Cultured secondary follicles isolated from animals at any weight had equivalently good survival. Secondary follicle start size and terminal follicle size were statistically greater in the obese cohort. Oocyte quality was poorer in cultured follicles from the obese mice. Within the thin cohort, cultured follicles collected during estrus and metestrus showed qualitatively better oocyte morphology than cultured follicles collected during diestrus. Confocal analysis revealed a larger spindle and polar body in the obese and thin, diestrus cohorts. Serum AMH levels were significantly lower and equivalent to levels of older animals in the diet-induced obese cohorts supporting the decreased fertility status. The diet induced obese group had characteristics consistent with a metabolic syndrome mouse model. Follicle growth and survival in culture was similar regardless of weight, but egg quality was significantly lower for cultured follicles isolated from the obese animals. Cycle stage and obesity are important for oocyte quality, the mechanism of decreased quality maybe abnormal polar body and spindle morphology.

Sources of Research Support: NIH/NICHD Structure-Function Relationships in Reproductive Science, U54 HD041857.

Nothing to Disclose: JEH-C, MX, JKJ, LDS, TKW

P2-379

P450-Side Chain Cleavage Enzyme Is an Ovarian Antigen in Murine Experimental Autoimmune Oophoritis.

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Approximately 4% of women with primary ovarian insufficiency (POI) develop a condition as a result of autoimmune oophoritis, which is associated with steroidogenic cell autoimmunity (SCA). Women with this condition are also at risk of developing autoimmune adrenal insufficiency. The human disorder can be detected by the presence of autoantibodies against steroidogenic enzymes. Murine experimental autoimmune oophoritis can be induced in B6A female mice by neonatal thymectomy on day three of life (NTX3). This has served as an animal model for autoimmune primary ovarian insufficiency. In this model, the autoimmune oophoritis is a T cell-mediated disease. There is an associated B cell activation that results in a spectrum of autoantibodies against multiple ovarian components. We previously identified a novel oocyte protein designated MATER using sera from these mice. Further, we demonstrated that induction of specific tolerance to MATER mitigates but does not completely abrogate the disease. It is clear that oocyte autoimmunity (OA) is one mechanism of the disorder, but possibly not the only mechanism. In this study, we aimed to identify the specific ovarian antigenic targets of steroidogenic cells. As determined by indirect immunofluorescence on mouse ovarian and adrenal sections, eight of fifteen NTX3 female mice exhibited SCA antibodies (~53%). Some of these sera also contained antibodies against oocytes. To identify the targets of the SCA, we produced recombinant proteins of three key ovarian steroidogenic enzymes: 17α-hydroxylase (17α-OH), P450-side chain cleavage (P450-SSC) and 3β-hydroxysteroid dehydrogenase (3β-HSD). By immunoblotting assay, we showed that the eight mouse sera containing SCA antibodies reacted with recombinant P450-SSC. None reacted with 17α-OH or 3β-HSD. As controls, sham-operated mouse sera, and NTX3 mouse sera containing antibodies against oocytes only did not react with the recombinant P450-SSC. We conclude that the P450-SSC is an ovarian antigenic target in this animal model. Whether this target plays a role in inducing the disease or is a result of antigenic epitope spreading remains to be determined. Our data agree with clinical findings that sera from women with SCA-POI contain autoantibodies against P450-SSC.

Sources of Research Support: Intramual program of NICHD, NIH.

Nothing to Disclose: Z-BT, WT, QW, AHD, LMN

P2-380
The BMP System in Zebrafish Ovary.

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Unlike the mammalian models, few studies have been conducted on the Bone Morphogenetic Protein (BMP) family in fish reproduction except BMP15. The present study was undertaken to explore the BMP family in zebrafish ovary. Spatiotemporal distribution of selected BMP ligands and receptors in the ovary was investigated. Localization studies revealed the presence of the BMP ligands (bmp2a, bmp2b, bmp4, bmp6 and bmp7) in the denuded oocytes while their type II receptors (bmpr2a and bmpr2b) were expressed exclusively in the follicular layers, indicating a potential paracrine signaling from the oocyte towards the follicular layer by various BMP ligands, as opposed to the tradition belief that the oocyte is passively controlled and nurtured by the follicular layer for its growth and development. The expression of bmp2a, bmp2b, bmp4 and bmp6 increased significantly during the transition from primary growth (PG) to pre-vitellogenic (PV) stage but decreased as folliculogenesis proceeded. On the contrast, the BMP receptors (bmpr2a and bmpr2b) increased in expression during folliculogenesis, reaching the peak levels prior to oocyte maturation. Using an innovative co-culture system involving Chinese hamster ovary (CHO) cells and zebrafish follicle cells, we studied the effects of zfBMP2b and zfBMP4 on the expression of zebrafish gonadotropin receptors and members of the activin family. Briefly, the CHO cells were transfected with constructs encoding bmp2b and bmp4 and the established cell lines were used to produce recombinant zebrafish BMP2b and BMP4. The recombinant CHO cells were pre-plated, followed by co-incubation with zebrafish follicle cells. The CHO cells secreting zfBMP2b and zfBMP4 would mimic the oocyte secreting factors in the zebrafish follicle and their effects on the potential target genes' expression in the follicle cells were studied by real-time qPCR. ZfBMP2b and zfBMP4 were found to be the long-sought growth factors that differentially up-regulated luteinizing hormone/choriogonadotropin receptor (lhcgr) and down-regulated follicle-stimulating hormone receptor (fshr). On the other hand, all activin beta subunits (inhbaa, inhbab, inhbb) were down-regulated while inhibin alpha (inha) and follistatin (fst) were up-regulated. The modulating effects of zfBMP2b and zfBMP4 on several key genes in folliculogenesis highlighted the significance of BMPs in zebrafish ovary and provided important insights into the functionality of BMPs as oocyte-derived factors.

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Nothing to Disclose: CWL, WG

P2-381

The Effect of Elevated Levels of GnRH in the Mouse Ovary on the Estrous Cycle.

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Introduction: Appropriate tissue-specific gene expression of GnRH is critical for maintenance of reproductive competence. Differential regulation of hypothalamic and ovarian GnRH expression is demonstrated in a knockout mouse model with a deletion of the mGnRH enhancer fragment between -2806 bp and -2078 bp of the mGnRH gene (GRE-/-). While this model demonstrates normal fertility, these mice have a shortened estrous cycle.
Objective: To further define the role of the enhancer element on estrous cyclicity by determining its effect on pituitary hormone expression and ovarian feedback mechanisms.
Methods and results: Knockout mice have been generated with a deletion of the mGnRH promoter fragment between -2806 bp and -2078 bp of the mGnRH gene. Evaluation of the estrous cycle of wildtype (GRE+/+) and knockout (GRE-/-) mice was performed by daily vaginal cytology and nightly blood draws for determination of serum LH and FSH. GnRH expression was evaluated in the hypothalamus and the ovary of GRE+/+ and GRE-/- mice by quantitative RT-PCR during the diestrus and proestrus phases. As expected, GnRH expression in the hypothalamus of the GRE-/- decreased 48% in diestrus and 36% in proestrus when compared to the GRE+/+ ($p \leq 0.05$). GnRH expression in the ovary was noted to be 5 fold greater in diestrus and 3 fold greater in proestrus in the GRE-/- mice ($p \leq 0.05$). GnRH, LH, and FSH receptors were also evaluated in the ovary by RT-PCR. GnRH-R expression was 4 fold greater in diestrus and ~2 fold greater in proestrus in the GRE-/- mice ($p \leq 0.01$). In proestrus, LH-R expression was decreased 41% in GRE-/- mice and FSH-R was decreased 62% in GRE-/- mice ($p \leq 0.05$) when compared to GRE+/+ mice. Additionally, while there was not a difference in the serum LH level between GRE-/- and GRE+/+, there was an increased frequency of LH surges in the GRE-/- mice (5 vs 7 days). There was a trend towards decreased FSH levels in the GRE-/- mice over the course of the estrous cycle.
Conclusion: Deletion of the cis-regulatory element of the GnRH promoter contained between -2806 bp and -2078 bp produces a unique model of dramatically elevated in vivo GnRH expression on the ovary and decreased expression in the hypothalamus. This has been shown to increase ovarian expression of GnRH receptor as well as blunt the expression of LH and FSH receptors in the setting of an overall shortened estrous cycle. This work further demonstrates a local physiological effect of GnRH on the ovary.

Nothing to Disclose: MY, HJN, AW, SR

P2-382

Effects of Aging on Antioxidant Gene Expression and Oxidative Damage in the Mouse Ovary.

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Aging is associated with increased production of endogenous reactive oxygen species (ROS), which may induce a wide range of oxidative damage in various organs. Oxidative stress has been implicated in various aspects of aging, but the role of oxidative stress in ovarian aging remains unclear. Our previous studies have shown that ovarian follicles and granulosa cells are vulnerable to ROS attack that initiates apoptotic cell death, suggesting that the age-related decline in ovarian function may arise through age-related declines in ovarian antioxidant capacity, resulting in oxidative damage and apoptosis. In this study, we test the hypothesis that ovarian antioxidant defenses decrease and oxidative damage increases with age in mice. Healthy wild type C57BL/6 female mice from the NIA Aged Rodent Colony aged 2, 6, 9, or 12 months were sacrificed on the morning of metestrus, and ovaries were collected. Quantitative real time RT-PCR was used to measure ovarian mRNA levels of antioxidant genes: glutamate cysteine ligase catalytic (*Gclc*) and modifier (*Gclm*) subunits, superoxide dismutase 1 and 2 (*Sod1, Sod2*), catalase (*Cat*), glutathione peroxidase 1 and 3 (*Gpx1, Gpx3*), glutathione reductase (*Gsr*), glutaredoxin 1 (*Glrx1*), thioredoxin1 and 2 (*Txn1, Txn2*), thioredoxin reductase 1 (*Txnrd1*), glutathione-S transferase mu1 and alpha4 (*Gstm1, Gsta4*) and microsomal GST1 (*Mgst1*). For in situ detection of lipid peroxidation, immunostaining using an antibody directed against 4-hydroxynonenal (4-HNE) was conducted. Female mice of all age groups had regular 4 to 5 day estrous cycles with similar percentages of cornified and leukocytic vaginal cytology. Ovarian expression of *Gpx1* increased and expression of *Glrx1* and *Txn2* decreased in a statistically significant manner with increasing age. Expression of other genes involved in antioxidant defenses remained unchanged with age. No statistically significant differences were found in 4-HNE immunostaining among the four age groups. However, there were nonsignificant trends towards increased lipid peroxidation with age in healthy primary, secondary, and antral follicles and increased lipid peroxidation in atretic secondary and antral follicles compared to healthy follicles. Our data do not support the hypothesis that a global decrease in antioxidant gene expression drives age-related declines in ovarian function in mice. However, our data suggest that changes in expression of *Glrx1*, *Txn2*, and *Gpx1* may be involved in ovarian aging.

Sources of Research Support: NIH Grant AG032087 awarded to UL.

Nothing to Disclose: JL, JL, UL

P2-383

Ovarian Androgen Production in the Prenatally Androgenised (PA) Adult Sheep: Increased Protein Expression of P450c17 in Theca Cells.

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Background:
Polycystic ovary syndrome (PCOS) is characterized by ovarian hyperandrogenism, associated with increased activity of P450c17 in theca cells in vitro. Animal models of PCOS (early prenatally testosterone-treated female monkeys) demonstrate both basal and hCG-stimulated androgen excess, suggesting that prenatal exposure to androgen can, in turn, affect the capacity of theca cells to secrete androgen1. The aim of this study was to examine, directly, ovarian expression of P450c17 in ovaries of prenatally androgenised sheep and in control animals.
Methods:
Immunohistochemistry for P450c17 in theca cells (TCs) was performed, using a specific antibody for P450c17 protein, on sections of archived ovary tissue obtained from 20 adult sheep ovaries at 8 months of age (9 control ovaries; 11 ovaries from PA animals) 2. A total of 2732 TCs were examined.
Results:
In ovaries from PA sheep a much higher proportion of theca cells stained positively for P450c17 than in sections from control animals: 49% (12.8-66%) against 23% (4.2-42%) in control sheep (median, range; Mann–Whitney U test p=0.03).
Conclusions:
In PA sheep ovaries a greater proportion of theca cells expressed P450c17 protein than in sections from control animals. These data provide direct support for data from in vivo studies in PA animals that suggest excessive ovarian androgen production is a consequence of prenatal exposure to excess androgen. It is not clear if this phenomenon reflects a direct ovarian effect of androgens on the fetal ovary or whether it results from associated abnormalities in gonadotropin secretion but it is clearly relevant in considering the possible etiology of androgen excess in PCOS

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(2) Forsdike et al, 2007. J Endocrinol 192: 421-8

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Nothing to Disclose: FC, SAS, RF, JR, KH, SF

P2-384

LH Receptor Activation Regulates Adiponectin Receptor Expression in Human Granulosa Cells Via a cAMP Pathway.

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INTRODUCTION: Adiponectin, an abundant, predominantly adipocyte-derived hormone, influences carbohydrate metabolism and energy homeostasis through two receptors, AdipoR1 and AdipoR2. In animal models, adiponectin also appears to influence ovarian steroidogenesis, folliculogenesis, and ovulation. The receptors AdipoR1 and AdipoR2 are present in the human ovary and may be differentially expressed during follicle development. In these studies, we investigated the role of LH receptor activation by hCG on the expression of AdipoR1 and AdipoR2 in human granulosa cells.
METHODS: Human ovarian granulosa cells were collected at the time of oocyte retrieval from women undergoing IVF. Cells were isolated over a density gradient and plated in McCoys media containing 5% FBS at 5%CO_2/37^oC for 48 hours. Medium was changed to low serum for 12 hours and then cells were subjected to one of four treatments for 24 hours: control, hCG (100 ng/ml), forskolin (30 µMol/L), or FSH (1 IU/ml). After 24 hours, RNA was isolated from the cells using Trizol reagent. Real-time (RT) cDNA synthesis reactions were performed using oligo (dT) M-MLV RT (Ambion, Austin, TX) with 0.5–1.5 µg of total RNA under the conditions described by the manufacturer. For real-time PCR, the TaqMan assay system was used (Applied Biosystems, Foster City, CA) with specific probes for AdipoR1 and AdipoR2 and TBP as a house keeping gene. Quantification was completed using the $\Delta\Delta C_T$ method.
RESULTS: Treatment with hCG increased expression of AdipoR2 expression nearly 4-fold ($P < 0.05$). Furthermore, treatment with forskolin and FSH increased AdipoR2 expression in a manner similar to hCG. In contrast, AdipoR1 expression was not significantly altered by any of the treatments.
CONCLUSIONS: In human granulosa cells, expression of AdipoR2 but not AdipoR1 appears to be regulated by increased cAMP induced by activation of the LHR, FSHR or directly with forskolin stimulation. Adiponectin signaling pathway cross-talk with gonadotropin signaling pathways may be an important area of interaction of energy homeostasis and reproduction.

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Nothing to Disclose: EPW, TT, KES, JEN, EAM

P2-385

RF-Amide-Related Peptide-3 (RFRP-3) Inhibits Gonadotropin-Stimulated Progesterone Biosynthesis in Human Granulosa Cells.

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BACKGROUND: RF-amide-related peptide-3 (RFRP-3), a mammalian homolog of avian gonadotropin-inhibitory hormone (GnIH), has pronounced inhibitory effects on the reproductive axis in many species. In mammals, RFRP-3 inhibits gonadotropin secretion via direct effects on hypothalamic GnRH neurons and on pituitary gonadotropes. In the present study, we examined whether RFRP-3 may act directly on human female reproductive tissue outside of the brain.
METHODS: Human granulosa-luteal cells were isolated from follicular fluid samples obtained from IVF-ET patients. Reverse transcription-PCR and Western blot analysis were used to examine the expression of RFRP-3 and its receptor [G protein-coupled receptor (GPR) 147] in primary cultures of human granulosa cells. We also investigated the effects of exogenous RFRP-3 on steroidogenesis, the expression of steroidogenic enzymes and intracellular cyclic AMP (cAMP) concentration in human granulosa cells.
RESULTS: Primary cultures of human granulosa cells express both RFRP-3 and GPR147. Treatment with RFRP-3 reduced FSH-, LH- and forskolin-stimulated progesterone production, but not that of 8-Br-cAMP. Basal progesterone production was not affected. These effects were abolished by the knockdown of GPR147 with siRNA or by an antagonist of RFRP-3, non-amidated RFRP-3. Likewise, RFRP-3 reduced the expression of steroidogenic acute regulatory protein (StAR) in response to FSH, LH and forskolin. In addition, RFRP-3 potently inhibited FSH- and forskolin-induced cAMP accumulation, and this effect was abolished by the knockdown of GPR147, non-amidated RFRP-3, and an inhibitor of inhibitory G protein α subunits (pertussis toxin).
CONCLUSION: RFRP-3 is expressed in human granulosa cells where it acts via its receptor, GPR147, to inhibit gonadotropin-stimulated progesterone production. RFRP-3/GPR147-mediated inhibition likely occurs at the level of adenylyl cyclase activation as a result of the activation of inhibitory G protein(s).

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Nothing to Disclose: HO, CK, GEB, TO, KT, TY, PCKL

P2-386

Brain/Gut Peptides, Tachykinins, Upregulate Oocyte Growth Via Protease Activation.

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Substance P, neurokinin A (NKA), NKB, and endokinins belong to the brain/gut peptide family, namely, tachykinin (TK) family, and are responsible for various central and peripheral functions including nociception, vasodilation, inflammation, and the processing of sensory information in a neuropeptidergic or endocrine/paracrine fashion. TKs and their receptors have also been shown to be expressed in the mammalian ovary. However, the biological roles of ovarian TKs have yet to be verified. In the previous study, we characterized prototypes of vertebrate TKs and their receptors, Ci-TK-I and Ci-TK-R, from the protochordate (ascidian), *Ciona intestinalis* (1). Recently, we have also explored a novel biological function of TKs in the ovary using *C. intestinalis* as a novel model organism (2,3). Immunostaining of the *Ciona* ovary demonstrated the specific expression of Ci-TK-R in test cells residing in oocytes at the vitellogenic stage. DNA microarray and real-time PCR revealed that Ci-TK-I induced gene expression of several proteases including cathepsin D, chymotrypsin, and carboxypeptidase B1 in the ovary. The enzymatic activities of these proteases in the ovary were also enhanced by Ci-TK-I. Intriguingly, the treatment of *Ciona* oocytes with Ci-TK-I resulted in progression of growth from the vitellogenic stage to the postvitellogenic stage. The Ci-TK-I-induced oocyte growth was blocked by a TK antagonist or by protease inhibitors. These results led to the conclusion that Ci-TK-I specifically enhances growth of the vitellogenic oocytes via upregulation of gene expression and enzymatic activities of the proteases such as cathepsin D, chymotrypsin, and carboxypeptidase B1. Moreover, immunostaining of the mouse ovary showed the specific colocalization of mammalian TK receptors (NK1, -2, and -3) and the aforementioned proteases in granulosa cells within prenatal follicles. Furthermore, 5-day treatment of mouse prenatal follicles with antagonists of TK receptors resulted in a complete suppression of oocyte growth. These results suggest that TKs plays a major role in oocyte growth in mammals and that the TKergic protease-assisted oocyte growth is essentially conserved throughout chordates. This is the first clarification of the biological roles of TKs in the ovary and the underlying essential molecular mechanism. Our present data are expected to pave the way to the understanding of oocyte growth at early stages and development of clinical treatments of oocyte quality.

(1) Satake H et al., (2004) J Biol Chem 2004; 279: 53798-53805
(2) Aoyama M et al., (2008) Endocrinology 2008; 149: 4346-4356
(3) Endocrine News 2008; 33: p.6

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Nothing to Disclose: HS, MA, TK, TS, TS, SI, KY

P2-387

Interaction of Prolactin and Bone Morphogenetic Protein System in Regulating Steroidogenesis by Rat Granulosa Cells.

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PRL directly inhibits gonadotropins secretion from the pituitary. Despite the evidence indicating that PRL also exerts inhibitory effect on gonadotropin actions in the ovary, the mechanism remains uncertain. To elucidate the mechanism by which PRL modulates follicular development in the ovary, we examined the functional roles of PRL in steroidogenesis using rat granulosa/oocyte co-culture by focusing BMP system. We first confirmed the expression of PRL receptors (PRLR), in which long and short forms of PRLR were detected in oocytes and granulosa cells. PRL effectively upregulated PRLR expression in granulosa cells in the presence of FSH, whereas FSHR expression was not affected by PRL. PRL treatment suppressed FSH-induced estradiol production in the presence or absence of oocytes, while FSH-induced progesterone was increased by PRL in granulosa cells. The PRL effects on FSH-induced progesterone were restored by co-culture with oocytes, implying the roles of oocyte-derived factors in suppressing progesterone in PRL-exposed granulosa cells. In accordance with the steroids data, FSH-induced aromatase expression was suppressed by PRL, while FSH-induced StAR, P450scc and 3βHSD levels were amplified by PRL. However, forskolin- and BtcAMP-induced steroid levels and FSH- and forskolin-induced cAMP were not affected by PRL, suggesting that PRL modulation on FSH-induced steroidogenesis was not solely due to cAMP-PKA regulation. Treatment with a BMP binding protein noggin facilitated PRL-induced estradiol reduction, and noggin increased PRL-induced progesterone in FSH-treated granulosa cells, suggesting that endogenous BMPs reduce progesterone but increase estradiol when exposed to high concentrations of PRL. Notably, PRL augmented BMP-induced Smad1/5/8 signaling by reducing inhibitory Smad6, and PRL also increased BMP ligands expression in granulosa/oocyte co-culture. In addition to STAT3/5 phosphorylation, PRL enhanced FSH-induced ERK and p38 phosphorylation in granulosa cells, in which ERK activation was preferentially involved in suppressing FSH-induced estradiol. Furthermore, noggin treatment enhanced the PRLR signaling including MAPK and STAT. Thus, PRL suppresses FSH-induced estradiol through augmenting FSH-induced MAPK activity with PRLR upregulation. Considering that BMPs suppressed PRLR in granulosa cells, it is likely that the BMP system in the growing follicles may play a key role in antagonizing PRL actions in hyperprolactinemia.

Nothing to Disclose: EN, FO, KI, TM, RY, NT, MT, JS, TO, HM

P2-388

Molecular Mechanisms of PSF Regulation of Forskolin-Stimulated P450scc Expression in Human Ovarian Cell Steroidogenesis.

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Steroidogenesis in granulose cells is a complex and highly regulated process determined, at least in part, by transcriptional activity of the P450scc gene. We previously reported that the transcription factor PSF binds to the promoter of the p450scc gene thereby inhibiting steroidogenesis. In order to understand the upstream signaling pathways and the transcriptional activation mechanisms of the P450scc gene, we have used the KGN human granulosa cell tumor line. We have shown, using the FSH-mimetic forskolin, a time and dose-dependent induction of phosphorylation of many PKA substrates. In particular, phosphorylation of CREB was maximal at 1 hr and returned to normal by 4 hr. In addition, protein expression was unchanged for PSF or Sp1, another transcription factor that binds to the p450scc promoter but activates expression. Using ChIP assays, we found that both Sp1 and PSF constitutively reside on the IGF-I response element (IGFRE) within the p450scc promoter in a forskolin-independent manner. On the other hand, pol II occupancy of the internal coding sequences of the p450scc gene was induced by forskolin. These observations suggest that recruitment of PSF and Sp1 on or off of the IGFRE is not a mechanism for regulation of transcription of this target gene. This suggests the possibility that posttranslational modifications of these transcription factors may be important in p450scc gene expression. Increased pol II occupancy in response to forskolin was followed by accumulation of p450 mRNA and secretion of progesterone. Treatment with siRNA to p450scc blocked forskolin induction of p450scc mRNA and progesterone secretion. Furthermore, siRNA to PSF reduced PSF mRNA and blocked forskolin induction of p450scc mRNA and progesterone secretion. These results have importance to the evolving picture of signaling pathways that impinge on transcription factor activity to ultimately regulate functional outcomes such as steroid secretion. To better understand these processes, we are studying kinases that mediate this information processing in granulosa cells as well as posttranslational modifications of PSF and Sp1.

Sources of Research Support: National Institute of Child Health and Human Development Grant HD-36092 (R. J. Urban).

Nothing to Disclose: LD, YB, JJ, RJU

P2-389

Rate of Follicular Atresia and Expression of Protein Bcl-2 in Autotransplanted Ovaries in Miniature Pig Model: Preliminary Results.

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Objective: An animal model of ovarian transplantation using miniature pig was used to study the impact of this procedure on ovarian physiology and fertility. Preliminary results of the investigation of survival mechanisms and apoptosis of ovarian cells are showed.
Methods: Forty female miniature pigs *(Sus escropha*), were submitted to laparoscopy for ovariectomy and ovarian auto-transplantation. Animals were sorted in five groups: Group I (control, n=8): Laparoscopic <st2:dm w:st="on">bilateral</st2:dm> ovariectomy; Group II (FSC, n=8): Fresh Subcutaneous Transplantation: ovarian slices of 2mm of thickness were transplanted to inguinal region right after removal; Group III (FIP, n=8): Fresh Intra-peritoneal Transplantation: slices of 2mm of thickness were transplanted to peritoneal region, right after removal; Group IV (CSC, n=8): Cryopreserved Ovaries Subcutaneous Transplantation: ovarian samples submitted to slow freezing protocol, kept in liquid Nitrogen for 7 days and transplanted in subcutaneous site; Group V (CIP, n=8): Cryopreserved Ovaries Intra-peritoneal Transplantation: ovarian samples submitted to slow freezing protocol, kept in liquid nitrogen for 7 days and transplanted in intra-peritoneal location. After 58 days, the ovaries were removed and processed for analysis; To assess the impact of different methods on follicle quality, they were counted and classified as healthy or atretic. Immunohistochemistry was performed to detect the presence and intensity of protein Bcl-2 antibody. Quantitative analysis of the intensity of the signal was made using Image J Plus software and the intensity is expressed in pixels. Statistical tests used were Kruskall-Wallis and Dumm (rate of atresia), ANOVA and Tukey (Bcl-2).
Results: Healthy follicles were found in lower number in cryopreserved ovaries transplanted in subcutaneous site (CSC) when compared to control group as well as, those transplanted into intra-peritoneal sites (CIP) ($p=0,044$). Signal intensity of Bcl-2 protein antibody was higher in control group (GI) and the lower in the fresh transplanted groups ($p<0.05$).
Conclusion: Miniature pig model seems to be a reliable tool to investigate ovarian transplantation. Our data show that different methods and sites of ovarian autotransplantation can induce different degrees of follicle atresia, and influence apoptosis pathways. It might be important for surgical and clinical choices.

Nothing to Disclose: LCVD, JI, GARM, JMS, MJS, RSS, SAYH, ECB

P2-390

Secretion of Pregnancy-Associated Plasma Protein-A by Human Granulosa-Lutein Cells Is Stimulated by hCG in the In-Vitro Cell Culture.

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Pregnancy-associated plasma protein-A (PAPP-A) is a metalloproteinase which specifically cleaves the insulin-like growth factor-binding protein 4 (IGFBP-4) liberating bound insulin-like growth factors (IGF) for receptor binding. It is assumed that PAPP-A is a regulator of local IGF bioavailability in a variety of biological systems. While in pregnant women high amounts of PAPP-A are produced by the placenta, in nonpregnant women the granulosa cells of ovarian follicles are important source of PAPP-A. There is evidence that PAPP-A produced by follicular granulosa cells is a crucial regulator of follicular development. In the present study we examined the effect of human chorionic gonadotropin (hCG) on the secretion of PAPP-A by in vitro cultured human human granulosa-lutein (GL) cells.
Human GL cells collected at the time of oocyte retrieval from six women undergoing in-vitro fertilization were cultured in 96-well plates in culture medium supplemented with increasing concentrations of hCG (0 – 500 mIU/mL). After incubation for 48 hours the concentration of PAPP-A and progesterone was determined in the spent culture medium using a novel highly sensitive ELISA technique.
The PAPP-A concentration in the spent culture medium ranged from 0.4 to 1.9 mIU/L under basal conditions. The PAPP-A production was stimulated by hCG in a dose-dependent manner reaching a maximum of 2.3 ± 0.4-fold increase at 50 mIU/L hCG. The stimulation of PAPP-A production was accompanied by a 2.5 ± 0.3-fold increase of progesterone production. PAPP-A and progesterone increment after hCG correlated strongly ($r = 0.886$, $p < 0.01$).
The present data indicate that the production of PAPP-A by human GL cells is regulated by hCG. hCG acts as agonist at the LH receptors of the GL cells as confirmed by its stimulatory effect on progesterone production. The results give further evidence for the hypothesis that PAPP-A plays a major role in luteinization process.

Nothing to Disclose: JR, FB, PW-T, DK, HvdV

P2-391

Expression of Luteinizing Hormone (LH) Subunit Genes in the Rat Ovary: Evidence from Two Animal Models.

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Luteinizing hormone (LH) belongs to the family of dimeric glycoprotein hormones, which includes FSH, thyroid stimulating hormone (TSH), and placental chorionic gonadotropin (CG). These hormones are composed of a common α-subunit(Cgα) and a specific β-subunit(LH-β). The present study was aimed to investigate the expression of LH subunit genes in the rat ovary using two animal models. Expression of Cgα and LH-β subuit genes in the rat ovary was demonstrated by RT-PCRs and southern hybridization analyses. The identity of LH-β mRNA from pituitray and ovary was further confirmed by 3'-RACE. To assess the production of immunoreactive LH-like peptide in the rat ovary, specific RIA was performed. Distributions of Cgα and LH-β peptides in rat ovary were verified using immunohistochemistry. To investigate the physiological regulation of the ovarian LH expression, hypophysectomized rat model and pregnant mare's serum gonadotropin (PMSG) injection model were employed. RT-PCRs and southern hybridization analyses revealed the presence of mRNAs for Cgα, pituitary type LH-β and testis type LH-β in the rat ovary. The size of the 3'-RACE fragments from both pituitary and ovarian LH-β transcripts was identical. Considerable amounts of immunoreactive LH-like peptides were detected in crude extracts from the rat ovary. In immunohistochemical staining, the theca cells, corporal lutea cells and oocytes were Cgα and LH-β positive, whereas cumulus cells and granulosa cells were negative. The mRNA level of Cgα was significantly lower ($p<0.05$), whereas the level of LH-β was significantly higher ($p<0.05$) in the hypophysectomized rat ovary compared to those from control animals, respectively. Administration of PMSG to immature rats resulted in significant increases of Cgα mRNA levels (24h, $p<0.001$; 48h, $p<0.01$; 72h, $p<0.001$) and a significant decrease of LH-β mRNA level (48h, $p<0.01$; 72h, 0.001). The mRNA levels of testis type LH-β in ovary were not changed after PMSG injection. The present study demonstrated that LH subunit genes are expressed and physiologically regulated in the rat ovary, and suggested that tha ovarian LH may participate in the regulation of reproductive physiology via paracrine/autocrine mechanism as well as endocrine mechanism.

Nothing to Disclose: SHL, SYL

P2-392

Demonstration of Expression and Regulation of Src Family of Kinases and Phosphodiesterase during Different Functional Status in the Macaque Corpus Luteum: Role in the Modulation of LHR Signaling during Luteolysis.

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In higher primates, hormonal regulation of corpus luteum (CL) function appears to be unremarkable, as it is primarily reliant on LH/CG for its function. However, knowledge of the processes whereby LH promotes development and maintenance of CL function remains limited. Studies are essential to selectively exploit various LH-activated signaling pathways critical to the control of CL function. Recent studies indicate stimulation of Src family of kinases [(SFKs), Fyn, Yes and Src] upon activation of LH/CG receptor (LHR) in MA-10 cells, but the mechanisms and functional consequences of activation of non-receptor tyrosine kinases are not fully understood in LH target tissues. With a view to examine the role of SFKs downstream of LHR signaling in the CL tissue, expression of SFKs was examined by semi-quantitative RT-PCR and immunoblot analyses during different functional status of the monkey CL. The mRNA expression for SFKs was detectable throughout the luteal phase, higher in CL tissues collected on day 1 of menses and after GnRH receptor antagonist (Cetrorelix; CET) treatment. Sequencing of amplified products revealed >90% homology to human and rhesus SFKs mRNA, confirming the presence of SFKs transcripts in the macaque CL. The immunoblot analyses revealed presence of higher levels of activated Src (phosphorylated at position Tyr416) from CL tissues collected during spontaneous and induced luteolysis. Further, to examine the role of phosphodiesterases (PDE), mRNA and protein expression of ovarian specific PDE4D isoform was examined throughout the luteal phase, during induced luteolysis and in LH replacement experiments. The presence of higher levels of activated Src in the regressed CL suggest its participation in the regulation of steroid synthesis, which may in part be modulated by PDE activity besides involvement of other components of LH/CG signaling pathway. Since, LH mediates its nuclear action primarily by activating canonical cAMP/PKA/CREB pathway, it was reasoned that activation of Src or PDE might modulate one of these components. However, examination of cAMP levels in the regressed CL did not show a significant decline, but in our previous study it was observed that the regressed CL had low PKA activity and pCREB levels (1) suggesting perhaps activated Src and/or PDE may mediate their actions downstream of cAMP. These results taken together, suggest participation of SFKs and PDE in the LHR signaling mediated regulation of macaque CL function.

S. Priyanka and R. Medhamurthy Mol Hum Reprod 2007 13:381

Nothing to Disclose: KS, MR

P2-393

Dynamics of Anti-Müllerian Hormone (AMH) Serum Concentrations during the Peripubertal Period in PCOS Daughters.

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Context: Polycystic ovary syndrome (PCOS) is a highly prevalent (5-10%) endocrine-metabolic dysfunction in premenopausal women, and is the most frequent cause of anovulatory infertility and hyperandrogenism in women. Ovarian morphology assessed by ultrasound and by histological examination has shown that polycystic ovaries are characterized by an excessive number of growing follicles compared with normal ovaries, suggesting an altered folliculogenesis in this syndrome. In previous studies, we have found that prepubertal and pubertal daughters of women with PCOS exhibit higher levels of anti-Müllerian hormone (AMH), a marker of growing follicles, compared with control girls. This suggests that the follicular alterations described in adult PCOS women may appear early during development. However, the dynamics of AMH serum concentrations during the different pubertal stages has not been described. Objective: To assess AMH serum concentrations of daughters of women with PCOS (PCOSd), during different pubertal stages, compared to daughters of control women (Cd). Design: Ninety six PCOSd and 45 Cd were studied during puberty and classified according to Tanner stage distribution. An oral glucose tolerance test with measurement of glucose and insulin was performed in all girls. Gonadotropins, sex steroids, SHBG, lipids, adiponectin and AMH were determined in the basal sample.
Results: PCOSd and Cd showed a similar dynamics of AMH concentrations during the different Tanner stages. Nevertheless, AMH levels were significantly higher in the PCOSd group compared to the Cd group in all Tanner stage: I (12.9 ± 3.4 vs 28.8 ± 5.3 pmol/L, mean ± SEM; p=0.02), II (19.4 ± 3.6 vs 35.6 ± 4.4 pmol/L, mean ± SEM; p=0.01), III (14.1 ± 2.7 vs 27.5 ± 4.8 pmol/L, mean ± SEM; p=0.02), IV (13.5 ± 2.7 vs 29.3 ± 6.7 pmol/L, mean ± SEM; p=0.04) and V (11.5 ± 2.0 vs 22.9 ± 3.9 pmol/L, mean ± SEM; p=0.02). Those PCOS with the highest AMH concentrations, using 2 SD above the respective Cd group mean value, showed higher post-stimulus insulin concentrations, higher insulin area under the curve, lower HDL, and lower adiponectin levels.
Conclusions: PCOSd showed higher AMH levels compared to Cd during all Tanner stages. Those PCOS with the highest AMH levels exhibited altered metabolic parameters since the beginning of puberty. These results suggest that metabolic factors may influence the size of the growing follicular mass in these girls.

Sources of Research Support: FONDECYT Grant 1071007.

Nothing to Disclose: TS-P, JP, BE, ALdG, MM, NC, VP, FS

P2-394

Role of Endogenous LH Secretion on Theca Cell Responsiveness to Provocative Gonadotropin Stimulation in Women with Polycystic Ovary Syndrome.

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Objective: In women with polycystic ovary syndrome (PCOS), excess androgen production is largely derived from the ovary. Consistent with earlier studies, we have previously reported that 17-OHP dose-responsiveness to recombinant human chorionic gonadotropin (r-hCG) in PCOS women is greater than that observed in normal women, suggesting an inherent defect in theca cell activity (2008 Endo Society, 2009 ASRM). However, androgen overproduction has also been attributed to increased LH secretion, which is characteristic of PCOS women. To examine whether endogenous gonadotropin secretion contributes to increased theca cell androgen production in PCOS women, we measured basal and stimulated serum 17-OHP levels following intravenous injection of r-hCG before and after administration of a GnRH antagonist.
Design: Prospective study at a general clinical research center in a tertiary academic medical center.
Methods: 9 PCOS and 10 normal controls received intravenous r-hCG, 25 micrograms. Blood samples were obtained prior to and 24 hours after r-hCG. After one month, each subject received a subcutaneous injection of GnRH antagonist (Cetrotide, 3mg). An identical dose of r-hCG was re-administered 48 hours later and blood samples were obtained as described above.
Results: Prior to GnRH antagonist treatment, 24 hour stimulated levels of 17-OHP in PCOS women were significantly higher than those of normal women. After GnRH antagonist, circulating gonadotropins and basal 17-OHP levels were reduced in both groups. Following hCG administration, the patterns of 17-OHP responses were strikingly similar, although serum 17-OHP levels at 24 hours in both groups were reduced compared to before gonadotropin suppression. Notably, 17-OHP responses in PCOS women treated with GnRH antagonist (reduced endogenous gonadotropins) remained higher than those observed in normal women prior to GnRH antagonist.
Conclusions: Consistent with our previous findings, theca cell responses are greater in PCOS women compared to normal women. After GnRH antagonist, 17-OHP responses to hCG were slightly reduced in both groups of women but were still markedly higher in the PCOS group compared to normal controls. These findings suggest that endogenous gonadotropin secretion influences basal 17-OHP release whereas an effect on stimulated theca cell androgen production appears to be minimal. This is consistent with a primary defect of theca cell activity in PCOS women.

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: MAR, MSC, RS, AH, RJC

P2-395

Endocrine Disruptors and Polycystic Ovary Syndrome (PCOS): Elevated Blood Levels of Bisphenol A in PCOS Women.

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INTRODUCTION: Bisphenol A (BPA) is used primarily in the synthesis of polycarbonate plastics and is key monomer in production of epoxy resins. It has been shown that the BPA blood levels are higher in men than in women, a finding that is attributed between androgen and BPA interactions on clearance and SHBG binding properties . In addition it has been found that the exposure of experimental animals to BPA adversely influences oocyte development and results in ovarian cystic morphology.
AIM OF THE STUDY: Aim of present study is the determination of BPA levels in serum of women with polycystic ovary syndrome (PCOS) compared to controls, age and body mass index (BMI) matched, as well as the investigation of association between BPA levels and hormonal and metabolic parameters of studied subjects.
METHODS: One Hundred normal and 71 PCOS (NIH criteria) women were studied. In all subjects anthropometric, hormonal and metabolic parameters, as well as, BPA blood levels were determined. Patients and controls were subdivided and matched respectively in two groups, according BMI, a lean subgroup (LC+LPCOS) and an obese subgroup (OC+OPCOS).
RESULTS: BPA levels were significantly higher in lean (1.12±0.10 vs 0.70±0.05, $p< 0.0007$) and obese PCOS women (0.97±0.08 vs 0.74±0.07, $p<0.044$) compared to controls respectively. Additionally, significant higher insulin and androgens levels were found between PCOS and controls subgroups. A significant correlation was found between testosterone ($r=0.188$, $p=0.03$), Δ4-androstenedione ($r=0.258$, $p=0.003$) and BPA serum levels.
CONCLUSION: Our findings demonstrate that, PCOS women have higher BPA blood levels compared to controls independently of BMI, and the demonstrated positive correlations between BPA levels and androgens imply that this endocrine disruptor may play a role in the pathophysiology of syndrome.

Nothing to Disclose: EK, AC, EP, FE, SL, MK, IK, DP, ED-K

P2-396

A Single Nucleotide Polymorphism in *STK11* Influences Insulin Sensitivity and Metformin Efficacy in Hyperinsulinemic Girls with Androgen Excess.

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Aim: Serine-threonine-kinase STK11 catalyzes the AMP-activated protein kinase complex. We tested the hypothesis that a gene variant in *STK11* contributes to variation in insulin sensitivity and metformin efficacy.
Population, Design, Outcomes: We studied the effects of a single nucleotide polymorphism (SNP; rs8111699) in *STK11* on endocrine-metabolic and body composition indices before and after 1 yr on metformin in 85 hyperinsulinemic girls with androgen excess representing a continuum from prepuberal girls with a combined history of low birthweight and precocious pubarche over to postmenarchial girls with hyperinsulinemic ovarian hyperandrogenism. Metformin was dosed at 425 mg/d in younger and 850 mg/d in older girls. *STK11* rs8111699 was genotyped. Endocrine-metabolic features were assessed in fasting state; body composition was estimated by absorptiometry.
Results: Genotype effects were similar in younger and older girls. At baseline, the mutated G allele in *STK11* rs8111699 was associated with higher insulin and IGF-I levels (both $p<0.005$). The response to metformin differed by *STK11* genotype: GG homozygotes (N=24) had robust metabolic improvements, GC heterozygotes (N=38) had intermediate responses, and CC homozygotes (N=23) almost no response. Such differences were found for 1-yr changes in body composition, circulating insulin, IGF-I, free androgen index and lipids (all $p<0.005$).
Conclusion: In hyperinsulinemic girls with androgen excess, the *STK11* rs8111699 SNP influences insulin sensitivity and metformin efficacy, so that the girls with the least favorable endocrine-metabolic profile improve most on metformin therapy.

Nothing to Disclose: AL-B, MD, EM, FEdZ, LI

P2-397

Abnormalities in the Mitogen Activated Protein Kinase (MAPK) Pathway in Adipocytes of Women with Polycystic Ovary Syndrome (PCOS).

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Introduction: PCOS is a common endocrine-metabolic abnormality of women, and ~70% of affected women demonstrate clinically measurable insulin resistance above and beyond that determined by their degree of obesity. We and others have previously demonstrated that alterations in the IRS/PI3K/Akt insulin signaling pathway contribute to the defective glucose metabolism and insulin resistance of PCOS. However, more recent studies suggest that the MAPK pathway, involved in cell growth and proliferation, could also contribute to PCOS insulin resistance. In this study, we investigated the total content and degree of phosphorylation of three major components of the MAPK pathway, p38, SAP/JNK and ERK1/2, in freshly isolated adipocytes from PCOS patients and matched controls
Methods: Lower abdominal subcutaneous adipose tissue was obtained from 13 women with PCOS and 12 body-mass index (BMI)-matched controls. Isolated adipocytes were untreated or stimulated with insulin. After protein extraction, total protein levels and basal and insulin-stimulated phosphorylation levels of p38, SAP/JNK and ERK1/2 were determined by Western blot analysis.
Results: There were no significant differences in total p38 or total SAP/JNK levels, or in basal or insulin-stimulated levels of phospho-p38 or phospho-SAP/JNK, in PCOS adipocytes vs. controls. Alternatively, lower total ERK1/2 and higher basal phospho-ERK1/2 levels (-13%, $p<0.02$, and +40%, $p<0.02$, resp.) were identified in PCOS adipocytes vs. controls.
Conclusions: Total content and degree of basal and insulin-simulated phosphorylation are similar in PCOS vs. control adipocytes for both p38 and SAP/JNK, two of the major components of the MAPK pathway. However, basal levels of phosphorylated ERK1/2 are significantly increased in PCOS, suggesting that constitutive activation of ERK1/2 may contribute to the insulin resistance of PCOS. Our results are consistent with other studies demonstrating that altered levels of phospho-ERK1/2 are present in PCOS skeletal muscle cells, and may contribute to increased androgen biosynthesis in ovarian theca cells. These data suggest a role for the MAPK pathway in the endocrine-metabolic abnormalities of PCOS.

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Nothing to Disclose: J-ML, Y-HC, GC, RA

P2-398

The Impact of Long Chain *n*-3 Polyunsaturated Fatty Acid (PUFA) Supplementation on Metabolic and Reproductive Hormone Profiles of Women with Polycystic Ovary Syndrome (PCOS).

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Since long chain (LC) *n*-3 PUFA supplementation has been reported to reduce plasma triglycerides, and have an anti-androgenic effect in prostate cancer, we investigated the effects in polycystic ovary syndrome (PCOS).
Twenty-two women with PCOS (NIH-criteria) participated in a double-blind placebo-controlled, cross-over trial. Subjects were randomly assigned to receive *n-3* PUFA (containing 1.8g EPA/DHA) or placebo for 6 weeks. After a further 6-week washout, subjects crossed over to receive the alternative treatment. At baseline and after each 6-week treatment, we evaluated IS (QUICKI, HOMA-IR), reproductive hormone profiles, and fasting and post-prandial plasma levels of glucose, insulin and lipids (NEFA, triglycerides, ApoB48).
LC *n-3* PUFA supplementation resulted in appropriate increase in plasma *n*-3 concentrations and reduction in *n*-6:*n-3* ratio. Weight, percent body fat, habitual dietary intake, IS (QUICKI, HOMA-IR), glucose, insulin and lipids were unchanged. However, there was a significantly greater reduction in DHEAS and bioavailable testosterone compared to placebo.
To further investigate the changes in androgens, we performed an *in vitro* assessment of androstenedione secretion from bovine theca cells following treatment with both *n*-6 and *n*-3 PUFA. There was a significant increase in androstenedione secretion following treatment with *n*-6 PUFA compared to *n*-3 PUFA and control.
Here we report the novel finding that *n*-3 PUFA supplementation in women with PCOS results in reduction in circulating androgens. The mechanism through which this effect occurs is not known and whether these relatively small biochemical effects are of clinical significance requires further exploration.

Change in androgens in response to n-3 PUFA compared to placebo

	Baseline (mean)	n-3 PUFA*	Placebo*	p value
Free androgen index	12.495	-2.369	1.359	0.06
Bioavailable testosterone (nmol/l)	2.159	-0.693	0.07	<0.05
DHEAS (µmol/l)	6.963	-1.132	-0.765	<0.01
Testosterone (nmol/l)	2.582	-0.173	0.014	NS
Androstenedione (nmol/l)	15.780	-1.48	-0.345	NS
SHBG (nmol/l)	27.2	0.605	-0.624	NS

*Data expressed as change from baseline

Nothing to Disclose: NP, AO, TK-T, NC, GB, HMR, JG

P2-399

Evaluation of Gallbladder Motility in Patients with Polycystic Ovary Syndrome.

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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. There are data indicating that insulin resistance (IR) and many hormonal changes observed in PCOS affect GB motility. However, patients with PCOS have not been evaluated in terms of GB motility until now. Therefore we aimed to evaluate GB motility in patients with PCOS.
Methods: Thirty-six 36 PCOS patients and 20 age and BMI-matched controls (mean age 23.4±3.9 vs 22.3±4.5 years, p=0.375; mean BMI 24.9±4.1 vs 26.8±4.0, p=0.177, respectively) formed the study group. The GB was measured in three dimensions, [longitudinal (L), width (W) and depth (D)], and the volume (V) was calculated using the ellipse formula (π/6xLxDxW). Following the determination of fasting GB volume, patients were given standard liquid aliment consisting of 29.5% (19.68 g) fat, 16.7% protein, and 53.8% carbohydrate. Then the GB volume measurement was repeated at 10^{th}, 20^{th}, 30^{th}, 40^{th}, 50^{th}, 60^{th}, 75^{th} and 90^{th} minutes (t0, t10, ...t90). Gallbladder ejection fraction (GBEF) was calculated [GBEF tx/ty (%)=(GB volume tx-GB volume ty)x100/GB volume tx) for each measurement period.
Results: Insulin, LH, total and free testosterone (TE) levels, HOMA index and LH/FSH ratio values were significantly higher 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 $cm^{3,}$ $p < 0.001$). A positive correlation was observed between V0 and fasting glucose, insulin, free TE and total TE levels and HOMA index value [(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; r=0.493, p=0.008), respectively]. PCOS group had lower GBEF at and after 20^{th} minute compared to controls. 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 negative correlation was detected between GBEF and BMI, free TE, total TE, 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).
Conclusion: We, for the first time in the literature, have showed that GBEF in decreased in PCOS patients, and this decrease was correlated with co-existing obesity, hyperandrogenism and insulin resistance.

Nothing to Disclose: SI, HNO, DB, YT, UO, GA, SG

P2-400

Brain Morphometric Abnormalities in Polycystic Ovary Syndrome Associated Obesity.

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Background: Women with polycystic ovary syndrome (PCOS) are at high risk for obesity. Neuroimaging studies have revealed abnormalities in cortical structures in obese individuals. We hypothesized that PCOS is a distinct subphenotype of obesity and, accordingly, may have unique neuroantomical changes.
Methods: T1-weighted MPRAGE scans were collected in 16 obese women with PCOS and in 9 reproductively normal obese control (obese non-PCOS) women of comparable age and weight as well as in 10 lean control (lean non-PCOS) women of comparable age. FreeSurfer software was used to measure cortical gray matter volume and thickness. FreeSurfer+Large Deformation Diffeomorphic Metric Mapping were used to generate a thalamic surface in all MPRAGE scans. Delineation of the mediodorsal nucleus on the thalamic surface using previously described methods enabled us to compare its deformation between groups. Data were analyzed using parametric or non-parametric analysis of variance with appropriate post-hoc tests. Significance was adjusted for multiple comparisons to $p<0.01$.
Results: Surface deformation of the right mediodorsal nucleus of the thalamus differed significantly ($p<0.01$), with obese PCOS having significantly more inward deformation than both obese and lean non-PCOS. Thickness of the left fusiform gyrus differed significantly ($p<0.0001$) with thinner dimensions in obese PCOS compared to both obese and lean non-PCOS. This region was also significantly thinner in lean compared to obese non-PCOS. Thickness of the left medial orbitofrontal cortex differed significantly ($p<0.01$) with decreased dimensions in obese non-PCOS compared to obese PCOS and lean non-PCOS. The right middle frontal gyrus and right precentral gyrus were significantly (both $p<0.01$) thinner in obese non-PCOS compared to lean non-PCOS. Right inferior frontal, right parahippocampal and right lingual gyri, and right cerebellar regions did not differ significantly between obese and lean non-PCOS, in contrast to previous studies in obese individuals(1).
Conclusion: There were neuroanatomical changes unique to obese PCOS when compared to obese and lean non-PCOS, and included surface deformation of the right mediodorsal nucleus of the thalamus and thickness of the left fusiform gyrus. In contrast, thickness of the left medial orbitofrontal cortex, which is part of the reward circuit, differed in obese non-PCOS. These findings suggest that there are differences in the neural mechanisms for obesity in PCOS.

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Nothing to Disclose: BM, KR, LW, DG, TP, JG, JC, AD

P2-401

Differential Regulation of Bisphenol A in PCOS Women and by Prenatal Testosterone Excess in Pregnant Nonhuman Primates.

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Bisphenol A (BPA) is a widely used estrogenic industrial chemical that can impair female mammalian reproduction (1), enhance ovarian androgen production (2), induce insulin resistance (3) and impair oocyte maturation (4-6), all features of polycystic ovary syndrome (PCOS) (7). To determine whether BPA levels differ between ovulatory normoandrogenic (NL) and PCOS women, BPA levels were measured in 1) blood and follicular fluid (FF) of 5 lean NL (BMI, 22.7±1.7 kg/m2; age, 31.4±3.9 yrs [SD]) and 5 age- and weight-matched PCOS women (BMI, 22.5±1.7 kg/m2, P=0.9; age, 30.2±3.2 yrs, P=0.6) undergoing in vitro fertilization (IVF), and in 2) urine of 50 NL and 51 PCOS non-IVF women. BPA levels also were measured in maternal blood and amnionic fluid of adult female rhesus monkeys receiving testosterone propionate (TP, 15 mg sc daily, or vehicle (CTL) from gestational days 40-80 [term, 165 days]) as a nonhuman primate PCOS model. Free (unconjugated) BPA represented over 95% of total (free+conjugated) plasma BPA in women and nonhuman primates. During IVF, free and total plasma BPA levels were significantly lower in PCOS (free, 0.59±0.29; total, 0.59±0.29 ng/mL) than NL women (free: 1.31±0.44, P<0.025; total: 1.42±0.45 ng/mL, P <0.01), without differences in % free BPA (P=0.1). Within follicles, BPA was almost exclusively conjugated, elevating conjugated BPA levels in FF (0.27±0.17) above those in plasma (0.09±0.08, P<0.01), without a PCOS effect (P=0.4). Total urinary BPA levels also were similar between PCOS and NL women (P=0.7). In monkeys, prenatal TP treatment tended (p=0.06) to lower maternal plasma free (1.30±0.54) and total BPA (1.36± 0.57 ng/mL) levels versus CTL (free: 2.82±1.29; total: 2.34±1.28 ng/mL) without affecting % free BPA (P=0.4). Amnionic total BPA levels positively correlated with maternal plasma free (R2=0.37, P<0.05) and total (R2=0.36, P=0.05) BPA levels, indicating fetal vulnerability to circulating maternal BPA levels. Both free and conjugated BPA levels, however, were similarly reduced to less than one-half of maternal values in TP-treated (0.6±0.03) and CTL monkeys (0.6±0.02, P=0.8 vs TP-treated). Therefore diminished plasma BPA levels in PCOS women are not due to enhanced urinary loss or follicular conjugation, but may represent preferential uptake of BPA into specific tissues, such as adipose, induced by androgen excess, while complex vulnerability to BPA exists in PCOS-like primates during maternal-fetal development.

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Nothing to Disclose: DAD, RA, VP, KK, SV, AFT, DHA

P2-402

Total Testosterone Assays in Polycystic Ovary Syndrome: Is There a Gold Standard?.

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Context: There is no standardized assay of testosterone in women. Liquid chromatography mass spectrometry (LC/MS) has been proposed as the gold standard by an Endocrine Society Position Statement.(1)
Objective: To compare assay results from a direct radioimmunoassay (RIA) with two LC/MS assays.
Design, Setting and Participants: Blinded laboratory study with masked duplicate samples. Baseline serum samples from 596 women with PCOS who participated in a large multi-center randomized controlled infertility trial (PPCOS) performed at academic health centers in the U.S. were re-run concurrently and independently at 3 laboratories with different testosterone assays: two academic (University of Virginia-UVA with RIA and Mayo Clinic with LC/MS) and one commercial (Quest with LC/MS).
Main Outcome Measure: Precision and correlation within and between assays and with baseline Ferriman Gallwey hirsutism scores.
Results: The median testosterone level by RIA at UVA was 50 ng/dL (25th-75th percentile 34-71 ng/dL) by LC/MS at Mayo 47 ng/dL (25th-75th percentile 34-65 ng/dL) and by LC/MS at Quest 41 ng/dL (25th-75th percentile 27-58 ng/dL). The correlations between the blinded samples which were run in duplicate were comparable. The correlation coefficient between LC/MS at Quest and Mayo was 0.83 (95% confidence interval 0.80-0.85), between RIA and LC/MS at Mayo was 0.79 (95% confidence interval 0.76 -0.82) and between RIA and LC/MS at Quest was 0.67 (95% confidence interval 0.63-0.72). The correlation coefficients for LC/MS at Quest were not significantly different from those from Mayo (P = 0.14) or UVA (P=0.23). Interassay variation for all assays was highest at the lower levels of total testosterone (<50 ng/dL). We examined the correlation between total testosterone levels and hirsutism score and found the highest correlation with the RIA (correlation coefficient = 0.24), vs LC/MS at Mayo (correlation coefficient = 0.15) or Quest (correlation coefficient = 0.17).
Conclusions: It is premature to anoint LC/MS as the gold standard for testosterone measurement. While testosterone levels with RIA are slightly higher than with LC/MS, all LC/MS assays may not be alike. Overall we found comparable correlations between LC/MS assays and our direct RIA. All assays have greater variability in the female normal testosterone range. We conclude that there is still utility, and potentially cost savings, in the measure of total testosterone by select RIA in hyperandrogenic women.

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P2-403

Increased Risk for Early Adult Cardiovascular Disease in Adolescent Girls with Polycystic Ovary Morphology.

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Hyperinsulinemia and obesity are identified as important features of polycystic ovary syndrome in adult women (PCOS) and could have impact for increased rate of CVD in these women. However, the mechanism by which obesity and excess insulin perturbs the cluster of other comorbidities associated with increased CVD is not fully understood. Identifying the subset of adolescent girls with early sighns of polycystic ovary morphology (PO-morph.) could provide valid information about the etiology of PCOS and also in assessing cardiovascular risk in very young women. The objective of the study was to investigate differences in insulin resistance, adiponectin and leptin concentrations and other cardiovascular risk factors in obese and lean adolescent girls with PO-morph.
METHODS: we studied 160 girls (age 12-18 yrs) with PO-morph. on ovarian ultrasound: 70 normal weight (CON, BMI 24±0.4) and 90 obese (OBS, BMI 30±0.6). In all girls we obtained antropometric measurements, physical examination, and took serum levels of androgenic, metabolic and hormonal profiles. Insulin resistance was estimated based on the indices driven from the results of standard OGTT.
RESULTS: All tested girls dad various degree of hyperandrogenemia and fasting glucose was similar in both obese and lean girls. However, there were significant differences in insulin levels and HOMA, the measure of insulin resistance. Results are in the table (mean ± SE).

Characteristic of PO-morphology in adolescent girls

Group	CON	OBS
systolic BP (mmHg)	104.5±2.4	128.5±2.5*
adiponectin (mg/ml)	11.0±1.6	5.4±1.2*
Proinsulin (pM)	12±1.6	21.4±2.6
Insulin sensitivity	8.8±0.3	4.4±0.2*
IR-HOMA	2.7±0.2	5.8±0.3*
Impaired glucose tolerance	11%	31%*
* , P<0.001		

CONCLUSION: All girls with polycystic ovary morphology have severe hyperinsulinemia after glucose stimulation. Adolescent OBS girls have markedly decreased adiponectin, increased leptin cocentration, severe hyperinsulinemia and insulin resistance. All these factors positively correlated with degree of polycystical morphological changes in their ovaries, which corraborate the role of hyperinsulinemia in early development of ovarian morphological changes. Measures of adiposity and marked elevations of biomarkers in the obese group may well forecast significant cardiovascular morbidities in the future.

Nothing to Disclose: MIB, MW-W, JS, WK

P2-404

Body Surface Area Determines the Cortisol Production Rate in Obese Patients with Polycystic Ovary Syndrome and Obese Healthy Women.

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Introduction. The pituitary-adrenal axis in obesity and polycystic ovary syndrome (PCOS) is marked by increased urinary excretion of cortisol and its metabolites. It is not as yet clear whether the increased cortisol production in PCOS is related to obesity *per se*. We investigated 15 obese PCOS women with a body mass index (BMI) between 30-54 kg/m^2 and 15 healthy obese controls, BMI 31-60 kg/m^2 , with a regular menstrual cycle. Patients and obese control women underwent 24 h blood sampling at 20 min intervals. Cortisol concentrations were measured with a sensitive assay. Data were analyzed with a new deconvolution program, approximate entropy (ApEn) and cosinor regression.
Outcome. Fasting progesterone, estradiol, SHBG and prolactin concentrations were identical in both groups, but testosterone levels were increased in PCOS (2.63 ± 0.35 *vs* 0.51 ± 0.14 nmol/liter, P <0.001). Basal, pulsatile and total cortisol production expressed per liter distribution volume, and per square meter body surface and as absolute amount per 24 h were similar in PCOS patients and matched healthy obese control women. However, the estimated absolute production per 24 h was significantly larger in patients and obese controls than that calculated for lean healthy controls. Burst frequency in PCOS was slightly diminished (13 ± 0.7 *vs* 15.3 ± 0.7 bursts/24 h, P=0.03) and this finding was corroborated by the decreased Weibull gamma parameter (11.9± 0.6 *vs* 14.2± 0.6, P=0.01). The regularity of cortisol secretion, quantitated by approximate entropy and the diurnal properties were identical.
Discussion. This study demonstrates equally increased cortisol production in PCOS women and obese healthy control women. Expansion of the distribution volume, in concert with central effects of leptin and possibly insulin can largely explain enhanced cortisol production in both PCOS and obesity. Increased adrenal androgen secretion in PCOS is therefore more likely caused by local and ACTH-independent mechanisms. The cause of the decreased pulse frequency is not yet clear.

Nothing to Disclose: FR, PK, AMP, HP

P2-405

A Combination of Simvastatin and Metformin Reduces Elevated Levels of Cytokines in Women with PCOS.

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Objective: Polycystic ovary syndrome (PCOS) is associated with a plethora of metabolic and endocrine derangements including chronic low-grade systemic inflammation manifested by, among other features, elevation of several pro-inflammatory cytokines. Recently, we have presented preliminary findings of a prospective randomized trial comparing the effects of simvastatin and metformin on PCOS after 6 months of treatment and demonstrating that both simvastatin and metformin improve hyperandrogenism, menstrual regularity and reduce ovarian size. In this report, we present the effects of these treatments on the levels of four cytokines: Tumor Necrosis Factor (TNFα), Interleukin 6 (Il-6), Interleukin 1β (Il-1β), and Interferon Gamma (IFN).
Materials and Methods: Forty-six women with PCOS were randomly assigned to the following treatments: simvastatin alone (S; 20 mg/day), metformin alone (M; 1,700 mg/day), or simvastatin + metformin (S+M) for six months. Serum was collected at baseline and after 6 months of treatment. None of the subjects used oral contraceptive pills or any other hormonal therapies. Levels of cytokines were determined using the Luminex system. Data analysis included Kruskal-Wallis and Wilcoxon Sign-Rank tests.
Results: After 6 months of treatment, compared to the baseline levels, TNFα declined following treatment with S alone by 34%; (P=0.02), M alone by 38% (P=0.04), and S+M by 31% (P=0.02). Il-6 was also reduced following all treatments: S by 66% (P=0.004), M by 54% (P=0.48), and the S+M by 65% (P=0.03). Il-1β did not significantly change following M treatment, but it declined by 32% following S treatment (P<0.05) and by 68% following S+M treatment (P=0.003). Finally, IFN did not significantly change following S alone or M alone, but declined by 37% following the combination treatment of S+M (P=0.006).
Conclusions: The present findings demonstrate that the use of Simvastatin and Metformin in women with PCOS results in a significant improvement of systemic inflammation. The most consistent reduction of inflammatory cytokines is apparent following combined S+M treatment.

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Nothing to Disclose: AC, BB, LP, RZS, AJD

P2-406

Polycystic Ovary Syndrome Is Associated with Depressive Disorder in Adolescents – A Prospective Cross Sectional Study.

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Background: Women with polycystic ovarian syndrome (PCOS) frequently report mood disturbance. While increased rates of depression in adult women with PCOS have been reported (1, 2), little is known concerning the prevalence of depression in adolescent PCOS girls though this is a time when prevalence of depression increases dramatically in the general population (3). One small study previously reported did not show a significant association with adolescent PCOS and depression (4). This is a prospective cross sectional study comparing the incidence of depression in adolescents with PCOS from a private and public tertiary referral unit in New Zealand with published normative data.
Methods: 102 girls with documented PCOS age 14-19 years completed the Reynolds Adolescent Depression Scale (RADS II) (5). The RADS long version was completed and the short form score extracted and compared with data from 3020 secondary school females of the same age and ethnic range who had participated in a national survey. The groups' RADS scores, as a number and as proportion depressed, were compared using linear regression, adjusting for the sample design of the Youth 2007 survey. As well as group (PCOS or Youth 2007), age, ethnicity and deprivation score were included as explanatory variables in both analyses. Within the PCOS group linear regression was used to investigate association of objective severity of symptoms, hormones, time since diagnosis and Body Mass Index (BMI) with level of depression.
Results: Girls with PCOS were more likely to have a higher RADs score (beta coefficient estimate for Youth 07 compared to PCOS -1.27, sd 0.61)
compared to the general school population (p=.04). There was strong evidence of an association of BMI with RADS score (p=.007) but no correlation of RADs score with severity of symptoms, hormones and time since diagnosis.
Conclusions: This is the first study documenting an increased prevalence of depression in adolescent girls with PCOS, with a significant positive association with BMI. Clinicians need to be proactive in recognising depression, as well as menstrual and androgen excess symptoms, in PCOS women.

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Nothing to Disclose: SMN, CMO, SNM, JMS, SRM

P2-407

The Hyperandrogenic Phenotypic Variability of Nonclassical 21-Hydroxylase Deficiency Is Not Determined by Cytochromes P450c17, 2C19, 3A4, 3A5, 3A7 Polymorphisms.

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Nonclassical 21-hydroxylase deficiency (21OHD) is a hyperandrogenic disorder caused by *CYP21A2* mutations, which predict moderate impairment of enzymatic activity in homozygosis (C/C) or in compound heterozygosis with those predicting severe impairment (A/C). The disease presents a wide phenotypic variability in patients carrying the same *CYP21A2* gentotype, varying from precocious pubarche, hirsutism with menstrual abnormalities and infertility suggesting an involvement of other genes on the phenotype. *CYP2C19*, *CYP3A4*, *CYP3A5*, *CYP3A7*, *CYP17* polymorphisms take part in interindividual differences in the testosterone metabolism and some of them modulate the PCOS phenotype. **Objective**: to analyze if polymorphisms in genes involved in the androgen synthesis and/or metabolism could account for the 21OHD phenotypic variability. **Patients**: 112 females with nonclassical form of 21OHD, diagnosed with ACTH-17OHP levels >10 ng/mL, at a mean age of 18 ± 14 yrs. **Methods**: The *CYP21A2* gene was sequenced and the genotype divided into A/C and C/C groups. The *CYP2C19*17*, *3A4*18*, *3A5*3*, **1D*, **6*, *3A7*1C*, *CYP17*A2* alleles were screened by sequencing or RFLP. We compared the chronological age at beginning of manifestations, Ferriman score, testosterone levels and the presence of menstrual abnormalities between the patient groups carrying wild type and polymorphic alleles in each nonclassical genotype (A/C and C/C). The Fisher´s exact, Likelihood Ratio Chi-Square tests, Mann-Whitney and Kruskal-Wallis tests were used. **Results**: There was no difference in the allelic distribution for all polymorphism tested regarding the age at beginning of manifestations, Ferriman score, hirsutism with or without menstrual abnormalities in both nonclassical genotypes. Mean testosterone levels were similar in both nonclassical genotypes and between patient groups carrying wild type and polymorphic alleles. **Conclusion**: In contrast to what was previously reported in PCOS, we ruled out an effect of P450c17 and liver P450 cytochrome polymorphisms on the wide hyperandrogenic phenotypic variability of nonclassical female patients.

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Nothing to Disclose: VOM, MLP, GM, JAMM, BBM, TASSB

P2-408

The Presence of Clitoromegaly in Nonclassical Form of 21-Hydroxylase Deficiency Is Modulated by the CAG Polymorphic Tract of Androgen Receptor Gene.

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Nonclassical form of 21-hydroxylase deficiency (21OHD) is a disorder characterized by hyperandrogenic manifestations including clitoromegaly. In nonclassical females there was no association between severity of enzymatic impairment predicted by genotype and presence of clitoromegaly. This fact suggests that individual differences in the peripheral androgen sensitivity could account for this phenotypic variability. Testosterone and its 5-alpha reduced metabolite, dihydrotestosterone, act on target tissues through the androgen receptor (AR). Allelic variants in *AR* and 5-α-reductase type 2 (*SRD5A2*) genes are known as modifier factors of androgen sensitivity. **Objective**: to evaluate if *AR* and *SRD5A2* gene polymorphisms are associated with clitoromegaly in a large cohort of nonclassical females. **Patients**: 86 females (mean 19 ± 14 yrs) diagnosed with ACTH-17OHP levels >10 ng/mL. **Methods**: The entire *CYP21A2* gene was sequenced. The *AR* CAG repeat region (nCAG) was amplified using *Hpa II* digested and undigested DNA, PCR products were electrophoresed and analyzed by GeneScan. The V89L and A49T polymorphisms of the *SRD5A2* gene were assayed by RFLP, using *RsaI* and *MwoI*, respectively. Mann-Whitney and Likelihood Ratio Chi-Square tests were used. **Results**: 10/86 females had clitoromegaly and there was no correlation with the severity of the *CYP21A2* genotype. Similar mean testosterone levels were found in children with and without clitoromegaly (44 ± 26ng/dL *versus* 37 ± 23 ng/dL) as well as in adults with or without clitoromegaly (130 ± 70 ng/dL *versus* 93 ± 63 ng/dL (p>0.05). Shorter CAG alleles (≤ 18 repeats) were found in 7/10 and in 10/76 patients with and without clitoromegaly, respectively. Both patient groups presented similar frequencies of skewed X-chromosome inactivation. The X-weighted-biallelic-mean of CAG alleles was lower in patients with clitoromegaly in comparison with those without (19 ± 2 *versus* 22 ± 2), (p=0.02). The T49 allele of the *SRD5A2* gene, associated with increase 5α-RD activity, was found in heterozygous state in two patients without clitoromegaly; whereas the L89 allele, associated with decreased 5α-RD activity, was not associated with the absence of clitoromegaly in heterozygosis or also in homozygosis (p>0.05). **Conclusions**: We demonstrated a modulator effect of the CAG polymorphic tract of the androgen receptor gene in the presence of clitoromegaly in nonclassical patients and we ruled out the influence of *SRD5A2* polymorphisms on this phenotype.

Sources of Research Support: FAPESP #05/04726-0, #08/51624-6.

Nothing to Disclose: VOM, LCK, LGG, JAMM, BBM, TASSB

P2-409

Improvement of Endothelial Function with Spironolactone Treatment in Non-Obese Women with Polycystic Ovary Syndrome.

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Accumulating evidence connects polycystic ovary syndrome (PCOS) not only with reproductive and metabolic abnormalities but also with increased risk of cardiovascular disease. Endothelial dysfunction is present in PCOS and represents an early, reversible marker of cardiovascular damage. We tested the hypothesis that treatment with spironolactone reverses endothelial dysfunction in PCOS. Thirty-three non-obese PCOS patients, compared with 20 control subjects, were evaluated. PCOS patients were given spironolactone 100 mg daily in 21 day long intervals followed by a 7 day pause for 6 months. Results are expressed as median (25-75th percentile). At baseline, endothelium-dependent flow-mediated dilation (FMD) was significantly lower in PCOS compared to controls: 5.9 (0.0-11.8) % vs. 10.2 (6.8-15.9) %, $p = 0.018$. This difference disappeared after 6 months of spironolactone treatment, when FMD in PCOS significantly increased to 8.3 (5.5-10.0) % ($p = 0.049$) and was no longer different from controls. Plasma androgen levels did not change while serum total and LDL cholesterol decreased significantly during treatment - from 4.7 (4.0-5.1) mmol/l to 4.4 (3.9-4.8) mmol/l and 2.5 (2.1-3.1) to 2.2. (1.9-2.4) mmol/l, $p < 0.01$ and $p < 0.05$, respectively. ApoB decline reached borderline significance - from 0.9 (0.7-0.9) g/l to 0.8 (0.6-1.0) g/l), $p= 0.07$. FMD change in PCOS patients with treatment was associated with basal total cholesterol ($b=-0.38$, $p = 0.046$) basal LDL cholesterol ($b=-0.48$, $p= 0.009$), and change in LDL cholesterol ($b=-0.36$, $p = 0.057$). In conclusion, our findings support the notion that hyperandrogenism of PCOS contributes to an adverse lipoprotein profile. Treatment with spironolactone normalized endothelial function and improved cholesterol levels in non-obese PCOS patients. Changes in lipid profile may be a direct consequence of inhibition of androgen action by spironolactone. Inhibition of mineralocorticoid receptors might have additional beneficial effects on endothelial function.

Nothing to Disclose: KBS, MS, MP, JP

P2-410

Effect of Low-Frequency Electro-Acupuncture on Serum Testosterone and Menstrual Pattern in Women with Polycystic Ovary Syndrome Compared to Physical Exercise: Randomised Controlled Trial.

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Objective: To compare the efficacy of low-frequency electro-acupuncture (EA) with physical exercise on serum testosterone and menstrual pattern in women with polycystic ovary syndrome (PCOS).
Design: Randomized controlled trial.
Setting: The Sahlgrenska Academy at University of Gothenburg and at The Sahlgrenska University Hospital, Sweden.
Participants: 74 women with PCOS.
Interventions: We randomly assigned women with PCOS to receive low-frequency EA, fourteen treatments during 16 weeks, (n=29), physical exercise during 16 weeks (n=30), and an untreated control group (n=15).
Main outcome measures: The primary outcome measures were changes in serum testosterone analyzed with gas and liquid chromatography/mass spectrometry and menstrual pattern, i.e. number of bleedings per month, from baseline to week 16 (i.e. within one week after last treatment) between low-frequency EA and physical exercise group. Secondary outcome measures were changes in testosterone and menstrual pattern between the low-frequency EA and the untreated control group, and between the physical exercise group and the untreated control group respectively, from baseline to week 16.
Results: Low-frequency EA decresased serum testosterone and improved menstrual pattern compared with the physical exercise group at week 16. Serum testosterone decreased by -25% and menstrual pattern improved from 0.28 bleedings per month to 0.69 between baseline and week 16 in the low-frequency EA group, while there were no significant changes in testosterone concentrations (-7%) and menstrual bleedings (0.26 bleedings per month to 0.41) in the physical exercise group. Low-frequency EA decreased testosterone concentrations and improved menstrual pattern compared with the untreated control group, in which testosterone (2%) and menstrual bleedings (0.23 bleedings per month to 0.19) were unchanged. Physical exercise improved menstrual pattern, but not testosterone, compared with the untreated control group.
Conclusion: Low-frequency EA was more effective than physical exercise and untreated control for improvement of serum testosterone and menstrual pattern in women with PCOS within one week after last treatment.

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Nothing to Disclose: EJ, GH, FL, AO, LN, POJ, CO, ES-V

P2-411

Comparing the Gene Expression Profiles of Adipose Tissues from Non-Obese and Obese Women with the Polycystic Ovary Syndrome (PCOS).

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Introduction: PCOS is a common endocrine-metabolic abnormality of women, and ~70% of affected women demonstrate clinically measurable insulin resistance above and beyond that determined by their degree of obesity. Adipose tissue dysfunction may play an important role in the impairment of insulin sensitivity in PCOS patients. In turn, obesity may alter/worsen any abnormality observed. In this study we compared the gene expression profile of adipose tissues of non-obese and obese PCOS, and body mass index (BMI)-matched controls.

Methods: Subcutaneous lower abdominal fat tissues were obtained from 7 obese (BMI≥ 30 kg/M^2) PCOS and 5 BMI-matched controls, and 4 non-obese PCOS and 3 controls (BMIs 21-28 kg/M^2). Gene expression profiling was performed by Affymetrix Human Genome U133 plus 2.0 arrays. GO analysis was performed in common genes that are up or down-regulated in the adipose tissue of PCOS individuals. Microarray results for selected genes were confirmed by q-RTPCR in independent samples not used in the microarray studies. **Results:** Microarray analysis identified 35 up-regulated genes and 13 down regulated genes whose expressions were altered in both non-obese and obese PCOS adipose tissues vs. matched controls. Among these we identified genes related to inflammatory response and insulin signaling pathways. TRAF3IP3, CCL5 and ARTS-1 related to the inflammatory response, were up regulated in both obese (1.58, 1.59, 1.55 fold changes respectively) and non obese PCOS (1.79, 1.80, 1.61 fold changes respectively) relative to controls. In contrast, IL6 was significantly down-regulated in adipose tissues of both obese (1.54 fold changes) and non-obese PCOS (19.77 fold changes). IGF2, EGR1, SNF1LK and FOSB related to insulin signaling, were significantly down-regulated in both obese (1.56, 1.54, 1.57 and 1.98 fold changes respectively) and non-obese PCOS (2.23, 11.65, 9.75 and 44.8 fold changes respectively). Microarray data were confirmed by qRT-PCR
Conclusion: In this study we demonstrated dysregulation of genes related to inflammation and insulin signaling in adipose tissues of both non-obese and obese PCOS patients. Abnormal expression of these genes could directly affect the pathophysiology of PCOS, and further characterization of these abnormalities may yield novel molecular targets for therapy and genetic study.

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Nothing to Disclose: H-SJ, Y-HC, J-ML, GC, RA

P2-412

Prevalence of PCOS in a Sample of Urban Australian Indigenous Women in Darwin.

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Introduction: PCOS, the most common endocrinological problem in reproductive aged women, has been found in population based studies to be present in 4-8% of women from Caucasian and Sri Lankan backgrounds(1-4). A higher risk of PCOS may occur in other populations, such as Indigenous Australian women, due to rising obesity, diabetes and components of metabolic syndrome. Supporting this possibility is a small study of Australian Indigenous women which reported a prevalence of 18%(5).
Patients and methods: The aim of this study was to assess the prevalence of PCOS in a group of urban Indigenous women living in Darwin, NT. All women between 15-44 years who volunteered for a community diabetes screening study were asked to participate in a women's reproductive health survey; 91% agreed. The women completed a general and reproductive health questionnaire, anthropometry and blood pressure measurements. Blood samples were taken for lipids, testosterone and SHBG and an oral glucose tolerance test performed. PCOS was defined using the NIH/NICCHD criteria of oligomenorrhoea (cycles>35 days) and hyperandrogenaemia (free calculated testosterone >34.2pmol/l; the 95th centile in a previous study for women without PCOS). 393 women consented to and completed both the reproductive health questionnaire and had androgens measured. Exclusion criteria were: menopause, previous hysterectomy or oophorectomy, current hormonal contraceptive use or cycle regularity question not answered (n=145).
Results: Of 248 women who met the inclusion criteria and were eligible for assessment of PCOS, 38 (15.3%) had PCOS. The frequency of PCOS did not change significantly with age but increased with BMI; 29.9% of women with a BMI >30kg/m2 had PCOS. The frequency of PCOS did not change with central obesity; probably because, overwhelmingly, it was the typical pattern of fat distribution in this group.
Conclusion: Urban Indigenous Australian women have some of the highest rates of PCOS described.

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Sources of Research Support: Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) Fotheringham Research Grant, 2002; National Health and Medical Research Council (NHMRC) Training Scholarship for Indigenous Australian Health Research, 2005-2009; Australian Academy of Science Doulgas and Lola Douglas Scholarship 2005-2009;Eli Lily;NHMRC Grant #236207; the Australian Government Department of Employment and Workplace Relations; the Clive and Vera Ramaciotti Foundation; the Vincent Fairfax Family Foundation; the Australia@Risk Partnership in Type 2 Diabetes; and Bayer HealthCare.

Nothing to Disclose: JB, KO, JC, RN

P2-413

Ovulatory Menstrual Cycles and Delayed Increase in Testosterone Levels in Women with PCOS Who Discontinue Hormonal Contraception.

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Many investigators wait for 3 months after stopping hormonal contraception before assessing hormone levels in subjects with polycystic ovary syndrome (PCOS). However, it is not clear if the 3 month time frame is sufficient. Further, ovulation suppresses gonadotropins and androgens temporarily, but ovulatory activity within the 3 month interval has not been assessed. We hypothesized that gonadotropin and androgen levels would increase, but not plateau, in the 3 months after stopping hormonal contraception and that subjects would experience ovulatory menstrual cycles.
Subjects with PCOS defined by the NIH criteria (n=12) were studied before and monthly after discontinuing hormonal contraception for a total of 3 months, with a subset of subjects followed for 6 months (n=8). All subjects underwent a monthly assessment including an interval menstrual history and physical exam. In addition, subjects underwent blood sampling for gonadotropins and sex steroids, and a transvaginal ultrasound was performed. Ovulation was documented by a progesterone elevation (≥2 ng/mL) or an elevated estradiol level coincident with a dominant follicle (>18 mm) on ultrasound approximately 2 weeks before menses. Follicular phase data were analyzed both with a paired *t* test comparing baseline and 3 month average values and one way repeated measures ANOVA.
LH (3.5±3.0 vs 25.0±11.4 IU/L; $p<0.01$) and FSH levels (4.1±3.0 vs 11.8±4.2 IU/L; $p<0.05$) increased and plateaued in the three months after stopping hormonal contraception. Testosterone levels also increased (30.8±14.8 vs 59.7±23.6 ng/dL; $p<0.05$) but continued to rise in individual subjects through month 6, while SHBG decreased over 2 months (124.0±75.2 vs 49.3±23.5 nmol/L; $p<0.05$). 91% of subjects had at least one spontaneous bleed within three months. Seven subjects had documented ovulation on the first (n=6) or second cycle (n=1) off hormonal contraception and one had ovulatory cycles based on normal cycle length (total 73%). Three subjects had no documented ovulation and late withdrawal bleeding (43 days - 3 mos), suggesting anovulation.
Although gonadotropins reach a plateau after three months in women with PCOS who stopped hormonal contraception, androgen levels continued to increase. Ovulation was also common. Therefore, a longer interval may be necessary before performing a hormonal assessment. Clinically, the ovulatory cycles may provide an opportunity for pregnancy in the first months after stopping hormonal contraception.

Nothing to Disclose: NSD, CKW

P2-414

Body Mass Index as a Predictor of Polycystic Ovary Syndrome Risk: Results of a Longitudinal Cohort Study.

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Background: Polycystic ovary syndrome (PCOS) affects 12-18% of women, yet is often under diagnosed[1]. Obesity is increasing, exacerbating PCOS, however the interaction between obesity and PCOS remains unclear.
Methods: From the large ongoing Australian Longitudinal cohort Study on Women's Health, women 18-23 years at survey 1 (1996) and responding to survey 4 (2006, n=9145, 65% of original cohort), were studied. PCOS diagnosis, body mass index (BMI), childwood weight and adult weight gain were self-reported. Cross tabulations, univariate analysis and logistic regression incorporating demographic variables were conducted.
Results: PCOS prevalence across all surveys was 5.8% (95% CI: 5.3% to 6.4%) and was closely associated with irregular menstrual cycles ($p<0.0001$). PCOS women on average reported a BMI of 2.0 kg/m^2 (95% CI: 1.5 to 2.4) higher than non-PCOS women at survey 1, and this difference increased to 2.9 kg/m^2 (95% CI: 2.3 to 3.5) at the fourth survey. Demographic characteristics of the two groups were similar. Women reporting PCOS also reported higher childhood weight and greater adult weight gain 2.3 kg (95% CI: 1.7 to 2.8) than women not reporting PCOS. For every additional kg/m^2 of BMI, the risk of reporting PCOS increased by 9.2% (95% CI: 7% to 11%).
Conclusion: Prevalence of self reported PCOS was 5.8% in an unselected community cohort, suggesting under diagnosis. Childhood weight, baseline and average BMI and longitudinal weight gain were all higher in PCOS. PCOS prevalence was higher with elevated BMI. With global progressive weight gain, PCOS represents a major and increasing public health challenge. This novel positive report of a strong relationship between BMI and PCOS, combined with known benefits of weight loss, support aggressive prevention and management of excess weight in young women.

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Nothing to Disclose: HJT, AAD, MG-H, CL, DJ, EP, DL, LM

P2-415

Obesity in Women with Polycystic Ovary Syndrome (PCOS): A Referral Bias?.

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Introduction: PCOS is a common endocrine-metabolic abnormalities, affecting 7-10% of women. While a greater degree of obesity has been described in populations of PCOS patients versus controls, whether such prevalence in PCOS represents an intrinsic abnormality in adipose storage and function (e.g. adipogenesis) or a referral bias is unclear.
Methods: We studied 675 women examined for a pre-employment physical from 1995-1999 (unselected population) and 292 PCOS women evaluated in a clinical practice (referral population) during the same years, to control for the rate of obesity in the population at the time.

Results: Prevalence of obesity (body mass index [BMI]>30 kg/M^2) in PCOS in the unselected population was 27.6%, similar to the 29.2% rate of the total unselected population. Alternatively, the prevalence of obesity among PCOS women in the referral population was 63.7%, a 2.3 fold higher rate of obesity. Distributions by weight class in the total unselected population, PCOS in unselected population, and PCOS in referral population are depicted in **Table**. Among referred PCOS women, the modified Ferriman-Gallwey hirsutism score (r=0.11, p=0.058), total and free testosterone levels (total: r= 0.13, p<0.03; free: r=0.20, p<0.0005), and severity of oligomenorrhea (r=0.16, p< 0.005), increased with BMI.
Conclusions: The rate of obesity among PCOS women identified in an unselected population was similar to the rate of obesity among all unselected women. Alternatively, the prevalence of obesity among PCOS women in a referral clinic was 3-fold higher, possibly reflecting a more severe PCOS phenotype. The present data suggest that the rate of obesity in PCOS may be overestimated, and that the higher obesity rate in PCOS patients within the clinical setting may represent the negative impact of overweightness on the severity of PCOS and its adverse effect on QOL.

Observed distribution (%) of weight classes in populations studied

Obesity Class	% Total unselected population (n=675)	% PCOS in unselected population (n=64)	% PCOS in referral population (n=292)	Odds of PCOS in referral population compared to unselected population
Underweight	5.3	4.6	1.0	0.22
Normal	41.8	43.1	16.8	0.39
Overweight	23.7	24.7	18.5	0.75
Class I Obesity	12.9	7.0	16.4	2.33
Class II Obesity	8.5	11.0	19.5	1.77
Class III Obesity	7.8	9.5	27.7	2.92

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Nothing to Disclose: RA, UE, MP, DAD, GC, BY

P2-416

Clinical, Biochemical and Metabolic Markers in Adolescents with Polycystic Ovarian Syndrome, with and without Ovarian Enlargement.

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Background: Since the Polycystic Ovarian Syndrome (PCOS) International Consensus, held at Rotterdam in 2003, ultrasound criteria (ovarian volume and follicle number) are now included in the definition of this disease. An ovarian enlargement of > 10cc was chosen as one of the criteria for the diagnosis of PCOS based on a literature review and discussion amongst experts. However, not all females with PCOS have ovarian enlargement, and researchers have called for more clarification on the connection between ovarian volume with clinical manifestations, biochemical measurements, and metabolic markers.
Objectives: The purpose of this study was to determine if among adolescents with PCOS, clinical, biochemical, and metabolic markers varied according to ovarian size (normal versus enlarged).
Methods: A retrospective chart review of adolescent girls with the diagnosis of PCOS was performed. They were divided into two groups, Group A: PCOS with at least one enlarged ovary (ovarian volume >10cc), Group B: PCOS with normal ovarian size (ovarian volume<10cc), measured on transabdominal ultrasound. Girls in Group A (n=26) ranged from 11.3-17.8 yrs (mean 15.7yrs) and in Group B (n=33) ranged from 10-18 yrs (mean 14.9 yrs), with mixed ethnic backgrounds. Clinical markers included BMI, acne, hirsutism, menstrual cycle (regular, irregular, amenorrhea, menorrhagia, premenarchal), and acanthosis nigricans. Biochemical parameters included total testosterone, free testosterone, and LH to FSH ratio. Metabolic markers such as insulin resistance (IR) and lipid profile were also examined between the two study groups.
Results: Ultrasound showed a mean ovarian volume of 13.2 cc in Group A and 6.2 cc in Group B. There was no significant difference between the two study groups with regards to clinical, biochemical, or metabolic parameters.
Conclusion: Polycystic ovarian syndrome in adolescents can have similar clinical and biochemical presentation with or without radiological ovarian enlargement. Additionally, metabolic markers do not correlate with the degree of ovarian volume enlargement. Based on these findings, severity of PCOS cannot be estimated with ovarian size on abdominal ultrasound in adolescents.

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Nothing to Disclose: EB, SM, BCS

P2-417

Atorvastatin Reduces Blood Pressure, Androstenedione, and DHEAS in Women with Polycystic Ovary Syndrome.

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Dyslipidemia may play a central role in Polycystic Ovary Syndrome(PCOS) as LDL-cholesterol is the primary precursor for sex steroid synthesis. Our objective was to determine the effects of atorvastatin on cardiometabolic and androgenic profiles in PCOS. We randomized 21 PCOS women with LDL ≥100 mg/dl to atorvastatin or placebo 40 mg daily for 6 weeks. Before and after treatment, blood pressure(BP), anthropometrics, brachial artery flow-mediated dilation(FMD) and conductance during reactive hyperemia, fasting blood work, and area under the curve (AUC) for glucose and insulin during oral glucose tolerance test(OGTT) were measured. Eighteen women completed the study. Results were analyzed by intention-to-treat. The two groups were similar in age and BMI (33.4±4.2 and 29.4±5.8 years; 40.2±11.1 and 36±10.4 kg/m^2). Compared to placebo, atorvastatin significantly reduced LDL, triglycerides, diastolic BP, androstenedione, and DHEAS (P ≤0.05 for all). In the atorvastatin group, there were significant reductions in C-reactive protein(CRP) and increases in peak conductance and AUC insulin (Table 1), but these differences did not meet statistical significance in the between groups comparision. In conclusion, atorvastatin improves cardiometabolic and reproductive aspects of PCOS and may be a multifaceted treatment for PCOS.

Table 1. Cardiometabolic and androgenic profiles before and after treatment

	Atorvastatin			Placebo		
	Before	After	P value	Before	After	P value
Systolic BP(mmHg)	119.3(15)	112(13.2)	0.04	114.5(14.4)	111.4(8.8)	0.47
Diastolic BP(mmHg)	71(14)	64.3(12.3)	0.02	64.6(8)	65.4(8.1)	0.67
LDL(mg/dl)	136.9(26.1)	68.5(19.3)	<0.001	131.3(21.6)	118.8(26.8)	0.09
Testosterone(ng/dl)	58.5(18.3)	47.1(21.4)	0.14	92.3(49.8)	75.7(43.6)	0.06
Androstenedione (ng/ml)	3.4(0.8)	2.5(0.9)	<0.001	3.8(1.2)	4.1(1.2)	0.36
DHEAS(ng/ml)	1661(829)	1326(854)	0.01	1702(681)	1740(782)	0.47
CRP(mg/L)	7.7(9.1)	4.3(5.4)	0.02	7.2(7.7)	6(7.3)	0.38
AUC Glucose	15620(2052)	16136(2569)	0.38	15309(3692)	15448(3165)	0.88
AUC Insulin	12435(9486)	17479(11929)	0.03	9338(5208)	9132(4466)	0.79
Peak Conductance(ml/sec/mmHg)	5(3.1)	6.9(2.8)	0.04	3.6(3)	4.3(3.3)	0.30
FMD(%)	11.8(6.9)	10.4(4.6)	0.58	9.8(5.8)	10.2(2.9)	0.85

Data presented as mean(SD)

Sources of Research Support: NIH K 12HD055882 and Pfizer.

Nothing to Disclose: NR-K, CS, CH, ARK, RSL

P2-418

Serum Testosterone Levels and Subjective and Objective Sexual Function in Women with Hypopituitarism.

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Objective: The psychological and physiological role of testosterone in women sexual function remains poorly understood. Cross-sectional studies have not found a correlation between subjective sexual function (such as arousal and desire; processes mediated centrally) and measurements of testosterone. Women with hypopituitarism have severely diminished ovarian and adrenal androgen production and thus represent an excellent model to study the consequences of androgen deficiency. We hypothesized that women with hypopituitarism would exhibit altered sexual function as a result of androgen deficiency.
Method: Total testosterone, objective sexual function (blood flow and somatosensory thresholds) and the subjective Female Sexual Function Index (FSFI) were measured in 29 women with documented hypopituitarism (median age 40.6±9.4 years, BMI 30.1±5.1 kg/m2) and 29 healthy volunteers (median age 28.3±8.4 year, BMI 23.6±4.2 kg/m2) in an IRB-approved study.
Results: Total testosterone levels were markedly diminished among women with hypopituitarism (4.74±4.3 ng/dL) compared to normal volunteers (37.0±22.4 ng/dL, $p<0.0002$). Patients had significantly lower sexual functioning in all domains of the FSFI compared to controls. In patients and controls together, higher levels of testosterone were significantly correlated with higher levels of sexual functioning in all domains and total FSFI. Quantitative somatosensory testing showed that patients are more insensitive to vaginal vibration (more vibration before detection), clitoral vibration, vaginal cold (lower temperature before detection) and vaginal heat (higher temperature before detection). However, when age is controlled for, there are no significant differences between groups. Only lower vaginal heat sensation was significantly correlated with higher levels of testosterone. Genital blood flow was similar between patients and controls and was not correlated with testosterone levels. Depression and fatigue correlated with low testosterone levels.
Conclusion: Testosterone deficiency in women with hypopituitarism leads to impairment in subjective, but not objective sexual function. Testosterone more likely affects central (brain) rather than peripheral processes. Genital sensation and genital blood flow are probably not testosterone-mediated. These data provide compelling rationale for placebo-controlled, randomized trials of testosterone replacement in women with hypopituitarism.

Sources of Research Support: NIH 1U54 HD41748.

Nothing to Disclose: AR, MM, JS, EZ, MLL, TCF

P2-419

Low Cardiovascular Event Rate in Post-Menopausal Women with Increased Cardiac Risk: Initial Findings from the Ongoing Blinded Libigel® (Testosterone Gel) Cardiovascular Safety Study.

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Objective: LibiGel® (testosterone gel) is being developed for the indication of hypoactive sexual desire disorder (HSDD) in estrogen-treated, oophorectomized postmenopausal women. To obtain FDA approval, the proposed dose of 300 mcg/d must demonstrate long-term cardiovascular (CV) and breast safety. In 2008 we reported on the design and defined endpoints of a long-term CV and breast safety study of testosterone gel in postmenopausal women with HSDD. Herein we report on the study progress.
Design: This is a Phase III, CV events-driven, adaptive design, randomized, double-blind, placebo-controlled, multi-center comparison of testosterone and identical placebo gel in postmenopausal women with HSDD and known CV risk. The primary safety outcome measure is the effect of treatment on the incidence of a comprehensive, adjudicated CV event composite of cardiac death, non fatal MI and stroke in addition to coronary revascularization, hospitalized angina and venous thrombo-embolic events. The co-primary safety endpoint is the incidence of invasive breast cancer. Events are reviewed/adjudicated by chartered events committees and a Data Monitoring Committee and the study is overseen by an Executive Committee.
Results: The study remains blinded and is ongoing. To date over 1,000 post-menopausal women, mean age 57.4 (range 48-77) years and elevated CV risk have been enrolled. Some study subjects have been on study drug for more than 2 years. The rate of protocol-mandated reporting of CV events in study subjects with known CV risk and HSDD is 0.52% including one MI and no deaths. To date 3 breast cancers have been reported in this blinded study.
Conclusions: In this large, placebo-controlled, safety study of low-dose testosterone in women, both the CV event and breast cancer rates have been very low to date in subjects who are at the higher end of CV risk continuum for the intended treatment population. These data demonstrate a lower-than-expected rate of serious CV events in the trial thus far. In addition to the effects of improved preventive measures as a standard of care accounting for a low CV event rate in at-risk postmenopausal women, the study supports a lack of effect of the study drug in this population.

Disclosures: MCS: Speaker, BioSante Pharmaceuticals, Inc.; Employee, BioSante Pharmaceuticals, Inc. SB: Collaborator, BioSante Pharmaceuticals, Inc. DB: Collaborator, BioSante Pharmaceuticals, Inc. JGZ: Collaborator, BioSante Pharmaceuticals, Inc.; Collaborator, BioSante Pharmaceuticals, Inc. WBW: Collaborator, BioSante Pharmaceuticals, Inc.

P2-420

Atorvastatin Improves 25OH Vitamin D Levels in Patients with Polycystic Ovary Syndrome.

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Background: It has been shown that women with polycystic ovarian syndrome (PCOS) are mostly 25-hydroxy vitamin D (25OHD) insufficient, and 25OHD replacement therapy may have a beneficial effect in these women. Both statin treatment and vitamin D supplementation have been shown to improve biochemical hyperandrogenemia, insulin resistance and markers of inflammation in patients with PCOS, raising the possibility that some of the statin effects are mediated through vitamin D. This randomised, double blind placebo controlled study was conducted to assess the effect of atorvastatin on 25OHD levels in patients with polycystic ovary syndrome.
Methods: Forty medication naïve patients with PCOS and biochemical hyperandrogenaemia were randomised to either atorvastatin 20mg daily or placebo. After 3 month an extension study for both patients on atorvastatin and placebo for another 3 months with the addition of metformin 1500mg was undertaken. The main outcome measure was a change in 25OHD levels.
Results: The baseline 25OHD nmol/L levels were comparable between two groups (45.9± 2.4 vs. 44.8± .8; pvalue=0.7). There was a significant increase of 25OHD levels with atorvastatin (45.9±2.4 vs. 60.8±3.5 nmol/L) compared to placebo (44.8±1.8 vs. 41.8±3.2 nmol/L)(pvalue-0.02). Three months treatment with metformin maintained the improvement of 25OHD with atorvastatin compared to baseline (45.9±2.4 vs. 61.8±3.5 pvalue<0.01). There were no significant changes in 25OHD levels in the placebo group after 12 weeks of metformin.
Conclusions: 12 weeks of atorvastatin increased 25OHD levels in patients with polycystic ovary syndrome. This effect of statins on 25 OHD levels hightlights their apparent pleotrophic effects.

Sources of Research Support: Unrestricted 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, JS, CA, AC, ESK, SLA

P2-421

Changes in Glucose Tolerance in Patients with Polycystic Ovarian Syndrome from India: A Follow up Study.

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Background: The prevalence of impaired glucose tolerance (IGT) and diabetes mellitus (DM) is high in women with polycystic ovarian syndrome (PCOS). There is scant data on follow up of glucose tolerance in these women.
Aims: To study changes in glucose tolerance in PCOS patients in follow up and to identify risk factors associated with any detected change.
Study design: Women diagnosed to have PCOS based on Rotterdam criteria underwent a repeat GTT at least 1 year after having a baseline GTT. No changes were made in their routine care (in our clinic all women are counseled about benefits and goals of lifestyle modification). All drugs except progesterone were stopped 3 months prior to the GTT. Detailed history and anthropometric measurements were recorded at baseline and on follow up. GTT categories were defined according to WHO criteria. Conversion was defined as change from normal glucose tolerance to IGT or DM and from IGT to DM. Reversion was defined as improvement in glucose tolerance category.
Results: Of the 104 women with baseline GTT data, 54 women (mean age 24.4$\pm$5.6 years, range 16-43 and median of 23.5 years) had a repeat GTT. After a median follow up of 23.5 months, range 12-82 months, there was a significant decrease in fasting blood glucose level (p=0.003). The change in weight, body mass index, blood pressure or waist hip ratio was not statistically different on follow up.
At baseline 39 (72%) had normal GTT, 14 (26%) had IGT and 1 patient had diabetes. Out of the 39 patients with normal GTT, 36 continued to have normal GTT, while 2 progressed to IGT and 1 developed DM. Out of the 19 patients with IGT at baseline, 9 reverted to normal GTT, 4 had persistent IGT and 1 progressed to diabetes. The single patient with DM reverted to IGT. There was no difference in age, weight, BMI, BP or WHR between patients who reverted from IGT to normal GTT and those who continued to have normal GTT. As the number of patients who showed worsening of GTT was small, no risk factors could be identified.
Conclusions: Our study does not show worsening of glucose tolerance in patients with PCOS as has been shown in Caucasian women. This difference could be due to 1) our patients were lean as compared to those in the earlier studies (mean BMI of 24.2$\pm$3.2 vs 30.3$\pm$ 4.5 Kg/m^2) and 2) all our patients were in regular follow up and their standard care with life style modifications was continued which could have had a beneficial effect on their GTT.

Nothing to Disclose: PD, SS, VB

P2-422

Cervical Insufficiency in Pregnant Women with Polycystic Ovarian Syndrome.

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INTRODUCTION: Cervical insufficiency or incompetence (CI) during pregnancy is an infrequent diagnosis (dx) and has historically been associated with non-hormonal factors such as multiple gestation, infection and prior cervical surgery. Patients diagnosed with polycystic ovarian syndrome (PCOS) are known to experience poor reproductive function including infertility, increased pregnancy loss, gestational diabetes and macrosomia. In a large community-based study of PCOS patients, we examined the frequency of CI including placement of cerclage (CER), and associations with method of conception, prior reproductive history, race/ethnicity, age and BMI. METHODS: Within Kaiser Permanente Northern California, a large integrated health care delivery system, electronic databases were used to identify pregnant women with PCOS having 2nd or 3rd trimester delivery during 2002-2006. Women with pre-existing diabetes mellitus, multiple gestation and prior cervical conization were excluded. Medications were ascertained using pharmacy records and chart review. Prior reproductive history, CI and prophylactic cerclage placement were ascertained by chart review. RESULTS: Among the 902 PCOS in the study group, CI was diagnosed in 32 (3.6%). An additional 7 women (0.8%) had a prior diagnosis of CI but not in the index pregnancy. A CER was placed in 16 (1.8%) women during this pregnancy. Some had CER placed based on past history but 19 (2.1%) PCOS women had newly diagnosed CI (progressive cervical dilation, significant funneling and shortening, but not pre-term labor or infection) occurring in a first continuing pregnancy. These included 4 with an unclear dx of CI vs. pre-term labor at the time of evaluation. South Asian and Black PCOS women were at especially high risk for CI (10.6% combined vs. 2.3% in other groups $p<0.01$). CI was also associated with a history of gonadotropin use to achieve the current pregnancy (50.0% used gonadotropins vs. 24.3% among the non-CI PCOS cohort ($p<0.01$)). There was no association of CI with age or BMI. CONCLUSION: Among a large cohort of PCOS women achieving ongoing pregnancy, CI occurred with a markedly higher than expected incidence, occurred more frequently among South Asian and Black women and in those treated with gonadotropins. Studies exploring the role of insulin resistance, androgen excess and higher circulating estrogen concentrations may be helpful in exploring mechanisms contributing to cervical incompetence in PCOS and other women.

Sources of Research Support: NIH / NICHD Grant R01 HD052966 awarded to JCL.

Nothing to Disclose: SLF, MPY, GAL, ELD, MKH, JRG, JY, JCL

P2-423

Effects of Combination Therapy with Bazedoxifene and Conjugated Equine Estrogens on the Endometrial and Vaginal Epithelium of Surgically Postmenopausal Monkeys.

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The addition of a progestin to menopausal estrogen therapy has been associated with increased breast cancer risk. Bazedoxifene acetate (BZA) is a new selective estrogen receptor modulator (SERM) currently under investigation as a potential replacement for the progestin component in combination hormone therapy. The objective of this study was to determine if the addition of BZA to conjugated equine estrogens (CEE) would antagonize the uterotropic effects of the estrogens on the endometrium while maintaining the beneficial effects of CEE treatment on vaginal epithelial maturation. Here, we present preliminary data from a randomized, placebo-controlled, 20-month, multi-organ system study. Ninety-eight adult cynomolgus monkeys (*Macaca fascicularis*) were ovariectomized and randomized into social groups to receive placebo (control) or treatment with BZA 20 mg, CEE 0.45 mg, BZA 20 mg + CEE 0.45 mg (doses expressed as woman's daily equivalents). Endometrial thickness and uterine area were measured by trans-abdominal ultrasound at baseline and following 6 months of treatment. Vaginal smears for cytology were obtained at baseline, 5-6 weeks, 3 months and 6 months post-treatment. Compared to CEE administered alone, the addition of BZA to CEE significantly antagonized the uterotropic effects of CEE on endometrial thickness and uterine size to the level of both control and BZA alone (BZA+CEE vs. CEE, $p < 0.0001$; BZA+CEE and BZA vs. placebo, $p > 0.05$). Initially, BZA attenuated the effect of CEE on vaginal maturation to the level of control and BZA alone following 5-6 weeks post-treatment (BZA+CEE vs. CEE, $p < 0.001$; BZA+CEE and BZA vs. placebo, $p > 0.05$). However, after 3 and 6 months of treatment, there was no significant attenuation of the CEE effect by BZA. Vaginal maturation was not significantly different between BZA and placebo at all time points. These data provide experimental evidence that: (1) Following 6 months of treatment, BZA inhibits the uterotropic effects of CEE at a woman's equivalent dose of 0.45 mg/day; (2) After 3 months of treatment, BZA does not attenuate the beneficial effect of CEE on vaginal epithelial maturation; (3) BZA administered alone has no significant effects on endometrial size and vaginal maturation of this primate model.

Sources of Research Support: In part by an investigator originated grant (TBC) from Wyeth Pharmaceuticals and in part by NIH T32 grant RR007009 (KFE).

Nothing to Disclose: KFE, CEW, SEA, JMC, HC, TBC

P2-424

Effects of Bazedoxifene/Conjugated Estrogens (BZA/CE) on Hot Flushes in Postmenopausal Women: Effect of Timing of Therapy Initiation.

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Objective: To assess the effect of timing of therapy initiation since menopause on hot flush relief with a tissue-selective estrogen complex (TSEC) of BZA/CE in postmenopausal women.
Methods: In this multicenter, double-blind, randomized, placebo (PLA)-controlled, phase 3 study (SMART-2), 318 healthy postmenopausal women (40–65 years) with moderate to severe hot flushes (7/day or 50/wk) took BZA 20 mg/CE 0.45 mg (n=127), BZA 20 mg/CE 0.625 mg (n=128), or PLA (n=63) once daily for 12 wks. Post-hoc analysis examined the effect of BZA/CE on the mean daily number and severity of moderate to severe hot flushes at baseline and up to 12 wks in women <5 years since menopause (YSM; n=198) and women ≥5 YSM (n=112); 8 women did not have evaluable data.
Results: Hot flush frequency (Table) and severity significantly decreased with BZA 20 mg/CE 0.45 mg or 0.625 mg at wks 4 and 12 in women <5 YSM and in women ≥5 YSM.

	Time point	BZA 20/CE 0.45	BZA 20/CE 0.625	PLA
<5 YSM	Baseline (BL)[a]	10.7±6.3	10.4±3.4	9.8±4.2
Change from BL[b]	Wk 4	-5.7±0.4*	-6.6±0.4‡	-4.2±0.6
Change from BL[b]	Wk 12	-7.5±0.4†	-8.0±0.4‡	-5.4±0.6
≥5 YSM	BL[a]	9.5±3.0	10.7±8.7	11.9±5.9
Change from BL[b]	Wk 4	-6.9±0.8‡	-6.5±0.8‡	-0.6±1.1
Change from BL[b]	Wk 12	-8.3±0.7‡	-7.9±0.6‡	-4.1±0.9

[a]BL value is mean no. of hot flushes. [b]Mean change from BL in no. of hot flushes. *P=0.035; †P=0.002; ‡P≤0.001 for changes from baseline for BZA/CE vs. placebo

Significant reductions in hot flush frequency and severity with both BZA/CE doses were seen as early as wk 4 in women <5 YSM, and as early as wk 2 in women ≥5 YSM. Reductions in hot flush frequency with PLA were larger in women <5 YSM
(-2.59 to -5.48) than in women ≥5 YSM (-0.58 to -4.09) throughout the 12 wks of therapy. Similar results were observed for hot flush severity (<5 YSM: -0.13 to -0.42 vs. ≥5 YSM: 0.17 to -0.19).
Conclusions: The TSEC containing BZA/CE alleviates hot flushes in women who initiate therapy <5 YSM and ≥5 YSM. The number of years since menopause when women initiate therapy affects the time of onset of relief of hot flushes. Women closer to menopause were also found to have a higher PLA response than women later in menopause. Further analyses of the treatment group baseline characteristics will be performed for the presentation to determine if any other differences between groups beyond time since menopause could have affected these findings.

Disclosures: JVP: Researcher, Wyeth (acquired by Pfizer Inc. in October 2009), Pfizer, Inc.; Consultant, Amgen, Lilly USA, LLC, Teva, Wyeth (acquired by Pfizer Inc. in October 2009).
Nothing to Disclose: KP, JP, SM

P2-425

Metabolic Effects of Bazedoxifene/Conjugated Estrogens (BZA/CE) in Postmenopausal Women: Effects of Age.

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Objective: To assess age effects on metabolic parameters with a tissue-selective estrogen complex (TSEC) of BZA/CE in postmenopausal women. Previously, BZA/CE did not affect glucose and decreased homocysteine (HCST) over 2 yrs (SMART-1).
Methods: In this randomized, double-blind, placebo (PLA)- and active-controlled trial, 3,397 women (age 40–75) took BZA (10, 20, or 40 mg) + CE (0.625 or 0.45 mg), raloxifene (RAL) 60 mg, or PLA daily for 2 yrs. Fasting serum insulin and glucose, and plasma HCST, thyroid-stimulating hormone (TSH), and C-reactive protein (CRP) were evaluated at baseline and at 6, 12, 18, and 24 mos (substudy), and between treatments for women age <50 yrs (n= 171) and women 50–59 (n= 663) in an exploratory analysis.
Results: In women <50 and women 50–59, changes in fasting insulin and glucose, CRP, and TSH with all BZA/CE doses were similar to PLA at all time points, except at month 12 with BZA 10/CE 0.45. HCST decreased significantly with some doses of BZA/CE vs. PLA at some time points in both subgroups; more significant differences were in women 50–59 than <50. The main differences between BZA/CE and RAL were in CRP and TSH for women <50 and TSH for women 50–59.

Mean Changes from Baseline at 24 Mos.

	BZA 20/CE 0.625	BZA 20/CE 0.45	PLA	RAL
≤50 yrs				
Insulin[a] (pmol/L)	-7.0±15.1	-8.5±14.9	-4.8±15.9	7.1±17.6
Glucose[a] (mmol/L)	0.0±0.3	-0.1±0.2*	0.1±0.2	0.4±0.3
HCST (µmol/L)	0.2±0.9	-0.3±0.9	0.6±1.0	0.3±1.1
CRP (mg/L)	-0.0±2.6*	-0.2±2.6*	-0.6±2.8	-7.9±3.1
TSH (mU/L)	1.0±4.0†	0.5±4.0†	0.3±4.2	-12.4±4.7
50–59 yrs				
Insulin[a]	-6.5±5.0	-4.7±4.5	2.2±4.9	2.7±4.9
Glucose[a]	0.0±0.1	-0.1±0.1	0.1±0.1	0.0±0.1
HCST	-1.0±0.4	-0.8±0.4	-0.2±0.4	-0.4±0.4
CRP	-1.2±0.8	0.5±0.7	0.5±0.8	0.3±0.8
TSH	0.5±0.6	0.4±0.5	0.1±0.6	-1.5±0.6

*P<0.05 and †*P*<0.01 for BZA/CE vs. raloxifene. No significant diff. between BZA/CE and PLA at 24 mos. [a]Fasting

Conclusions: No statistical differences between BZA/CE and PLA were clinically relevant, and metabolic changes in this subgroup analysis were similar between women <50 and women 50–59, and to those reported for the total population. More improvements in HCST with BZA/CE were seen in older than younger women.

Disclosures: SM: Employee, Wyeth (acquired by Pfizer Inc. in October 2009). KP: Employee, Wyeth (acquired by Pfizer Inc. in October 2009). JP: Employee, Wyeth (acquired by Pfizer Inc. in October 2009). AC: Employee, Wyeth (acquired by Pfizer Inc. in October 2009).

P2-426

The Effects of Bazedoxifene/Conjugated Estrogens on Breast Pain in Postmenopausal Women.

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Background: A novel tissue selective estrogen complex (TSEC) partners bazedoxifene (BZA) with conjugated estrogens (CE) for the treatment of menopausal symptoms and prevention of postmenopausal osteoporosis. Here we report the effects of BZA 20 mg with CE 0.45 or 0.625 mg on self-reported breast pain in postmenopausal women.
Methods: These analyses were based on data from the SMART (Selective estrogens, Menopause And Response to Therapy) trials, which were randomized, double-blind, placebo-controlled phase 3 studies of 3-month to 2-year duration and enrolled healthy, postmenopausal women with an intact uterus (SMART-1, SMART-2, SMART-3). The proportion of subjects reporting ≥1 day of breast pain at baseline and over the first 12 weeks (and each 4-week interval) based on daily diary data (SMART-1, SMART-2) was evaluated; the probability of reporting breast pain, effect of baseline breast pain, and mean number of days with breast pain were also assessed. Breast pain reported as treatment-emergent adverse events (TEAEs; data from SMART-1, SMART-2, SMART-3) was evaluated.
Results: The percentage of subjects reporting ≥1 day (as measured by daily dairy) of breast pain over the first 12 weeks of treatment with BZA 20 mg/CE 0.45 or 0.625 mg (SMART-1, 15.4% and 14.4%, respectively; SMART-2, 16.1% and 17.4%, respectively) was not significantly different from that with placebo (SMART-1, 11.8%; SMART-2, 14.3%). In addition, there was no significant difference in the probability of reporting ≥1 day of breast pain among subjects treated with BZA 20 mg/CE 0.45 or 0.625 mg versus placebo. However, subjects with breast pain at baseline were significantly more likely to report breast pain during the study (odds ratio [95% confidence interval], SMART-1: 10.3 [6.0, 17.6]; SMART-2: 4.4 [1.1, 17.5]). Among subjects who reported ≥1 day of breast pain, the mean number of days with breast pain was not significantly different between either BZA/CE group and the placebo group. The incidence of breast pain reported as TEAEs and study withdrawals due to breast pain was similar among subjects in the BZA/CE and placebo groups.
Conclusions: A TSEC pairing BZA 20 mg with CE 0.45 or 0.625 mg did not increase breast pain compared with placebo in postmenopausal women. These results add to the body of evidence supporting the lack of breast stimulation with BZA/CE in postmenopausal women.

Disclosures: HY: Employee, Pfizer, Inc. SM: Employee, Pfizer, Inc.

P2-427

Progesterone Therapy Preserves Endothelial Function in Healthy Women 1-10 Years Since Final Menstrual Flow: A 12-Week Randomized, Masked Placebo-Controlled Trial.

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Endothelial function is fundamental to cardiovascular health. We previously showed (Mather, 2000) that intra-arterial progesterone and estradiol similarly improved endothelial function in healthy menopausal women.
Objective: To compare endothelial function on oral micronized progesterone (OMP) and placebo.
Trial Design: 4-wk baseline then 12-wk masked therapy.
Methods: Randomized, masked, placebo-controlled trial of OMP conducted at an academic center between June 2003 and Oct 2009. We recruited community women with moderate to severe VMS. Eligibility criteria included: within 10 y of LMP, no ovarian hormone therapy for 6 mo, non-smoking, non-obese, no diabetes, hypertension, or heart disease. Women further needed normal ECG and fasting lipids and glucose. Enrolled women completed questionnaires, measurements (BMI, waist circumference, BP), and fasting levels. Endothelial function, at baseline and 12-wk therapy, was measured by venous occlusion plethysmography following established protocols in response to 3 dose increases of the endothelium-independent vasodilator sodium nitroprusside (SNP) and 3 dose of acetylcholine (ACH), an endothelium-dependent vasodilator.
Interventions: OMP (3x100 mg Prometrium® before sleep) or placebo daily. Drug use was noted daily.
Outcome: The planned primary outcome was % increase in forearm blood flow (FBF%) from saline control (SAL) to the highest ACH dose (ACH3); absolute change between ACH3 and SAL (FBFΔ) was also analyzed.
Randomization and masking: Randomization was computer-generated, and dispensed by an independent pharmacist. Participants and all study personnel were blinded.
Results: Paired baseline and therapy plethysmography data were available for 34 women randomized to OMP (n=18) and placebo (n=16). Women were aged 54.9±3.9 (mean±SD), 4.0±2.6 y from last flow, BMI 25.2±3.1, waist circ. 79.8±6.7 cm. Most (97%) were Caucasian. Baseline FBF% was 419±45% and FBFΔ was 11.9±1.3. Both FBF% and FBFΔ were higher in those randomized to OMP (487±189% and 13.6±5.4) than placebo (408±278% and 10.2±6.0). These differences were not statistically significant (95%CI for FBF% [-232,74] P=0.30; FBFΔ [-5.7,0.7] P=0.12; ANCOVA with baseline covariate).
Withdrawal and Adverse Events: No serious adverse events occurred.
Conclusion: Endothelial function is preserved with oral micronized progesterone therapy for vasomotor symptoms in healthy women 1-10 y since final menstrual flow.
Trial Registry: www.clinicaltrials.gov NCT00152438.

Sources of Research Support: Private individual donations to CeMCOR and in kind donations from the British Columbia Endocrine Research Foundation. Active drug and placebo provided by Schering (Canada) and by Besins Healthcare.

Nothing to Disclose: CLH, TGE, EGN, VS, JCP

P2-428

Circulating Dehydroepiandrosterone Sulfate Levels in Women with Bilateral Salpingo-Oophorectomy during the Menopausal Transition.

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Annual samples from the Study of Women Across the Nation (SWAN) over 10 years reveal a detectable rise in circulating dehydroepiandrosterone sulfate (DS) concentration that is ovarian stage- but not age-related and occurs in most middle-aged women. The current study follows logically from that observation and compares annual circulating DS concentrations in SWAN subjects who underwent bilateral salpingo-oophorectomy (BSO) to the pattern of circulating DS in women who progressed through the menopausal transition naturally. From the 1179 women in the SWAN cohort eligible for longitudinal DS evaluation, eighty-one underwent BSO during the pre- or early-perimenopause stage of the menopausal transition and were potentially available for study. Of these eighty-one BSO subjects, twenty had sufficient annual samples for evaluation of the post-BSO trajectory of circulating DS. A detectable rise in circulating DS was observed in fourteen (70%) of the twenty BSO women, which is similar to the proportion (85%) of women with intact ovaries who had a detectable DS rise. The rise in mean circulating DS (5-8%) was similar in both BSO and non-BSO women ($p>0.05$), indicating that the DS inflection of weak adrenal androgens occurs in the absence of ovaries and is therefore likely of adrenal origin.
Conclusions: These data suggest that the change in ovarian function in the early menopausal transition is associated with a change in adrenal delta-5 steroid production and that individual differences in adrenal steroid production may be primarily responsible for individual differences in total circulating sex steroid production during the menopausal transition. Since the predominant peripheral conversion products of dehydroepiandrosterone in women are androgens, and not estrogens, these data also suggest that the androgen-estrogen balance may be largely determined by underlying adrenal function, rather than a result of the decline of ovarian function that precedes menopause. Further, the decline in ovarian function which appears to be requisite for inducing the increase in adrenal steroid production can be either gradual, as with natural menopause, or abrupt, as with surgical menopause, as demonstrated here. Moreover, once initiated, this induction is sustained for several years and continues past the menopause in women with intact ovaries. The time course appears to be similar in intact and BSO women, despite the lack of additional change in ovarian signals following ovariectomy.

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Nothing to Disclose: SC, GAL, NS, DSM, CC, GG, AP, MV, BLL

P2-429

Wide Distribution of the Serum Levels of DHEA and Sex Steroids in Postmenopausal Women – Relative Role of the Ovary?.

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A problem with dehydroepiandrosterone (DHEA), the exclusive source of sex steroids after menopause, is that its serum concentration is already decreased by 60% on average at time of menopause and continues to decrease thereafter. Moreover, since there is no feedback control to maintain DHEA at fixed levels, it is of importance to examine the variability of serum DHEA as well as the relative role of the ovary. Serum levels of DHEA and eleven of its metabolites were measured by gaz or liquid chromatography/mass spectrometry in 442 intact and 71 ovariectomized postmenopausal women aged 42 to 74 years. With a mean ± SD concentration of 1.98 ± 1.29 ng/ml, serum DHEA in intact postmenopausal women is highly variable with 5^{th} and 95^{th} centiles at 0.51 and 4.28 ng/ml, respectively, for a 8.4-fold difference. A comparable variability is observed for the eleven metabolites of DHEA. The 22.3% higher serum DHEA in intact compared to OVX women is accompanied by parallel changes in all the other steroids, thus indicating that all sex steroids derive from DHEA in postmenopausal women, with no direct secretion of active estrogens or androgens by the postmenopausal ovary. The highly variable serum DHEA provides an explanation for the lack of signs of hormone deficiency in some women while the majority suffer from symptoms or signs. The present data also indicate that the postmenopausal ovary secretes approximately 18% of total DHEA in this age group, thus possibly explaining the negative effect of oophorectomy on longevity, especially from coronary heart disease, in agreement with the better recognized correlation between low DHEA and cardiovascular mortality in men.

Nothing to Disclose: FL, CM, JB

P2-430

Associations of Estrogens and Androgens with Fat Partitioning and Muscle Characteristics in Postmenopausal Women.

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Background: Among postmenopausal women, declining estrogen has been thought to facilitate fat partitioning from the periphery to the intra-abdominal space, whereas changes in the androgen environment may have detrimental outcomes pertaining to muscle quality and quantity. However few studies have examined associations among hormone concentrations and indices of body composition. The objective of this longitudinal study was to identify independent associations of estrogens and androgens with body composition and fat distribution in postmenopausal women. We hypothesized that lower estrogen concentrations would be related to greater intra-abdominal adipose tissue (IAAT) and lesser thigh fat independent of total adiposity, and that lower testosterone concentrations would be related to lesser muscle mass and greater intermuscular adipose tissue (IMAT) independent of total adiposity.
Methods: 81 healthy postmenopausal women who were either using or not using hormone replacement therapy (HRT) were evaluated at baseline and 2 yr. Total and regional body composition [fat mass, appendicular skeletal muscle (ASM), and lean body mass (LBM)] were measured by dual energy X-ray absorptiometry (DXA). IAAT, thigh muscle, IMAT, and total thigh fat were analyzed by computed tomography (CT) scanning. Serum concentrations of estradiol (E2), estrone (E1), estrone sulfate, total testosterone (T), and free T were assessed.
Results: In all women combined, IAAT increased over two years ($P<0.01$). Leg lean and ASM decreased over 2 years within the HRT users ($P<0.01$ and $P<0.001$, respectively, paired t-test), but not within the non-HRT users ($P=0.79$ and $P=0.96$, respectively, paired t-test). In the non-HRT users, all estrogens were significantly and inversely associated with IAAT and were significantly and positively associated with thigh fat; E2 and E1 were positively associated with leg fat; and all androgens were significantly, inversely associated with IMAT. In the HRT users, free T was significantly, positively associated with leg lean and ASM. Free T was 50% lower in HRT users vs non-users ($P<0.001$).
Conclusion: In postmenopausal women, lower circulating estrogen may contribute to greater fat partitioning to the intra-abdominal cavity and away from the periphery. Lower androgens may contribute to reductions in both muscle quantity and quality, which may be of particular concern for women using HRT, who have lower free T.

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Nothing to Disclose: AKM, AJM, RAO, BAG

P2-431

Influence of Menopausal Hormone Replacement Therapy on Hemostatic and Inflammatory Markers.

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INTRODUCTION
Mortality due to cardiovascular diseases (CVD) in women increases progressively with age. Premenopausal women have substantially lower cardiovascular mortality rates than men at the same age range, while after menopause these rates become very similar.
Estrogenic deficiency contributes to endothelial dysfunction, due to increased inflammation in the vascular wall, lipoprotein oxidation, decreased endothelial nitric oxide production, smooth muscle cell proliferation, platelet activation and thrombus formation, favoring atherosclerosis. Although estrogens may have a cardio protecting effect the relationship between hormone therapy (HT) after menopause and CVD remains controversial.
The serum levels of inflammatory and hemostatic markers can be used to evaluate the risk of CVD, and their levels are positively related with its extension and severity.
Objective: To evaluate the serum levels of hemostatic and inflammatory markers in postmenopausal women in use of hormone therapy (TH) and in controls without HT.
Methods: This is a descriptive sectional study of 43 postmenopausal women, of which 16 were in use of continuous transdermal 17 beta estradiol associated to oral cyclic micronized natural progesterone or dihydrogesterone and 27 were non-users of these drugs. The users had a mean age of 55.4 years, mean body mass index (BMI) of 24.9 Kg/m2, and mean waist/hip ratio of 0.89. For the non-users, the mean age was 55.4 years, mean BMI was 28.7 Kg/m2 and the mean waist/hip ratio 0.88. Blood samples were collected from both groups for measurements and comparison of ferritin, lipoprotein (a), C-reactive protein (CRP), antithrombin III, fibrinogen, von Willebrand´s factor, functional C protein and S protein.
Results: Lower mean levels of CRP (p=0.022) and fibrinogen (p=0.017) were observed among users of HT. There were no significant statistical differences between the average levels of the other markers.
Conclusions: Our findings seem to demonstrate a favorable inflammatory and hemostatic profile with the use of transdermal estradiol associated to oral cyclic micronized natural progesterone or dihydrogesterone. New clinical studies are necessary to evaluate the impact of these findings on cardiovascular events.

Nothing to Disclose: VE, PEM, PT, AAA, VMK, IM, RMRM

P2-432

Impact of Progesterone Administration on Sleep Quality, GH and TSH in Postmenopausal Women.

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We have previously shown that in normal young women, luteal endogenous progesterone could stimulate GH secretion and enhance sleep inhibitory action on TSH secretion (1). The present study aimed to investigate whether, after menopause, oral administration of progesterone would produce similar effects. Eight postmenopausal women, 48-74 y.o., participated in random order in two different studies, separated from each other by ≥ 1 month. Each study included a 3-week period, during which the subjects took daily, immediately before bedtime, a capsule of either 300 mg of progesterone (PRG) or placebo (PL). On the last day, blood was sampled at 15-min intervals for 24 hours for hormonal determinations and sleep was polygraphically recorded. The present report focuses on the effects of PRG administration on GH, TSH, and sleep quality. Paired comparisons were calculated using the Wilcoxon signed-rank test. Over the scheduled sleep period (23-07 h), mean (± SEM) GH secretion (estimated by a deconvolution procedure) was enhanced under PRG treatment (154 ± 44 µg vs 103 ± 25 µg, P = 0.05), while over the scheduled wake period (07-23h), it was similar in both conditions (239 ± 91 µg vs 241 ± 90 µg). IGF-I levels (measured on 2-3 morning plasma samples) tended to increase under PRG (103 ± 23 µg/l vs 90 ± 20 µg/l, P = 0.09). Conversely, 24-h TSH levels tended to decrease under PRG (0.91 ± 0.13 mU/l vs 1.19 ± 0.22 mU/l, P = 0.07), while 24-h free T4 levels were similar under PRG (10.3 ± 0.4 ng/l) and PL (10.1 ± 0.4 ng/l, P = 0.53). Under both conditions, relatively stable daytime TSH levels were followed by the expected circadian evening rise (onset at 20.00 h ± 24 min under PRG vs 20.38 h ± 23 min under PL, P = 0.23), but the sleep-related inhibition of TSH secretion occurred earlier under PRG: over the 23-01 h period, a significant negative slope of the linear regression of TSH values was evidenced under PRG (P = 0.05) but not under PL (P = 0.12), resulting in significantly lower TSH levels under PRG over the 23-07 h period (0.98 ± 0.18 mU/l vs 1.39 ± 0.24 mU/l, P = 0.05). The total duration of wake after sleep onset was markedly decreased under PRG (71 ± 18 min vs 152 ± 35 min, P = 0.05) and the mean total delta activity (reflecting duration and intensity of slow-wave, i.e deep, sleep) was increased by 44% (P = 0.04). These results indicate that after menopause, administration of progesterone may improve sleep quality and modulate GH and TSH secretion.

(1) Caufriez A et al, Clin Endocrinol (Oxf) 2009; 71:535

Nothing to Disclose: AC, RL, ML-B, MK, GC

P2-433

TSH Levels in Women in the Pre- and Menopause Transition: A Population-Based Study.

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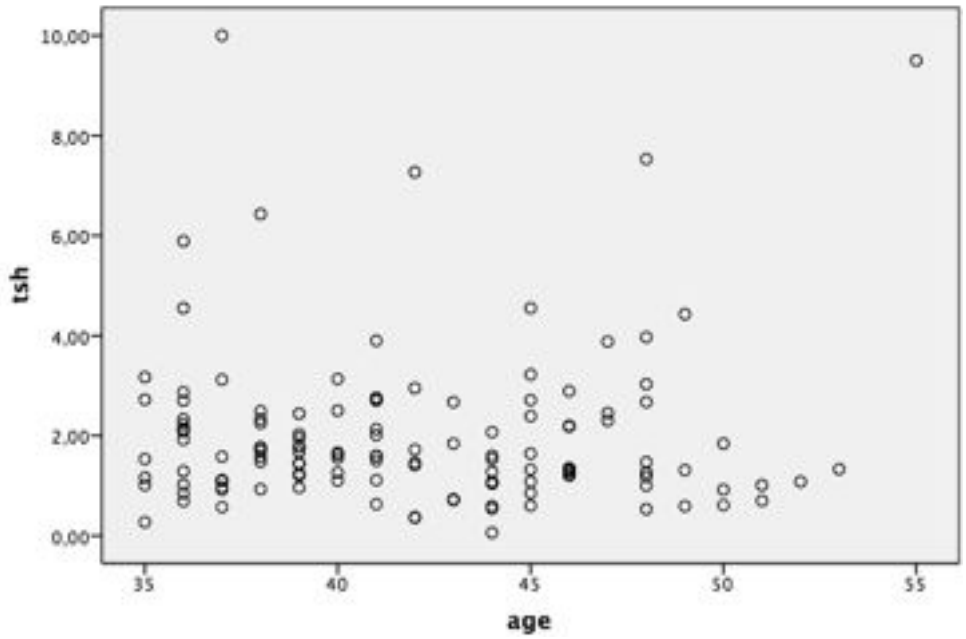
Background: thyroid dysfunction, mainly hypothyroidism, is more frequent among women over the age of 50 and an association between the decline of ovary hormones and thyroid function could be supposed to occur.
Objectives: the aim of this study was to assess thyroid stimulating hormone (TSH) levels and its association with age and FSH levels in a population-based sample of women in the pre-menopause and menopause transition.
Methods: data were collected in the first cross-sectional study, performed between 1995 and 1997 in Passo Fundo, a city of 170,000 inhabitants of Caucasian ethnic background, in Southern Brazil. Two hundred and ninety eight participants were randomly selected from 16.958 women between 35 to 55 years who had menstruated at least once in the past 12 months. Menopause status was established on the basis of the characteristics of menses or time since amenorrhea, as previously described (1). TSH concentrations were measured by immunometric quimioluminescence (Immulite®) sensitivity 0.002µIU/mL, reference ranges 0.4 to 4.0 µIU/mL, FSH and LH were measured by immunoradiometric assay (Biodata®) sensitivity 0.25 IU/L for FSH and 0.15 IU/L for LH. Pearson and Spearman correlation tests and ANOVA were used.
Results: one hundred and twenty six women had TSH measured. The mean age of all participants was 40.12±7.1 years (mean ± SD) and TSH 1.99 ±1.59 µIU/mL. Seventeen women had TSH ≥ 3.0 µIU/mL and 6 had TSH ≥ 5 µIU/mL. When stratified by age categories: 35 to 39 (n=50), 40 to 44 (n=39), 45 to 49 (n=33), and 50 to 55 (n=8) the mean TSH levels was 2.07 ± 1.63, 1.73 ± 1.25, 2.15 ± 1.47 and 2.12 ± 3.0, respectively (p=0.85). Age, systolic blood pressure, FSH, and LH levels were higher in menopause transition group (p<0.05) in comparison to premenopausal women. No correlation was found between TSH and FSH levels, (Spearman: r= -0.14: p=0.11) or age (Pearson; r=0.03: p=0.74).

Fig 1. TSH levels and age in pre- and menopause transition



Conclusions: TSH levels are not modified along with age, FSH levels and pre- or menopause transition status in women between 35 to 55 years old.

Oppermann K et al.,Menopause 2003;10:209

Nothing to Disclose: KO, HRKL, SCF, PMS

P2-434

Male Gender Identity in Complete Androgen Insensitivity Syndrome.

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Introduction
In XY individuals with complete androgen insensitivity syndrome (CAIS), the external genitalia are unremarkable (normal female) at birth. That is why CAIS patients are invariably assigned and reared as girls. There is no documented case of gender change in individuals with CAIS.
Case report
In 1995, our patient was diagnosed with CAIS at age 17 following investigations for primary amenorrhoea. The diagnosis was based on an unambiguously female phenotype, a 46,XY karyotype and a 2660delT androgen receptor gene mutation, leading to a premature stop in codon 807. There were no other CAIS individuals known in the family. Bilateral gonadectomy and hernia repair was performed in 1996 and gonadal tissue was described as immature testicular tissue. A short time of oestrogen treatment induced a negative emotional reaction and treatment was stopped.
Since the age of three, childhood-onset cross gender behavior had been noticed. After a period of psychotherapy, started in 2001, persisting male gender identity was confirmed. Hormonal therapy with Testosterone enanthate 200 mg intramuscularly every 2 weeks was started in 2003, however without inducing any of the desired secondary male characteristics. A subcutaneous mastectomy was performed in 2005; a relief for the patient. The patient was referred to our team for phalloplasty. Metaidoioplasty was not feasible, since there had been no effects from testosterone treatment on clitoral growth.
We saw this individual at age 28 with clear male gender role, presentation and locomotion, mastectomy scars, and female external genitalia without clitoral hypertrophy. We noticed a low voice, which the patient attributed to continuous exercise. There was no psychiatric co-morbidity and there was an excellent real life experience. The patient had a female partner. A short period of treatment with dihydrotestosterone induced no virilisation. The patient received in 2008 phalloplasty by left forearm free flap to the perineal region and scrotoplasty. Even without virilization, testosterone treatment was continued and bone density remained normal.
Conclusion
To our knowledge, this is the first case of CAIS who chose gender reassigment to male. The patient clearly qualifies as female-to-male transsexual and was treated according to the Standards of Care by the World Professional Association for Transgender Health. However, we do not believe that female sex of rearing as a standard procedure should be questioned in CAIS.

Nothing to Disclose: GGRT, GDC, SM, PH, FKF, JVB, MC

P2-435
Mild Androgen Insensitivity (MAIS) and Osteoporosis.

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Sex steroids have an important role in skeletal physiology. Androgens alone are not suffficient to maintain bone mineralization as revealed in cases with estrogen receptor mutation, and those with aromatase defeciency. Androgens have been linked to age related bone mass changes and androgenic steroids are known to increase the bone mineral content and reduce fracture rate. It is argued that ultimately androgen related bone accretion depends on conversion to estrogens and that androgens have dimunitive role per se. Lessons learnt from small studies in androgen insensitivity syndrome(AIS) have not been entirely revealing, and often complicated by the timing of gonadectomy and the appropriateness of estrogen replacement. Based on current literature it would be assumed that as long as estrogen levels are appropriate and no disruption of estrogen receptor signal is present, skeletal mineralization should be appropriate.
We describe a case with Mild androgen insensitivity syndrome(MAIS)who has significant osteoporosis.A 43yr old male was referred to our clinic for evaluation of unilateral gynecomastia of unknown duration.In the past he had undergone orchiectomy and radiation therapy for seminoma and remained in remission. History was remarkable for seizure disorder treated with phenytoin, mysoline, carbamezapine and phenobarbital at various times. Physical exam was remarkable for left gynecomastia(non-tender) and absent left testicle.Patient was started on monthly testosterone injections(100mg IM).His labs are as shown in the table before and after androgen replacement.

Laboratory Investigations

	Before androgen replacemnet	After androgen replacement
Plasma testosterone(194-833ng/dl)	654	955
LH(1.3-12.9 mIU/ml)	42	5.4
Estradiol(<50pg/ml)	15	19
FSH(0.9-15mIU/ml)	41.3	-
DHT(30-85 ng/dl)	53	-
SHBG(10-80nMol/L)	142	-

Prolactin and TSH was normal.
Over ensuing months patient had complete resolution of gynecomastia. His initial BMD revealed a lumbar T score of -2.8 necessitating initiation of therapy with alendronate. His calcitropic hormones(25 hydroxy vitamin D,1:25 dihydroxy vitamin D and iPTH were normal). Although antiseizure medications are associated with osteoporosis, an entirely normal calcium-PTH axis would not be supportive. We believe that androgen resistance may have a role to play. Androgen action at a critical bone accretion state may be crucial and independent of concurrent estrogen status.

Nothing to Disclose: PJ, NS, SG, RK

P2-436

Unique Phenotypic Features Associated with a Novel Missense Mutation in the Ligand-Binding Domain of the Androgen Receptor Gene.

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Introduction
Androgen insensitivity syndrome is caused by mutations in the androgen receptor (AR) gene, most commonly in the ligand binding domain. There is genotype-phenotype variability. A mutation at a given locus may result in partial androgen insensitivity syndrome (PAIS) with some degree of ambiguous genitalia, or complete androgen insensitivity syndrome (CAIS) resulting in a female phenotype.
Clinical Case
A 14 year 7 month old female presented for endocrine evaluation of tall stature and primary amenorrhea, with a height above the 99th percentile (+3.59 SD). She had Tanner 4 breasts, Tanner 3 pubic hair, and normal external female genitalia with no clitoromegally. There were no inguinal masses palpable. Laboratory studies were significant for elevated testosterone.

Table 1

Test (female normals, units)	Pre-Surgical	Post-Surgical/Pre-Estrogen Therapy
FSH (0.8-8.5 mIU/mL)	3.7	171
LH (0.8-7.6 mIU/mL)	12.8	55.9
Estradiol-17β (0.3-33 pg/mL)	39	<20
Total Testosterone (<41 ng/dL)	874	<20
DHT (4-22 ng/dL)	32	

Pelvic ultrasound revealed gonads located in both upper inguinal canals with a 1.1 cm mass in the right. Chromosomal analysis showed a 46, XY karyotype. Androgen receptor mutation analysis revealed a G>T base pair substitution in exon 4, in the ligand-binding domain, resulting in a missense mutation of Serine to Isoleucine at position 703. Given the risk of malignancy on the basis of abnormal imaging, a gonadectomy was performed. Pathology results were consistent with testicular tissue bilaterally, the right with a benign 1cm Sertoli cell adenoma. The specimen of the left included a rudimentary fallopian tube.
Conclusion
Our case presents a number of unique features which add complexity to the phenotypic descriptions of the AIS spectrum. CAIS is generally described as having absence of pubic and axillary hair, due to the role of skin androgen receptors in folliculogenesis. Our patient presented with pubic hair development yet no other features to indicate PAIS. Additionally, mullerian structures are expected to regress in AIS as the androgen receptor has no role in the production or action of antimullerian hormone (AMH). Pathology results in this case revealed rudimentary mullerian structures, a finding only rarely described in the literature. Finally, while there are other mutations previously reported at amino acid 703 resulting in both PAIS and CAIS, we have identified a novel mutation (S703I) resulting in CAIS.

Nothing to Disclose: SS-B, VLC, TAL, PZ

P2-437

A Unique Exonic Splicing Mutation in the CYP17A1 Gene as Cause of Steroid 17α-Hydroxylase Deficiency.

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Background: 17α-hydroxylase/17,20-lyase deficiency(17OHD), caused by mutation in CYP17A1 gene, is characterized by hypertension,hypokalemia and abnormal development of genitalia. To date, more than 50 mutations in the CYP17A1 gene have been reported to cause either combined or isolated 17α-hydroxylase/17,20-lyase deficiencies.
Clinical case: A 2.5-yr-old girl with 46,XY disordered sex development showed nearly normal basal cortisol level(6.80μg/dl at 8am), ACTH level (54.30pg/ml , 12.0~78.0 pg/ml) and reduced sexual steroids (testosterone <0.08 ng/ml and DHEA 0.1μg/dl). But in a status of stress, plasma ACTH elevated remarkably (4765.8 pg/ml, 12.0–78.0pg/ml). No overt hypertension and hypokalemia was observed. The levels of basal and stimulated corticosterone (181.87-565.31-522.53 nmol/L) were increased whereas the reaction of cortisol (16.2-18.8-16.7 μg/dl) was absent. ACTH test and hCG test suggested the residue activity of 17α-hydroxylase/17,20-lyase.Mutation analysis revealed a compound heterozygous CYP17A1 mutations, with Y329fs in one allele and silent nucleotide variation (c. 1263G>A: GCG> GCA) in another allele. In vitro expression analysis of an allelic minigene confirm that although the novel nucleotide variation did not cause alteration of the coded amino acid, it induced a splicing acceptor site, resulting in the aberrant splicing of mRNA and missing a part of exon 8. The translation products include deletion of seven or six amino acids from codon 415 without causing frame-shift. As shown by the activity assays and molecular modeling, the deleted protein abolish the 17α-hydroxylase and 17,20-lyase activity completely. But RT-PCR also proved existence of a small fraction of normal, functionally intact enzyme that could explain the partial masculinization in this patient.
Conclusion: This is the first description of an exonic splicing mutation in the CYP17A1 relevant to the phonotype of 17OHD. Different from the 46, XY homozygotes for Y329fs we identified in four Chinese kindreds, this patient showed some degree of masculinization of the external genitalia.The codon 416-423 near the heme-binding region of P450c17 provides strong evidence for the key role of the amino acids in 17α-hydroxylase/17,20-lyase function. Early genetic diagnosis gives advantage to the selection of gender and initiation of glucocorticoid therapy.

Nothing to Disclose: JQ, BH, B-LL, C-MP, HZ, Y-LL, W-LW, M-DC, H-DS

P2-438

The Use of GnRH Analogue Stimulation Testing To Identify Isolated FSH Insufficiency in a Man with Oligozoospermia Followed by Successful Treatment with Human Menopausal Gonadotropin.

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Background: Isolated FSH deficiency is a well recognised entity with multiple cases reported(1),these patients usually have unequivocally low basal FSH levels.FSH treatment has been used in men with oligo/azoospermia and normal gonadotropin levels with conflicting reports of success.A high FSH and low inhibin level suggestive of primary testicular pathology have been reported to predict a poor response to FSH therapy(2).However GnRH analogue stimulation testing is not routinely performed in otherwise well androgenized men with oligo/azoospermia to detect a relative FSH insufficiency if basal gonadotropin levels are normal.
Case report: A 28 year old male presented with primary sub-fertility.There was no complaint of loss of libido or erectile dysfunction .On examination he was well androgenized and had normal body proportions .The testicular volume was 20ml bilaterally and he had a normal sense of smell .His semen analysis showed a sperm count of 6 million/ml(normal>20million/ml).His serum testosterone was 1.87 ng/ml(normal:1.7-10ng/ml). The basal LH and FSH values were 3.92IU/l(normal<10) and 2.8IU/L(normal<10) respectively. Stimulation testing was done with 100 µg of GnRH analogue Triptorelin (decapeptyl) and sampes for FSH and LH drawn at 30 and 60 min.The Peak LH response was 41.5IU/L and FSH reponse was 4.62 IU/L(normal>14 IU/l)(3).An MRI of sella was normal. A provisional diagnosis of isolated FSH insufficiency was made and patient treated with hMG starting with a dose 12.5mg alternate days. After 2 months of treatment sperm count increased to 18 million with 50% motility.The dose was increased to 37.5 mg on alternate days ,after two more months of treatment the sperm count increased to 35 million/ml with 60% motility. Subsequently his wife conceived normally and she delivered a full term normal male child.
Conclusion: We propose that GnRH analogue stimulation testing be done in oligo/azoospermic men with normal basal gonadotropin levels to identify isolated FSH insufficiency. human menopausal gonadotropin or recombinant FSH therapy may be used successfully in such patients as illustrated by this case report.

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2) Carlo Foresta, Riccardo Selice, Andrea Garolla and Alberto Ferlin Follicle-stimulating hormone treatment of male infertility. Current Opinion in Urology 2008, 18:602–607
3) Kauschansky A, Dickerman Z, Phillip M, Weintrob N, Strich D. Use of GnRH agonist and human chorionic gonadotrophin tests for differentiating constitutional delayed puberty from gonadotrophin deficiency in boys. Clin Endocrinol (Oxf). 2002 May;56(5):603-7

Nothing to Disclose: GB, VAS, AR

P2-439

Acute Visual Loss Due to PDE5 Inhibitor Induced Non Arteritic Anterior Ischaemic Optic Neuropathy in a Young Man with Hypogonadism and Erectile Dysfunction.

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Background: Phosphodieterase5 (PDE5) inhibitors are one of the commonly prescribed medications world wide for erectile dysfunction (ED). Their cardiovascular effects are well known. However their role in causing Non Arteritic Anterior Ischaemic Optic Neuropathy (NAAION) is still debated. PDE5 inhibitor related NAAION usually occur in men >50years old with other risk factors which include hypertension, diabetes, hyperlipidaemia, atherosclerosis, stroke, glaucoma, small cup-to-disc disc ratio and smoking. We report a case a young patient without any other known risk factors developing NAAION after use of tadalafil (Cialis ®).
Presentation: A 28year old male with ED and obesity was diagnosed with hypogonadism. He was started on testosterone gel 50mg od but was changed to Testosterone Undecanoate due to lack of efficacy. ED persisted after optimal testosterone replacement therapy and was tried on tadalafil 10 and then 20mg twice weekly with only partial response. He was changed to tadalafil 5 mg daily with good response.
Two months after starting on regular tadalafil, patient presented to the Ophthalmology Casualty Department with acute painless visual loss of left eye. Examination of the left eye showed vision of 6/36 on Snellen's chart, extensive field defects involving nasal half and inferior temporal quadrant and a left positive Relative Afferent Pupillary Defect. The nasal half of the optic disc was swollen and oedematous. The right eye was normal. Tadalafil was immediately stopped and prednisolone 40mg was started. There a subjective improvement in vision in one week. At 8 weeks the vision normalised to 6/4, the field defects completely resolved and disc swelling subsided. The steroid was stopped.
Discussion: NAAION is the most common acute optic neuropathy found in people aged 50 years and older. Very rarely PDE5 inhibitors are reported to cause NAAION. It is hypothesised that the mild hypotensive effect of PDE5 inhibitors accentuates physiological nocturnal hypotension resulting in ischaemia of the optic nerve head.
Conclusion: Almost always, patients with PDE5 inhibitor induced NAAION are above 50 years and have other risk factors. To our knowledge, this is the youngest and first known case of NAAION, with use of tadalafil being the only risk factor. As PED5 inhibitors are extensively used in clinical practice, we recommend that the prescribing Physicians must be aware and inform the patient about this very rare but serious ocular complication.

Nothing to Disclose: VM, THJ, HMJH

P2-440
Neuroendocrine Tumor of Prostrate. A Case Report.

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54 year old male smoker presented with pain at the tip and shaft of penis and testicular pain following catheter insertion. He had two prior Emergency Room visits for acute urinary retention and clinic visits for treatment of prostatitis. He had a 10 month history of progressive dysuria,burning micturition, urinary hesitancy, lower abdominal pain, rectal itching, and 10 kg weight loss in 2months. The physical exam revealed exquisite tenderness in infraumbilical and rectal areas, and a tender irregularly surfaced hard prostrate with a palpable mass along medial portion of prostrate. Initial labs revealed Calcium: 10.0 mg/dl, Alkaline phosphatase: 214 mg/dl, PSA 8.91(<4.0 ng/ml), CEA: 1.9 (0.0-5.0 ng/ml), Alpha Feto Protein: 4 (0-6ng/ml), The CT scans of the chest, abdomen and pelvis revealed a heterogenous enlarged prostrate with L 3 vertebral body mixed blastic & lytic lesion, numerous large ill-defined low-density lesions in the liver, right illiac chain and retroperitoneal lymphadenopathy, and a 12mm pulmonary nodule in right upper lung field.
The biopsy during TURP revealed convergence of the small cell carcinoma morphology with that of poorly differentiated prostatic carcinoma. The “hybrid” appearing tissue sample (including features of small cell carcinoma) had immunohistochemical stains positive for PSA and negative P 63 stains(III c). The immunohistochemical stains were focally positive for neuron specific enolase and CD 56, strongly positive for PSA, A colonoscopy was also done and it revealed a tumor at about 13 cm from the anal verge. The rectal biopsy showed poorly differentiated malignant neoplasm with immunohistochemical stains positive for Cytokeratin AE1/3, elevated Ki-67. Accordingly, this patient's malignancy is best considered to be one neoplasm – poorly differentiated prostatic carcinoma with an extensive small cell carcinoma component – which has spread to involve the rectum. Lab workup also revealed Chromogranin A: 10H nmol/L (0-5), PSA: 8.91(<4.0 ng/ml).
Neuroendocrine (NE) differentiated small cell carcinoma is a very rare tumor comprising 0.5%-2% of all prostatic carcinomas. Suttman et al reported a mixed adenocarcinoma and neuroendocrine carcinoma of the rectum associated with an adenocarcinoma of the prostate as a second primary malignancy, while our case is vice versa. Also, our case had both mixed blastic and lytic bony lesion favoring both adenocarcinoma and small cell carcinoma respectively.

Nothing to Disclose: NK, GS, PS, TT

P2-441

Wilms' Tumor Gene 1 (WT1) Mutation in a 46, XY Phenotypic Male with Elevated Gonadotropins Despite Normal Testosterone Concentration.

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Patients with WT1 gene mutations usually present in childhood with progressive nephropathy, intersex, and Wilms' tumor. We here report an adult patient with a WT1 missense mutation, unambiguous male phenotype who was referred for hypogonadism.

19 year old adult male was referred for the management of hypergonadotropic hypogonadism. During infancy, he underwent surgical correction of hypospadias, left orchiopexy and right ochiectomy with prosthesis placement. Focal segmental glomerulosclerosis was diagnosed at age 3. At age 15 the patient presented with gynecomastia and delayed puberty and was treated with mammoplasty and testosterone therapy.

Despite only intermittent adherence with androgen therapy, he developed normally and reported normal libido, sexual function and muscle strength. Physical examination revealed a normal phallus, normal testis (one native testis, one prosthesis), and sparse beard growth. After not taking his androgen replacement, lab tests revealed: Total Testosterone (T): 419 ng/dL (250-1100) with elevated gonadotropins—LH: 31.8 IU/L (1.5-9.3) and FSH: 66.2 IU/L (1.6-8). In addition, Prolactin: 31 ng/ml (2-18) and subsequently, Alpha Subunit: 1.8 ng/ml (<0.6) were elevated. Other pituitary testing was normal. MRI: normal pituitary size and signal intensity. Chromosomes: 46, XY genotype. Testosterone therapy was restarted achieving T: 777 ng/dL but LH, FSH and α-subunit remained elevated suggesting central androgen resistance. DNA sequencing for WT1 mutation revealed a C>T transition at nucleotide 1180.

Clinical presentations of WT1 mutations are variable and are associated with two well described phenotypes relevant to our patient: Denys-Drash and Frasier Syndromes. In these disorders, typified by gonadal dysgenesis, genotypic males present as phenotypic females or display ambiguous genitalia at birth. Our patient, who presented with a male phenotype, is decidedly uncommon. The presence of persistently elevated gonadotropins despite testosterone replacement therapy has been previously reported in only one patient that we were able to identify(1). This gives rise to the possibility of an unclassified syndrome of hypothalamic-pituitary resistance to sex steroids. In young adult males presenting with hypogonadism, WT1 mutations should be included in the differential diagnosis. More investigation is required to understand the etiology of gonadotropin elevation in the face of adequate sex hormone replacement therapy.

(1)Melo, KFS et. al., JCEM 2002; 87:2500

Nothing to Disclose: SMK, SMM, KHH

P2-442

Pharmacokinetics and Efficacy of a 1.62% Testosterone Gel for the Treatment of Hypogonadal Men: Secondary Efficacy Results from a Phase III Study.

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OBJECTIVE:To test the efficacy and safety of a 1.62% testosterone gel (T-gel) in hypogonadal men over 182 days. DESIGN:A double-blind, randomized 6:1, placebo-controlled study in 274 (enrolled) males (serum total testosterone (TT)<300ng/dL). Doses were titrated over 42 days targeting a TT of 300–1000ng/dL and then maintained until Day 182. Subject/dose allocation was (based on last titrated dose) 17/1.25g; 60/2.5g; 66/3.75g, and 91/5g. On Days 14, 56, 112, and 182, blood was taken for 24h pharmacokinetic (PK) analysis. An efficacy endpoint was TT C_{max} during PK Days. Safety evaluations included prostate exam, prostate specific antigen (PSA), hematocrit, liver enzymes, sex hormones, and adverse events. Subjects were discontinued if the mean of 2 post-Baseline PSA level measurements was >4.0 ng/mL and/or showed an increase >0.75 ng/mL. RESULTS:

	n/N(%) in 1.62% T-Gel Group Within Testosterone C_{max} Ranges(ng/dL)			
Day	**≤1500**	**1501-1799**	**1800-2500**	**>2500**
14	203/210(96.7)	1/210(0.5)	5/210(2.4)	1/210(0.5)
56	178/183(97.3)	2/183(1.1)	1/183(0.5)	2/183(1.1)
112	159/179(88.8)	8/179(4.5)	10/179(5.6)	2/179(1.4)
182	156/169(92.3)	6/169(3.6)	6/169(3.6)	1/169(0.6)
Overall	696/741(93.9)	17/741(2.3)	22/741(3.0)	6/741(0.8)

n=#subjects achieving range; N=#subjects with evaluable PK

For all PK days combined, 93.9% of the subjects on T-gel had C_{max} values ≤1500 ng/dL, and 5.3% were in the range of 1501–2500 ng/dL. In comparison, ≥99.2% of placebo subjects had C_{max} values ≤1500 ng/dL and 0.8% were within 1501–2500 ng/dL. Nine subjects on T-gel had C_{max}<300 ng/dL on Day 182. Ten subjects had 11 (2 non-PK Day and 9 PK Day) observations of TT>2500 ng/dL during the study. Mean C_{max} for subjects on testosterone were within the eugonadal range for all study days. Six subjects had treatment-emergent serious adverse events (TESAEs) and no deaths occurred. A similar % in both groups experienced a TESAE. There were 34 subjects with treatment-emergent elevated PSA values in the T-gel groups and none in the placebo group. Seventeen subjects discontinued due to increased PSA. One prostate cancer was diagnosed and deemed unrelated. CONCLUSION:1.62% T-gel treatment was efficacious, resulting in an acceptable distribution of C_{max} values. TT values >2500 ng/dL were rare, brief, and inconsistent, and explainable by patient- and/or site-specific variables. The treatment was safe and well tolerated.

Disclosures: JMK: Investigator, Solvay Pharmaceuticals, Inc., Endo Pharmaceuticals, Coloplast. JJB: Employee, Solvay Pharmaceuticals, Inc. JLG: Employee Sep 2005 through Aug 2009, Solvay Pharmaceuticals, Inc., GlaxoSmithKline. MGM: Employee, Solvay Pharmaceuticals, Inc. SF: Employee, Solvay Pharmaceuticals, Inc. CM: Employee, Solvay Pharmaceuticals, Inc.

P2-443

The Efficacy and Safety of a 1.62% Testosterone Gel for the Treatment of Hypogonadal Men: Efficacy Results from a Phase III Study.

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OBJECTIVE: To demonstrate the efficacy/safety of a 1.62% testosterone gel (T-gel) in hypogonadal men for 182 days. DESIGN: A double-blind, randomized, placebo-controlled study in males (testosterone (T) <300ng/dL). 274 subjects enrolled and 196 completed the study (168 T-gel, 28 placebo). During a 42-day titration phase, doses were adjusted to produce serum T levels within the eugonadal range (300-1000ng/dL). The min/max T-gel doses were 1.25g/5.00g/day. Day 42 subject/dose allocation was 17/1.25g; 60/2.50g; 66/3.75g, and 91/5.00g. Subjects were kept at their Day 42 dose until Day 182. 24-hour pharmacokinetic (PK) analysis occurred on Days 14, 56, 112, and 182. Safety evaluations included prostate exam, prostate specific antigen (PSA; free and total), hematocrit, liver enzymes, sex hormones, and adverse events (AEs). Subjects were discontinued from the trial if the average of 2 post-Baseline PSA level measurements was >4.0ng/ml and/or showed an increase >0.75ng/mL. RESULTS: On Days 56, 112, and 182, 81.6-82.5% of treated subjects attained an average serum T concentration (C_{av}) within the eugonadal range.

Number (%) Achieving Eugonadal Range for Total Testosterone C_{av} by Day & Treatment

	1.62%T-gel		**Placebo**	
Day	**n/N(%)**	**95%CI**	**n/N(%)**	**95%CI**
14	138/210(65.7)	(58.9,72.1)	11/37(29.7)	(15.9,47.0)
56	151/183(82.5)	(76.2,87.7)	11/32(34.4)	(18.6,53.2)
112	146/179(81.6)	(75.1,87.0)	10/27(37.0)	(19.4,57.6)
182	139/169(82.2)	(75.6,87.7)	8/28(28.6)	(13.6,48.7)

P<0.0001 for all days. CI=confidence interval (via exact methods);n=#subjects achieving total T 300-1000ng/dL;N=#subjects with evaluable PK

37.0% or fewer subjects on placebo had a C_{av} within the normal range on any study day (*P*<0.0001 on all Days). Ten subjects had 11 observations of total T>2500 ng/dL at some point in the study. These were rare, brief, inconsistent, and unsustained, and explained by patient- and/or site-specific variables. Six subjects had treatment-emergent serious AEs and no deaths were reported. Similar percentages of serious AEs were reported in both treatment groups. There were 34 subjects with treatment-emergent elevated PSA values in the T-gel treatment groups and none in the placebo group. Due to increased PSA, 17 subjects discontinued from the study. One prostate cancer was diagnosed and deemed unrelated. CONCLUSION: The 1.62% T-gel showed consistent efficacy over a 182 day period. No new safety issues were identified and it was considered safe and well tolerated.

Sources of Research Support: Solvay Pharmaceuticals, Inc.

Disclosures: JMK: Investigator, Solvay Pharmaceuticals, Inc., Endo Pharmaceuticals, Coloplast. JJB: Employee, Solvay Pharmaceuticals, Inc. JLG: Employee Sep 2005 through Aug 2009, Solvay Pharmaceuticals, Inc., GlaxoSmithKline. MGM: Employee, Solvay Pharmaceuticals, Inc. SF: Employee, Solvay Pharmaceuticals, Inc. CM: Employee, Solvay Pharmaceuticals, Inc.

P2-444

The Effect of Varying the Application Site on the Bioavailability of 1.62% Testosterone Gel in Hypogonadal Males.

Jodi Miller PharmD, MS[1], Margaret Britto PhD[2], Sherahe Fitzpatrick MD[1], Cecilia McWhirter MS[1], Samuel A Testino, Jr PhD[1], John J Brennan PhD[1] and Troy L ZumBrunnen PharmD[1].

[1]Solvay Pharma, Inc Marietta, GA and [2]Quintiles, Inc Overland Park, KS.

OBJECTIVE: The objectives of this study were to determine the single and multiple dose pharmacokinetics (PK) and the relative bioavailability of testosterone after application of 1.62% testosterone gel (T-gel) to the abdomen, upper arms/shoulders, or an alternating schedule of both application sites in hypogonadal males.
DESIGN: Thirty-six hypogonadal male subjects were enrolled in a single center, open-label, randomized, three-way crossover study. Subjects received 5.0 g of 1.62% T-gel once daily for each of three 7-day treatment regimens, with a 5-day washout period between treatments. Whole blood samples were collected at baseline (Day -1), and on treatment Days 1 and 7 of each treatment for PK assessments.
RESULTS:

			GLM		GLM Ratio (%) (95% Confidence Interval)		
	Day	**Tmt A**	**Tmt B**	**Tmt C**	**A/B**	**B/C**	**A/C**
AUC_{0-24} (ng*h/dL)	1	8490	12600	9100	68 (60,77)*	138 (122,156)*	93 (83,105)
	7	10500	16000	15400	66 (59,73)*	104 (93,116)	68 (61,76)*
C_{max} (ng/dL)	1	472	765	501	62 (53,72)*	153 (132,177)*	94 (81,110)
	7	610	1000	942	61 (53,71)*	106 (92,123)	65 (56,75)*

*P<0.05. GLM=geometric least-squares mean. Treatment (Tmt) A: abdomen for 7 days; Tmt B: upper arms/shoulders for 7 days; Tmt C: abdomen for 3 days then upper arms/shoulders for 4 days.

Following all treatments with 1.62% T-gel, mean observed testosterone concentrations were within the eugonadal range of 300-1000 ng/dL by 2 h postdose on treatment Day 1. Bioavailability was lower for abdomen than upper arms/shoulders following both single and multiple dosing (33% and 38% lower mean AUC_{0-24} and C_{max} on Day 1; 34% and 39% lower mean AUC_{0-24} and C_{max} on Day 7). Application site rotation provided levels of testosterone exposure comparable to abdomen and upper arms/shoulders on Days 1 and 7, respectively, in accordance with the application site being used on that day. For all treatments, mean time-averaged testosterone concentrations (C_{av}) were within the eugonadal range on both treatment days.
CONCLUSION: Abdominal application of 1.62% T-gel provided approximately 30-40% lower testosterone bioavailability compared to upper arm/shoulder application, but an alternating application schedule of 1.62% T-gel to upper arms/shoulders or abdomen does not appear to impact achievement of eugonadal testosterone levels, despite the differences in bioavailability.

Sources of Research Support: Solvay Pharmaceuticals, Inc.

Disclosures: JM: Employee, Solvay Pharmaceuticals, Inc. MB: Researcher, Solvay Pharmaceuticals, Inc. SF: Employee, Solvay Pharmaceuticals, Inc. CM: Employee, Solvay Pharmaceuticals, Inc. SAT: Employee, Solvay Pharmaceuticals, Inc. JJB: Employee, Solvay Pharmaceuticals, Inc. TLZ: Employee, Solvay Pharmaceuticals, Inc.

P2-445

The Effect of Concomitant Application of Moisturizer Lotion or Sunscreen on the Bioavailability of 1.62% Testosterone Gel in Hypogonadal Males.

Jodi Miller Pharm D, MS[1], Margaret Britto PhD[2], Sherahe Fitzpatrick MD[1], Cecilia McWhirter MS[1], Samuel A Testino, Jr PhD[1], John J Brennan PhD[1] and Troy L ZumBrunnen Pharm D[1].

[1]Solvay Pharma, Inc Marietta, GA and [2]Quintiles, Inc Overland Park, KS.

INTRODUCTION AND OBJECTIVE: This study evaluated whether moisturizer lotion or sunscreen alters systemic testosterone exposure when applied 1 hour after application of 1.62% testosterone gel (T-gel) in hypogonadal subjects. METHODS: Eighteen hypogonadal male subjects were enrolled in an open-label, randomized, 3 period, 3 treatment, crossover study. Subjects were dosed with 2.50 g of 1.62% T-gel once daily to the upper arms/shoulders for 7 days during each of 3 consecutive treatment periods, for a total of 21 days. During each 7-day treatment period, 6.0 g of moisturizer lotion (treatment A) or 6.0 g of sunscreen (treatment B) was applied 1 hour after T-gel dosing. Treatment C was the reference with no concomitant application. Whole blood samples were collected at baseline (Day 1), and the last (7^{th}) day of each treatment period (ie, study days 7, 14, and 21) for pharmacokinetic (PK) assessments. RESULTS:

Results and Comparisons of Testosterone PK on the 7th Day of Each Treatment

	GLM			**GLM Ratio (%) (95% Confidence Interval)**	
	Tmt A	**Tmt B**	**Tmt C**	**A/C**	**B/C**
AUC_{0-24} (ng*h/dL)	8790	8400	7720	114 (101, 129)*	109 (96, 123)
C_{max} (ng/dL)	542	522	462	117 (102, 136)*	113 (98, 131)

*$P<0.05$. AUC_{0-24}=area under the serum concentration-time curve; C_{max}=maximum serum concentration; GLM=geometric least-squares mean; Tmt=treatment.

Application of moisturizer lotion increased the area under the serum concentration-time curve (AUC_{0-24}) by 14% and maximum testosterone serum concentration (C_{max}) by 17% compared with 1.62% T-gel alone. Application of sunscreen had no effect on the AUC_{0-24}, but increased C_{max} by 13% compared to 1.62% T-gel alone. Individual and mean time-averaged serum concentrations (C_{av}), and mean C_{max} values were within the eugonadal range (300-1000 ng/dL) for all treatments. 1.62% T-gel appeared to be safe and well tolerated in this population during this study. CONCLUSIONS: Both moisturizer lotion and sunscreen applied to the same application site as 1.62% T-gel slightly increased testosterone exposure compared to 1.62% T-gel administered alone. However, under all treatment conditions, testosterone levels remained in the normal range. Thus, the testosterone increases observed were considered not clinically significant.

Sources of Research Support: Solvay Pharmaceuticals, Inc.

Disclosures: JM: Employee, Solvay Pharmaceuticals, Inc. MB: Researcher, Solvay Pharmaceuticals, Inc. SF: Employee, Solvay Pharmaceuticals, Inc. CM: Employee, Solvay Pharmaceuticals, Inc. SAT: Employee, Solvay Pharmaceuticals, Inc. JJB: Employee, Solvay Pharmaceuticals, Inc. TLZ: Employee, Solvay Pharmaceuticals, Inc.

P2-446

Evaluation of the Effects of Application Site Washing on Systemic Testosterone Exposure after Application of 1.62% Testosterone Gel in Hypogonadal Males.

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OBJECTIVE: This study investigated the effect of washing the application site on testosterone exposure and on residual testosterone remaining on the skin surface after topical application of 1.62% testosterone gel (T-gel).
DESIGN: Twenty four hypogonadal males (with total testosterone levels < 300 ng/dL) were enrolled into an open-label, randomized, 3-way crossover study. Subjects received 5 g 1.62% T-gel applied once daily to the shoulders/upper arms for 7 days during each of 3 treatment periods. On the 7th dosing day of each period, the skin was washed (soap/water) at one of the following times: 2, 6, or 10 h postdose. Pharmacokinetic serum samples were collected at baseline, and on Days 6 (no washing), and 7 (with washing) of each treatment period. Skin stripping for determination of residual testosterone was also performed on Days 6 and 7.
RESULTS: Washing at 2 or 6 h postdose reduced testosterone bioavailability slightly (10-14% decrease in AUC_{0-24}; no effect on C_{max}). Washing at 10 h postdose had no effect on AUC_{0-24} or C_{max}. Mean time-averaged testosterone concentrations (C_{av}) were within the eugonadal range (300-1000 ng/dL) on Days 6 and 7 for all treatments. The amount of testosterone recovered from skin stripping samples decreased by 84.0%, 87.2%, and 81.3% after postdose application site washing at 2, 6, and 10 h, respectively, compared to no postdose washing.
CONCLUSION: Washing at 2, 6, or 10 h after 1.62% T-gel application has a small, clinically irrelevant effect on systemic exposure. Thus, showering or bathing at least 2 h after gel application should have minimal impact on testosterone absorption, while reducing the amount of testosterone available on the skin by at least 80%, which may be important for lowering the potential for skin transfer of testosterone to others.

Sources of Research Support: Solvay Pharmaceuticals, Inc.

Disclosures: JM: Employee, Solvay Pharmaceuticals, Inc. MB: Researcher, Solvay Pharmaceuticals, Inc. SF: Employee, Solvay Pharmaceuticals, Inc. CM: Employee, Solvay Pharmaceuticals, Inc. SAT: Employee, Solvay Pharmaceuticals, Inc. JJB: Employee, Solvay Pharmaceuticals, Inc. TLZ: Employee, Solvay Pharmaceuticals, Inc.

P2-447

Single and Multiple Dose Pharmacokinetics of Testosterone in Hypogonadal Males after Application of 1.62% Testosterone Gel.

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OBJECTIVE: This study investigated the safety, single and multiple dose pharmacokinetics (PK), dose proportionality and accumulation of testosterone (T) after application of 5 dose levels of 1.62% testosterone gel (T-gel) in hypogonadal men. DESIGN: Fifty six hypogonadal men (serum total T<300ng/dL) were enrolled in an open-label, randomized, parallel group study. Subjects were dosed with either 1.25, 2.50, 3.75, 5.00 or 6.25g of 1.62% T-gel once daily on the shoulders/upper arms or abdomen for 14 days. Serial blood samples for measurement of testosterone PK were collected at baseline (Day -1), and following single (Day 1) and multiple dosing (Day 14). RESULTS: After a single dose of 1.62% T-gel, mean T concentrations increased above 300ng/dL within 8 hours. Mean time-averaged concentration (C_{av}) was within the eugonadal range for all doses on Day 1 and Day 14. Mean maximum serum concentration (C_{max}) was within the eugonadal range for all doses on Day 1, and for the 1.25-3.75g doses on Day 14.

Mean (SD) Testosterone PK Parameters

		T-Gel Dose (g)				
	Day	**1.25**	**2.50**	**3.75**	**5.00**	**6.25**
C_{av}(ng/dL)	1	318 (80)	389 (124)	373 (125)	521 (134)	471 (119)
	14	354 (137)	377 (183)	463 (117	624 (309)	623 (170)
C_{max}(ng/dL)	1	404 (104)	498 (162)	533 (210)	737 (229)	654 (194)
	14	463 (159)	503 (220)	721 (181)	1030 (340)	1020 (335)

C_{av}=mean time-averaged concentration; C_{max}=max serum concentration. Testosterone eugonadal range:300-1000ng/dL

At doses of 5.00 and 6.25g, there was a greater incidence of individual C_{max} values exceeding 1000ng/dL. Area under the serum concentration-time curve (AUC_{0-24}) and C_{max} increased with dose from 1.25 to 5.00g after single dosing and over the entire dose range of 1.25-6.25g after multiple dosing. Steady state was achieved by Day 2 for all treatments. No accumulation of testosterone was seen with the 1.25g and 2.50g doses after 14 days, and less than 2-fold accumulation was seen with the 3.75g to 6.25g doses. The 1.62% T-gel was considered safe and well-tolerated in this study. There were no adverse events (AEs) of severe intensity, serious AEs, or deaths during the study. CONCLUSIONS: These findings demonstrate that administration of a 1.62% T-gel over a dose range of 1.25-6.25g to hypogonadal males provided serum T concentrations within the eugonadal range. Additionally the 1.62% T-gel was considered safe and well-tolerated over the dose range and time period of the study.

Sources of Research Support: Solvay Pharmaceuticals, Inc.

Disclosures: JM: Employee, Solvay Pharmaceuticals, Inc. MB: Researcher, Solvay Pharmaceuticals, Inc. SF: Employee, Solvay Pharmaceuticals, Inc. CM: Employee, Solvay Pharmaceuticals, Inc. SAT: Employee, Solvay Pharmaceuticals, Inc. JJB: Employee, Solvay Pharmaceuticals, Inc. TLZ: Employee, Solvay Pharmaceuticals, Inc.

P2-448

Intramuscular Testosterone Undecanoate for Substitution in Male Hypogonadism – The Experience of 12.4 Years Elucidates Beneficial Effects on the Newly Defined Metabolic Syndrome and Reveals a High Degree of Safety.

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Background:
A reliable form of androgen substitution therapy in terms of favorable kinetics and tolerance as well as effective restoration of androgenicity is paramount for hypogonadal men. The intramuscular injection of the long-acting ester testosterone undecanoate (TU) offers a convenient modality for testosterone substitution.
Methods:
We report data from 227 patients (117 with primary, 79 with secondary hypogonadism and 31 with late-onset hypogonadism) aged 15 to 71 years (mean 38±12 years) receiving altogether 3692 intramuscular injections of 1000 mg of TU during a maximal treatment time of 12.4 years.
Results:
The medication was well tolerated and local irritation at the injection site was rare and moderate and did not exceed a duration of 3 days. Serum trough levels of testosterone were generally within the low normal range, indicating sufficient substitution. Individual dosing intervals ranged from 10 to 14 weeks. The proportion of men fulfilling the new joint consensus criteria of the International Diabetes Federation and the National Cholesterol Education Program for definition of the Metabolic Syndrome decreased from initially 86% to 45% (Chi-square for trend:$p<0.001$). Regarding the single components of this clinical entity, especially waist circumference decreased from 106.0±10.1 to 94.9±8.7 cm ($p<0.001$) within a time frame of 8 injections. Concentrations of lipoprotein subfractions, blood pressure and fasting glucose levels were positively influenced in a similarly significant manner. PSA concentrations did not exceed 4.0 µg/L, except for two measurements (each 5.5 µg/L) in cases of subsequently confirmed prostatitis. Hematocrit was significantly elevated under treatment but remained within the normal range, except for occasional measurements (maximal value 54.4%).
Conclusion:
Intramuscular injections of testosterone undecanoate represent a feasible, safe and well tolerated modality of androgen substitution in hypogonadal men of a wide age-range, substantiated by more than one decade of experience, facilitating a decrement of metabolic/cardiovascular risk factors.

Disclosures: FS: Employee, Bayer Schering Pharma. EN: Advisory Group Member, Bayer, Inc.
Nothing to Disclose: MZ

P2-449

IPASS: Data from an Ongoing Study of the Tolerability and Effectiveness of Injectable Testosterone Undecanoate for the Treatment of Male Hypogonadism in 937 Men.

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[1]Univ Clins Germany Muenster, Germany ; [2]Bayer Schering Pharma AG Berlin, Germany ; [3]Bayer Vital GmbH Leverkusen, Germany ; [4]Ctr for Diabetes and Endocrinology, Barnsley Hosp NHS Foundation Trust Barnsley, UK and [5]Sexual Med and Andrology, Univ of Florence Florence, Italy.

Background: To provide preliminary data from an ongoing study assessing the safety and effectiveness of injectable long-acting testosterone undecanoate (TU 1000mg) in hypogonadal men in daily clinical practice. This substance is typically injected intramuscularly every 10 to 14 weeks.
Methods: Interim results from an ongoing world-wide multi-center non-interventional, surveillance study assessing the safety and effectiveness of injectable long-acting TU in hypogonadal men in daily clinical practice aiming to involve up to 1.500 men are presented. Data from 937 men (aged 49.1 ± 13.9 years) receiving 3653 injections were available for the interim analysis. In a single patient, TU therapy was administered for typically 9–12 months. Patient data from 18 countries in Europe, Asia, Latin America and Australia were acquired. Parameters of erectile function, libido, vigour/vitality, mood and ability to concentrate were assessed by physician interview using items and five-point Likert scales from validated tools. Physical and circulatory parameters as well as hematocrit, PSA levels, glucose control and lipid profiles were recorded.
Results: Overall, scores of mental and sexual satisfaction (libido, vigour, overall mood, ability to concentrate) increased markedly (all $p<0.0001$), while mean waist circumference decreased significantly from 101 cm to 96 cm ($p<0.0001$). These results were stable upon stratification between men receiving previous androgen therapy and those who were not previously treated. The percentage of patients who reported “low” or “very low” levels of sexual desire/libido decreased from 62% at baseline to 11% after four TU injection intervals. PSA levels and hematocrit remained within the normal range, no case of prostate cancer was observed.
Conclusion: These interim results from an ongoing study of injectable long-acting TU in the already largest worldwide sample of hypogonadal men document that this form of therapy is well tolerated in daily clinical practice, extending previous findings in regard to safety and effectiveness. Clinically relevant and beneficial efficacy is documented especially regarding sexual functions and waist circumference.

Disclosures: AM: Employee, Bayer, Inc. JUH: Employee, Bayer, Inc.
Nothing to Disclose: MZ, THJ, MM

P2-450

Safety Study of Long-Acting Parenteral Testosterone Undecanoate over 42 Months.

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Objectives: To investigate the safety of the administration of long-acting parenteral testosterone undecanoate (TU) to hypogonadal, mainly elderly men.
Design and Methods: 122 men aged 34 – 69 years (mean ±SD = 59.5 ± 6.0), with baseline testosterone 5.9 – 12.1 nmol/L were treated with parenteral TU. These 122 patients were followed for 42 months.
Results: Plasma levels of testosterone rose from 9.3 ± 1.7 nmol/L to 18.7 ± 2.1 nmol/L reaching their maximum at 9 months, never exceeding reference values. Further results are presented in table 1. There was a slow but steady increase in prostate volume, not paralleled by an increase in serum prostate specific antigen (PSA) of similar magnitude. Serum PSA rose slightly over the first 24-36 months treatment, then values stabilized at levels of 5-10% higher than baseline to rise again after 42 months. PSA never exceeded 4 ng/mL. The residual volume of the bladder decreased over the first 24 months and then stabilized. The scores on the International Prostate Symptoms Score decreased over the first 24 months and then stabilized.
The hematocrit increased significantly and had reached its maximum values after 12 months. Over the 42 month study period, at any time point, nine patients had a hematocrit above 52%, the upper limit of normal. No specific measures were taken (dose reduction of testosterone, venipuncture). An elevated hematocrit was never found at two occasions in the same patient.
Conclusions: Over a period of 42 months testosterone treatment with TU appeared acceptably safe. There was an increase in prostate size and PSA but not in bother. Longer and larger scale studies are needed. Monitoring individual patients as recommended (Wang et al, 2009) is necessary.

Wang C et al, Eur J Endocrinol 2009: 55:121-30

Disclosures: FS: Employee, Bayer Schering Pharma. AAY: Speaker, Bayer Schering Pharma, Berlin, Germany, Pfizer Germany, Ferring Pharmaceuticals. LJG: Speaker, Bayer Schering Pharma.
Nothing to Disclose: AH

P2-451

Effect of Dietary Fat on the Testosterone (T) Pharmacokinetics (PK) of a New Oral Testosterone Undecanoate (TU) Formulation in Hypogonadal Men.

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Introduction: TU is a highly lipophilic, orally active T prodrug for the treatment of hypogonadism. Bioavailability of TU is affected by food and dietary fat content. This study investigates dietary fat effects on the PK of a new proprietary formulation of oral TU designed to improve bioavailability by enhancing intestinal lymphatic absorption.
Subject and Methods: In an open-label, 2-center, 5-way crossover study with washout periods of 4–10 days, a single oral dose of TU 300 mg was administered to 16 hypogonadal men with serum T level 7.1±0.88 nmol/L (mean±SEM). Subjects were randomized to receive TU fasting or 30 mins after ~800 calories meals with 10%, 20%, 30% or 50% of calories as fat. Serial blood samples were collected for 24 hrs after drug to determine serum T by LC-MS-MS.
Results: There was a dietary fat-dependent response in mean serum T average concentration (C_{Avg}), maximal concentration (C_{Max}) and area under the serum T curve (AUC) (Table). Using the 30% group as the reference diet, serum T PK parameters for the 20% group were bioequivalent; differences with the 50% group were relatively small (<30%). Serum T levels (C_{avg}) were within the reference range in 56.3%, 87.5%, 93.8%, 100% and 87.5% of subjects in fasting, 10%, 20%, 30% and 50% fat diets, respectively. TU at 300mg orally given across a broad range of diets (10-50% fat) gave increased absorption over fasting; serum T levels were in the normal range in >85% of men. Time to reach peak serum T levels (T_{max}) occurred 4-7 hrs in fed states and reached lower limit of the reference range by about 12 hrs; thus twice-daily dosing is necessary to achieve desirable 24 hr serum T.

24 hr serum T PK parameters of TU 300 mg administered fasting or with food (geometric mean [95% CI])

	Fasting	10% Fat	20% Fat	30% Fat	50% Fat
C_{Avg} (nmol/L) AUC/24	10.0 [4.4;22.6]	14.2 [6.6;28.1]	16.6 [10.0;34.7]	18.8 [12.9;30.6]	22.3 [13.5;41.6]
AUC (nmol*h/L)	240 [105;541]	342 [158;674]	399 [241;832]	451 [309;734]	536 [323;999]

Conclusions: The new TU formulation yielded excellent serum T levels and resulted in greater absorption of T than older formulations. Twice-daily administration of this formulation with food across a range of 10%-50% dietary fat will produce physiologic T levels in most hypogonadal men. Even in a fasted state, significant levels of serum T were observed. Further clinical testing of this TU formulation for T replacement is planned.

Sources of Research Support: Clarus Therapeutics, Inc.

Disclosures: RED: Founder, Clarus. SF: Clinical Researcher, Clarus. RS: Consultant, Clarus. CW: Consultant, Clarus.
Nothing to Disclose: AY, EA, MH, LH, AL, RB

P2-452

Testosterone Replacement in Older Men and Correlations to Serum Cytokines and Lipid Profiles.

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Background: Low serum testosterone has been correlated with increased inflammation and risk of cardiovascular disease. However, the reports of anti-inflammatory and cardiovascular effects of testosterone replacement have been inconsistent.
Methods: We analyzed serum from twenty-four older men with low-normal endogenous testosterone concentrations (70 ± 2 yrs; serum testosterone levels < 500 ng/dL) collected during a 5-month randomized double-blinded placebo controlled study to examine the efficacy of testosterone treatment in a continuous or monthly cyclic fashion. Subjects were dosed on a weekly basis receiving either continuous testosterone (100 mg testosterone-enanthate, IM injection) for the entire 5-month period (TE, n=8), monthly cyclic testosterone treatment (one month 100 mg testosterone-enanthate, IM injection alternating with one month placebo, MO, n=8), or placebo for the entire period (PL, n=8).
Results: Serum total testosterone increased in TE and following each T treatment in MO but not in PL ($P<0.01$). Serum cytokine concentrations remained within normal ranges, with no group differences over time. Serum triglycerides, total cholesterol, HDLC ratio, HDL, LDL, VLDL, and HDL/LDL ratio were similar in all groups at all times. At baseline, serum testosterone correlated inversely with IL-6, IL-10, Granulocyte-macrophage colony-stimulating factor, and age. During the 5 month treatment period, total testosterone correlated with PSA and hematocrit, and inversely with triglycerides, HDLC ratio, and VLDL ($P<0.05$).
Conclusions: While there were no significant changes in serum cytokines or chemistries over time, testosterone concentrations correlated highly with markers of cardiovascular health in serum of older men when followed over 5 months of treatment. Conversely, correlations between testosterone and cytokines disappeared during the treatment period. These data support similar reports by others and indicate that some effects of testosterone may be subtle and that within-subject changes can remain unnoticed in lab results during testosterone treatment periods of 5 months or less.

Sources of Research Support: NIH/NIA Grant # R01 AG022023 (RJ Urban). Studies were conducted on the General Clinical Research Center (GCRC) at The University of Texas Medical Branch, funded by grant M01 RR 00073 from the National Center for Research Resources, NIH, US Public Health Service and by The Claude D. Pepper Older Americans Independence Center, funded by grant P30 AG024832.

Nothing to Disclose: ELD, GLS, KMR, SLC, WJD, CRG, MS-M, RJU

P2-453

Durability of the Effects of Testosterone and Growth Hormone Supplementation in Older Community Dwelling Men: The HORMA Trial.

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Testosterone (T) and rhGH are potential function promoting treatments (Rx) for aging-associated loss of muscle mass and performance, but the durability of beneficial and adverse effects (AEs) after stopping Rx is unknown. **Methods**: We determined if beneficial anabolic effects and AEs of T and rhGH persist after stopping Rx. In this double-masked RCT, T gel (5g or 10g/day) and rhGH (0, 3, or 5ug/day) were administered to 122 men 65-90 years old for 16 wks; 112 completed Rx and 108 had follow-up 3-months after Rx was stopped (wk 28). **Results**: Wk 28 T and IGF-1 levels were similar to baseline. Total lean body mass (LBM), appendicular skeletal muscles mass (ASMM), 1-repetition maximum (1-RM) composite strength of upper and lower body muscles, and fat mass at wk 28 did not differ from baseline. For participants with LBM and ASMM gains > median wk17 changes, 39-45% of benefits in lean mass ($p<0.0001$) and 31-36% of 1-RM improvements ($p\leq0.05$) were retained at wk 28 (Table). Similarly, 44-48% of fat mass reductions persisted ($p<0.0001$). Durable benefits were associated with higher hormone levels at wk 16 and better life-style (diet & activity). Hct and PSA returned to baseline but lipid changes were more durable. Baseline Framingham 10-year cardiovascular disease (CVD) risks were low (13-14%), did not worsen during Rx, and improved by wk 28 ($p<0.0001$). The hypothalamic-pituitary-gonadal axis fully recovered. **Conclusions**: Substantial improvements in LBM, ASMM, fat mass and muscle strength were retained 3-months after stopping hormone supplementation in participants with greater than median changes during Rx. Metabolic and CVD risks were low. These data justify further studies of anabolic combinations in older individuals with functional limitations.

Changes at wk 28 if outcomes > median at wk 17

	Change (Δ) in Body Composition		Δ in 1-RM Composite Stength	
Median at wk 17	Δ at wk 17 kg	Δ at wk 28 kg	Δ at wk 17 %	Δ at wk 28 %
	if >median	if >median at wk 17	if >median	if >median at wk 17
Total LBM				
> 1.5kg (N=58)	3.19 (1.48)‡	1.43 (1.47)* 45%	30.2 (33.0)	9.2 (32.1)** 31%
ASMM				
> 0.8kg (N=60)	1.69 (0.80)	0.66 (0.91)* 39%	31.0 (32.4)	11.3 (35.6)†36%
Total fat				
≤-1.1kg (N=55)	-2.62 (1.50)	-1.15 (1.94)* 44%	N/A	N/A
Trunk fat				
≤-0.7kg (N=56)	-1.79 (0.94)	-0.86 (1.22)* 48%	N/A	N/A

‡ mean (SD); * $p<0.0001$; ** $p=0.05$; † $p=0.03$

Sources of Research Support: In part from the National Institutes of Health R01 AG18169 and local NCRR GCRC M0I RR00043 at USC, the U.S. Department of Agriculture (USDA) ARS Cooperative Agreement 58-1950-9-001, the NCRR GCRC grant M01 RR000054 at Tufts University, the Mass Spectrometry Research Resource at Washington University (NIH RR000954, DK020579, and DK056341), and NIH grants U01AG14369 and 1R01DK70534 at Boston Medical Center, Boston University School of Medicine. Study therapies were provided by Solvay Pharmaceuticals Inc, Genentech Inc, and Tap Pharmaceutical Products Inc; industry sponsors provided no monetary support.

Disclosures: FRS: Consultant, Merck & Co.; Committee Member, Pfizer, Inc. EFB: Investigator, Solvay Pharmaceuticals, Inc. RR: Chief Scientific Officer, Novartis Pharmaceuticals.
Nothing to Disclose: SB, JH, KEY, CC-S, ETS, MK, MD, CM, CH, YS, SPA

P2-454
Gonadal Status and Physical Performance in Older Men.

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Male aging is characterized by a decline in testosterone and physical performance. Low testosterone is one of potential factors facilitating the development of disability. Many consensus were developed to define hypogonadism. However, no study has fully investigated the relationship between gonadal status and determinants of physical performance in older men. **Methods.** 410 65 yr and older men of InCHIANTI study (Tuscany, Italy) with complete data on testosterone, muscle strength, cross-sectional muscle area (CSMA), mini mental state examination (MMSE), short physical performance battery (SPPB), and depressive status. Testosterone was assayed using RIA with MDC of 0.86 ng/dL.CSMA was evaluated by using a recent peripheral quantitative computerized tomography. Global cognitive performance was assessed with MMSE. Handgrip strength was measured by dynamometer. Depression was evaluated by the 20-item CES-D. SPPB was used to summarize physical performance. Generalized linear models were used to test the relationship between gonadal status and determinants of physical performance in Model 1 (adjusted for age and BMI), and Model 2 (Model 1 plus IL-6, physical activity, HOMA, chronic disease).
Results. According to serum levels of total testosterone, 3 different groups of men were created: 1) severely hypogonadal (N=20) with testosterone <230 ng /dl; 2) moderately hypogonadal (N=75) (testosterone >230 and <350 ng/dL), and 3) eugonadal (N=297) with testosterone > 350 ng/dL. Mean age was 82.0± 8.4 years in the severe hypogonadal group, 76.1±7.4 in the moderately hypogonadal group; and 73.8 ± 6.3 in the eugonadal group (p for trend<0.001). BMI was 26.6 ± 3.4 (mean±SD) in eugonadal group, 26.9 ± 3.4 in moderately hypogonadal group and 27.5± 3.2, in severely hypogonadal group (p for trend=0.39). In age and BMI adjusted analysis (Model 1), a significant difference in hemoglobin levels, MMSE score, hand grip strength and SPPB score (p for trend<0.001) was observed among 3 groups, with severely hypogonadal men having lower hemoglobin, MMSE, muscle strength and physical performance. No significant trend was found for CES-D score, muscle mass and 4 meter walking speed. In Model 2 the trend was still significant for hand grip strength (p for trend=0.004) and haemoglobin levels (p for trend<0.0001) but not for other measures.
Conclusions. In older men, gonadal status is independently associated with some determinants (haemoglobin and muscle strength) of physical performance.

Sources of Research Support: National Institute on Aging Contracts N01-AG-916413, N01-AG-821336, N01-AG-5-0002, and NIA Grant R01 AG027012. This research was supported in part by the Intramural Research Program, National Institute on Aging, NIH.

Nothing to Disclose: MM, FL, ML, AV, CC, FA, SB, SB, RV, JMG, LF, GV, SP, GC

P2-455

Testosterone Therapy Improves Body Composition and Metabolic Parameters in Obese Aging Men: Results of a Placebo-Controlled Randomized Controlled Trial.

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Background
Testosterone (T) therapy decreases total body fat and increases skeletal muscle in older men (1), and prevents visceral fat accumulation in non-obese aging men (2). The magnitude of T effect is influenced by baseline adiposity and serum T, yet detailed studies in obese cohorts are lacking, and data about T effects on visceral and subcutaneous (sc) adipose tissue are conflicting.
Methods
Obese men aged 40-70 yrs with T levels <15nM (4.5ng/mL), BMI 30-40kg/m2 and waist circumference (WC)>102cm participated in a double blind RCT of T undecanoate (Nebido® 1000mg IM at wks 0, 6, 16, 26, 36, 46) or placebo (P); all had ongoing weight loss advice (diet/exercise). Body composition (DEXA, CT), glucose, insulin, HOMA index and sleep studies were measured at wks 0 and 52. Serum T (mass spectroscopy), lipids, PSA, Hb and hematocrit were measured throughout.
Results
40 men aged 53.3±7.4yrs (mean±SD) entered and 35 completed the study (T n=19, P n=16). At wk 0 the groups had similar BMI (T 33.6±2.4 vs. P 33.7±2.1kg/m2) and WC (113.1±6.2 vs. 113.7±7.2cm). Serum T increased with T undecanoate (3.3±1.0 to 6.6±3.0ng/mL; P=0.0001) and was unchanged with P. Total body weight, BMI and WC did not change. Total body fat (36.8±4.7 to 33.0±5.7kg; P<0.0001) and percent fat mass (35.0±0.6 to 31.3±0.7%; P<0.0001) fell with T and were unchanged with P. On cross-sectional CT sc abdominal (40.6±1.6 to 36.8±1.8cm2; P=0.0003) and thigh (19.9±1.8 to 17.0±1.4cm2; P=0.003) fat decreased with T; visceral fat did not change. Fat free mass (68.5±1.3 to 71.8±1.5kg; P<0.0001) and skeletal muscle mass (33.8±0.7 to 35.7±0.8kg; P<0.0001) increased with T and were unchanged with P. Total (5.4±0.2 to 4.9±0.2mmol/L; P=0.009) and LDL (3.7±0.2 to 3.2±0.2mmol/L; P=0.01) cholesterol fell with T but TG, HDL-cholesterol, glucose, insulin and HOMA index did not change with either treatment. Hb (14.8±2.4 to 16.3±2.4g/dL; P<0.0001) and hematocrit (43.0±0.7 to 47.2±0.7%; P<0.0001) increased with T; PSA and sleep parameters were not affected by treatment. There were no treatment-related withdrawals.
Conclusion
Compared to lifestyle modification advice, 12-months of T therapy in obese aging men reduced total body fat by 10% and increased skeletal muscle mass by 6%. Subcutaneous abdominal fat decreased by 9% but visceral fat was unchanged. T reduced total and LDL-cholesterol but did not influence TG, glucose or insulin. These initial favorable results require confirmation in larger studies of longer duration.

1.Page S et al., JCEM 2005;90:1502
2.Allan C et al., JCEM 2008;93:139

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Nothing to Disclose: CAA, BJGS, EAF, RIM

P2-456

Hypogonadal Patients Receiving Testosterone Treatment for up to 5 Years Are Not at Higher Risk of Prostate Cancer Compared to Age-Matched Controls.

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Background: The risk of prostate cancer is still the major concern when treating hypogonadal men with testosterone.
Methods: 154 testosterone deficient patients (average age 58 ± 1.7 years and mean follow-up of 38 months, range: 18-59 months), receiving injectable TU 1000 mg (Nebido®, Bayer-Schering, Berlin, Germany) 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). TRUS-guided biopsies were performed when indicated by PSA velocity > 0.75 mg/L, or elevation over 4.0 mg/L.
Results: At baseline, hypogonadal patients showed lower PSA values and lower prostate volumes (0.68 ± 0.4 mg/L and 25.6 ± 1.4 ml, respectively). Subjects in the control group had PSA levels of 2.42 ± 1.2 mg /L, and prostate volume 38.4 ± 2.42 ml at baseline. Hypogonadal patients whose PSA velocity in the observation period was > 0,75 mg /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 a significantly higher Gleason score of 3+3 till 4+5 and up to 80% tumor cells in a core. In all subjects in both groups, no abnormalities in rectal palpation were noticed.
Conclusions:
1) Subjects with T-deficiency have lower prostate volumes and PSA levels than eugonadal ones.
2) Testosterone therapy does not increase CaP incidence.
3) The group on testosterone treatment had smaller tumors and less malignancy (better differentiation).

Disclosures: AAY: Speaker, Bayer Schering Pharma, Berlin, Germany, Pfizer Germany, Ferring Pharmaceuticals. FS: Employee, Bayer Schering Pharma.
Nothing to Disclose: AH

P2-457

A Liquid Chromatography Tandem Mass Spectrometry-Based Method for Analysis of Total Testosterone in Human Serum.

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Measurement of hormone levels such as testosterone can be used to help assess various disease states in men, women, and children. Steroids are usually present in blood at low concentrations so methods are needed that provide rapid and reliable analysis at high sensitivity. Traditional methods of analysis, such as immunoassays, can suffer from poor accuracy and reproducibility.
This poster presents the results from an inter-laboratory collaboration where we provide data from an easy to implement liquid chromatography tandem mass spectrometry method for measuring total testosterone in serum. The samples were provided from a well characterized data set derived from a mixed population, which represent a wide clinical range of testosterone concentrations. The goal of this initiative is to improve diagnosis, treatment, and prevention of diseases and disorders through standardization of total testosterone measurement in serum.
Typical assay performance for 200 ul of serum provides LOQ of 0.01 ng/ml <10% RSD with a linear range exceeding 2 orders of magnitude. Results are consistent for what is required for a clinical assay that measures total testosterone in serum.

Nothing to Disclose: KG, LS, JM

P2-458

Total Testosterone (TT) Measurement Is a Good Screening Test for Male Hypogonadism, but Should Not Be Used To Make a Definitive Biochemical Diagnosis.

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Background : The 2006 Endocrine Society guidelines suggest that the best initial evaluation for a biochemical diagnosis of hypogonadism is the measurement of morning TT level and confirmation “by repeating the measurement and in some patients by measurement of free or bioavailable T”.[1] However, it is unclear how well TT levels predicts free T levels. We determined the sensitivity and specificity of low TT levels for predicting low calculated free T levels in a cohort of 3672 men who were evaluated for hypogonadism at the VA Puget Sound during 1997-2007.
Methods : TT and SHBG levels were determined using an immunoassay, and the free T levels were calculated using the Vermuelen formula. In a similar group of veterans, the calculated free T levels from this assay correlated well with results from equilibrium dialysis followed by liquid tandem chromatography-mass spectroscopy (R2 = 0.93).[2] We generated a receiver operator curve (ROC) to determine the sensitivity and specificity of low TT for predicting low calculated free T levels (less 3.4 ng/dl), the clinical gold standard for biochemical hypogonadism.
Results :
TT levels performed well as a predictor of free T levels (area under ROC = 0.93). Sensitivity for low TT was fair, but specificity was poor except at very low TT (table).

Sensitivity and specificity of low TT for low calculated free T

Threshold TT (ng/dl)	Sensitivity	Specificity
<100	33.9%	99.9%
<150	58.8%	98.9%
<200	76.5%	92.6%
<250	87.6%	81.9%
<280*	91.0%	73.7%
<300	93.0%	67.9%
<350	96.8%	53.5%
<400	98.2%	41.0%
<450	98.8%	31.3%
<500	99.3%	23.1%

*Lower limit of normal for TT = 280 ng/dl

Summary : 1) TT < 280 ng/dl is fairly sensitive but nonspecific for predicting low calculated free T; 2) Specificity is low until TT is < 150 ng/dl; 3) TT > 450 or 500 ng/dl excludes low calculated free T in virtually all men.
Conclusions : In most clinical settings, TT > 450 ng/dl excludes male hypogonadism, but low TT lacks specificity for the biochemical diagnosis of male hypogonadism.

1. Bhasin S, et al., J Clin Endocrinol Metab 2006; 91:1995-2010
2. DeVan ML, et al., Clin Chem 2008;129:459-463

Disclosures: LH: Clinical Researcher, GlaxoSmithKline, Solvay Pharmaceuticals, Inc.
Nothing to Disclose: BDA, JMH, TJW, AS, AMM

P2-459

Magnitude and Prevalence of Altered Gonadotropic Function in a Male Veteran Population.

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Advancing age is associated with a number of acute and chronic disorders which independently or jointly with medications could accentuate age-related decline in gonadal function. In this study blood samples collected from 1230 ambulatory men (age range: 20-90 yrs) during their routine clinic appointments were used for the measurements of total testosterone (ng/dL), FSH (mIU/mL), LH (mIU/mL), E2 (ng/dL), prolactin (ng/mL), SHBG, and albumin. Free and bioavailable testosterone (cFTe/cBioTe) and E2 (cFE2/cBioE2) were calculated by using total testosterone, E2, SHBG, and albumin. Results were significant for decreased cFTe (<5 ng/dL) in a total of 252 (20.5%) with cFTe <1 in 32, 1-1.99 in 13, 2-2.99 in 39, 3-3.99 in 43 and 4-4.99 in 126 men. Low cFTe was associated with increased LH (>8.9) in 59 (4.8%), and more commonly normal LH in 193 men (15.7%). Selective increases in serum FSH (>18), and prolactin (> 14.6) concentrations were identified respectively in 26 (2.1%) and 70 (5.7%) subjects. Correlation analysis in 768 men with within-the-normal range circulating cFTe, LH and FSH, and prolactin concentrations revealed significant positive correlation (R:P values) of age with FSH (0.27:<0.0001), LH (0.25:<0.0001, and SHBG (0.25:<0.0001). While total testosterone and cFE2 did not correlate with age, there was a significant negative correlation of age with cFTe (-0.3:<0.0001) and cBioTe (-0.35: <0.0001). Contrary to the age influence, increasing BMI had a negative impact on LH (-0.15:<0.0001), and FSH (-0.12:0.001), associated with decreased cFTe (-0.1:0.008), cBioTe (-0.09:0.02), and SHBG (-0.34:<0.0001). Alternatively, total E2 (0.14:0.0003), cFE2 (0.21:<0.0001), and cBioE2 (0.2:<0.0001) were positively correlated with BMI. In conclusion, aging in men for the most part is characterized by within-the-normal range increments in gonadotropins, and corresponding decrements in the free and bioavailable testosterone. This finding is consistent with age-related gradual decline in testicular function, which infrequently becomes clinically relevant with markedly increased serum LH concentrations. The more common occurrence of low testosterone associated with relatively low gondotropins (16%) suggests a central mechanism, most probably exacerbated by excess weight, acute and chronic illnesses, and/or medications to include narcotics.

Sources of Research Support: Salem V.A. Medical Center Research Institute.

Nothing to Disclose: AV, BD, DL, JDV, AI

P2-460

The “Fertile Eunuch Syndrome”: Genetics and Natural History.

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Background: Isolated GnRH Deficiency presents as either normosmic Idiopathic Hypogonadotropic Hypogonadism (nIHH) or with anosmia, Kallman Syndrome (KS) and has a broad clinical spectrum, ranging from severe (microphallus, cryptorchidism & complete absence of puberty) to the “fertile eunuch” variant, a milder form with normal testicular volume, active spermatogenesis, yet undervirilization and hypogonadal serum testosterone (T) levels. Here, we characterize the genetics, clinical heterogeneity and the course of the largest series of fertile eunuchs described to date.
Methods 772 men with isolated GnRH deficiency were screened to identify subjects with: (i) Sperm in their ejaculate and/or testicular volume ≥ 12 ml, ii) without previous gonadotropin therapy. Subjects underwent detailed clinical phenotyping and baseline neuroendocrine profiling (q10 x 12^{o} overnight sampling for LH). Genetic screening for mutations in *KAL1, GNRHR, FGF8, FGFR1, KISS1R, PROK2, PROKR2* and *NELF* was performed. Subjects were re-evaluated with neuroendocrine studies and/or serial serum T levels (off treatment) to assess for reversal.
Results: Twenty-three men (12 nIHH; 11 KS) were fertile eunuchs. At diagnosis, their mean age was 24 ± 6 yrs; serum T: 76 ± 9 ng/dL; and testicular volume 12 ± 2 ml. Of 16 subjects with baseline neuroendocrine studies, nine (56%) showed an apulsatile LH secretion pattern whilst the rest showed pulsatile LH secretion. The pulsatile group showed higher mean serum T, LH and FSH levels **(Table I)**. Mean inhibin B levels of the cohort was normal (179 ± 52 pg/ml). Of 12 subjects who were re-evaluated, 8 showed reversal of GnRH deficiency (sustained serum T > 270 ng/dL). Four subjects harbored mutations in known IHH genes (*GNRHR* [n=3] and *FGFR1* [n=1]). Interestingly, reversal of GnRH deficiency occurred in two of these subjects.
Conclusions: 1.The fertile eunuch variant occurs in a small subset (3%) of GnRH deficient men. 2. This subset shows a high frequency of reversal (66%) compared to 10% of all men with IHH[1]. 3. Men with a fertile eunuch presentation should be serially assessed for reversal. 4. Reversibility in the setting of deleterious mutations suggests involvement of non-genetic factors.

Biochemical characteristics: pulsatile vs.apulsatile LH secretion groups

	Pulsatile group (n=7)	Apulsatile (n=9)	p value
Serum T(ng/dL)	100 ± 15	65 ± 11	0.04
LH (IU/L)	6 ± 1	2 ± 0.5	<0.01
FSH (IU/L)	4 ± 1	2 ± 0.5	0.06

1. Raivio T et al, Reversal of idiopathic hypogonadotropic hypogonadism. N Engl J Med. 2007 Aug 30;357(9):863-73.

Sources of Research Support: NICHD Grant 5U54HD028138-19.

Nothing to Disclose: RB, XH, AAD, GPS, SBS, NP, WFC

P2-461

Mechanisms of Androgen Receptor-Modulated Negative Feedback on the Gonadal Axis in Healthy Men.

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[1]Mayo Clin Rochester, MN ; [2]Univ of Virginia Charlottesville, VA and [3]Univ of Sydney Sydney, Australia.

Context. Testosterone (T) exerts negative feedback on the hypothalamo-pituitary (GnRH-LH) unit, but the mechanisms mediating feedback are not established.

Site. Center for Translational Science Activities.

Subjects. Twenty-four healthy men ages 20-73 yr, BMI 21-32 kg/m^2.

Design. Prospective, placebo-controlled, randomized, double-blind crossover study.

Methods. Placebo, flutamide (CNS-permeant antiandrogen) and bicalutamide (CNS-impermeant antiandrogen) were administered for 4 days.

Rationale. Incrementally greater LH responses to flutamide than bicalutamide were assumed to reflect involvement of CNS more than pituitary/peripheral androgen receptor- (AR) dependent pathways.

Endpoints. Pulsatile, basal and entropic (pattern-regularity) measures of LH secretion.

Results. Flutamide but not bicalutamide: (a) elevated the ratio of mean LH/mean T concentrations ($P = 0.013$); (b) augmented LH pulse frequency ($P = 0.011$); (c) diminished the mass of LH secreted per burst ($P = 0.001$); (d) potentiated the age-related abbreviation of LH-secretory bursts ($P = 0.009$); (e) suppressed exogenous GnRH-induced LH release ($P = 0.015$); and (f) decreased the regularity of GnRH-stimulated LH release ($P = 0.012$): **Figure 1**. Furthermore, the effect of flutamide exceeded that of bicalutamide in reducing LH secretory-pattern regularity ($P < 0.001$) and in amplifying total (basal plus pulsatile) LH secretion ($P = 0.002$), basal LH secretion ($P = 0.010$), and mean LH and T concentrations ($P < 0.001$): **Figure 2.** BMI reduced basal LH secretion.

Conclusion. The LH secretory phenotype induced by flutamide closely resembles that of aging per se, raising the possibility that aging is associated with impoverished T feedback on CNS sites.
Figure 1:

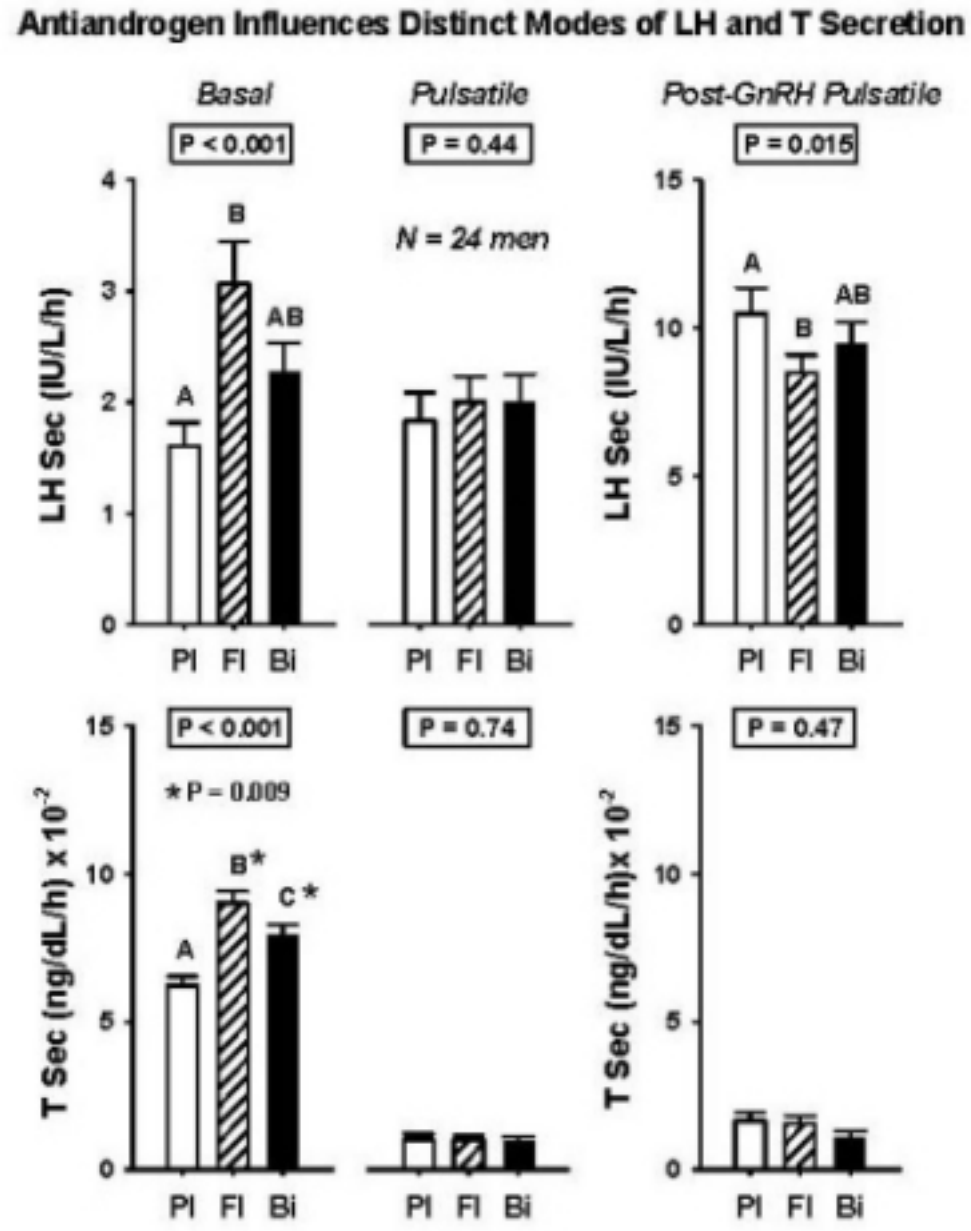
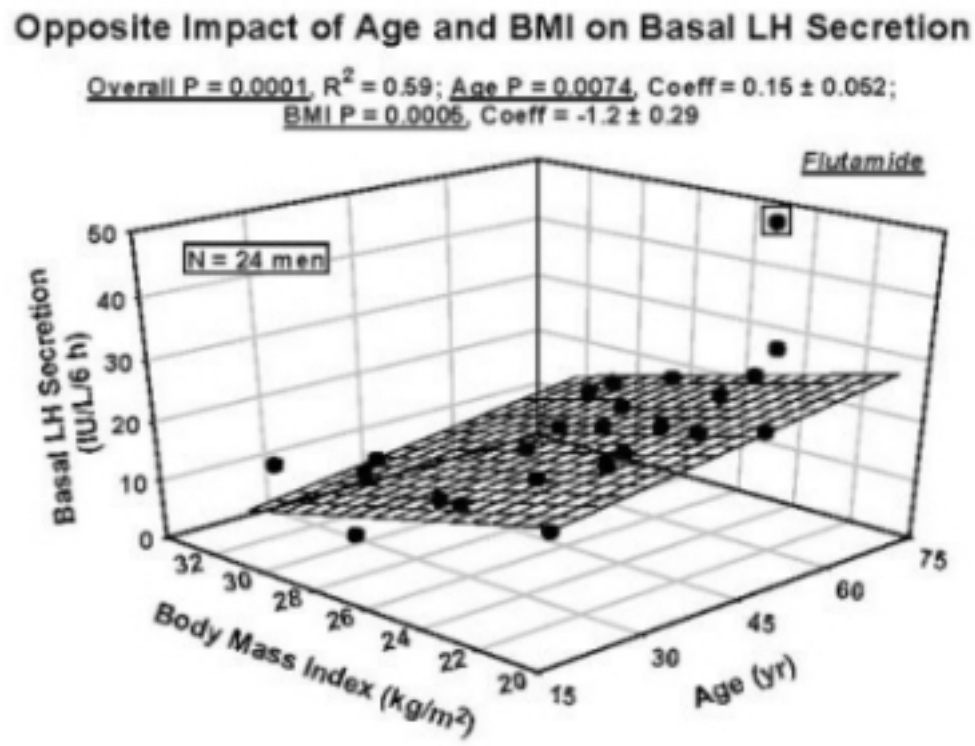

Antiandrogen Influences Distinct Modes of LH and T Secretion
Basal
Pulsatile
Post-GnRH Pulsatile
P < 0.001
P = 0.44
P = 0.015
N = 24 men
LH Sec (IU/L/h)
A
B
AB
Pl Fl Bi
P < 0.001
P = 0.74
P = 0.47
* P = 0.009
T Sec (ng/dL/h) x 10-2
B*
C*

Figure 2:



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P2-462

Male Functional Hypogonadotropic Hypogonadism (MFHH): A Distinct Clinical Entity?.

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Background: There is a tight link between reproduction and metabolism across species. In women, energy deficit (i.e. excessive exercise/weight loss) leads to a functional inhibition of the reproductive axis, termed as Hypothalamic Amenorrhea (HA) (1-3). Whether a similar clinical condition exists in men is unclear.
Methods: We studied 7 men presenting with symptoms of hypogonadism and $\geq$1 factors known to predispose to HA; i.e. excessive exercise (n=4), weight loss (n=3), psychological stress (n=3). Subjects discontinued treatment and underwent detailed genotyping and phenotyping, including measurements of reproductive and metabolic hormones, an overnight frequent sampling study of LH, and DEXA scan for body composition. 35 age-matched healthy adults served as controls.
Results: All 7 men had spontaneous and full pubertal development, evidenced by a growth spurt, normal testicular size (22 ± 2 ml) and Tanner V pubic hair. Yet, 1 subject reported a history of delayed puberty and 4 had eunuchoidal proportions. All 7 men had Hypogonadotropic Hypogonadism (HH) (serum T - mean ± S.D.: 168 ± 45 vs. 534 ± 130 ng/dL, $p < 0.001$, serum LH: 7.2 ± 1.5 vs. 10 ± 2.7 IU/L, $p < 0.05$). 6 subjects had pulsatile LH pattern while 1 was apulsatile. All men had lower BMI (20.7 ± 2.5 vs. 25 ± 3.5 kg/m^2, $p < 0.01$) and lower body fat mass (9.8 ± 2.4 vs 17.6 ± 7.2%, $p < 0.01$) (4). Notably, upon reassessment, 2 subjects demonstrated a sustained adult serum T level (> 270 ng/dL) after resolution of predisposing factors. No subjects had mutations in known loci of GnRH deficiency.

Patients with MFHH vs controls

	MFHH (n = 7) (Mean ± SD)	Healthy Men (n = 35) (Mean ± SD)	P value
Age (years)	23 ± 5.7	26 ± 3.5	0.071
BMI (Kg/m ^{2})	20.7 ± 2.5	24.9 ± 3.5	< 0.01*
Testicular Volume (ml)	21.8 ± 1.9	22.8 ± 3.2	0.425
Testosterone (ng/dL)	167.7 ± 45.1	534.0 ± 130.4	<0.001*
Estradiol (pg/ml)	12.4 ± 2.3	37.5 ± 8.7	<0.001*
LH (IU/L)	7.2 ± 1.5	9.9 ± 2.7	<0.05*
Pulse Freq (#/12 hrs)	4.4 ± 2.5	5.1 ± 1.8	0.404
LH Amplitude (IU/L)	5.6 ± 1.5	7.2 ± 2.5	0.143
FSH (IU/L)	5.0 ± 2.1	5.3 ± 1.9	0.723

* = Statistically significant

Conclusion: In men, HH can occur in the setting of energy deficits and psychological stress. We propose that this presentation represents a distinct clinical entity akin to HA. A follow-up study is underway to assess reversibility and to establish the clinical diagnosis of MFHH.

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Nothing to Disclose: NRC, AAD, PWB, MTC, GPS, KWK, SBS, LP, WFC, NP

P2-463

Decreased Gonadotropin Secretion in Male Hypogonadism Due to Sickle Cell Anemia (SCD-SS).

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Background: Sickle Cell Anemia affects over 70,000 patients in the US; most of them are African Americans. Hypogonadism is thought to occur in 5 to 12% of SCD-SS patients; however, this needs to be reassessed in view of improved management and life expectancy of SCD-SS patients.
Methods: We performed a pilot study and randomly sampled 14 adult male SCD-SS patients, aged 18-50 years, for serum testosterone, LH and FSH and classified into hypogonadal (HG, serum testosterone<250 ng/dL) and normogonadal (NG, serum testosterone ≥250 ng/dL) groups.
Results: Mean testosterone levels were 125.2 ± 65ng/dL in the HG group (n=6) and 587.5 ± 175 ng/dL (p<0.001) in the NG group (n=8). Serum LH levels were significantly lower in the HG than in the NG group (2.8 ± 1.4 vs. 5.1 ± 1.2 mIU/mL; p= 0.011). Serum FSH levels were decreased in the HG group (2.9 ± 2.1 vs. 5.7 ± 2.1 mIU/mL), however, the difference was at the limit of significance (p=0.050). The mean hemoglobin in the HG group was significantly lower than that in the NG group (8.6 ± 0.7 vs. 9.9 ± 0.7 gm/dL, p=0.034). Serum ferritin and total bilirubin were not significantly different. Interestingly, the HG group was significantly, 9 years older than the NG group (30.7 ± 5.3 years, range 20-38 years vs. 21.5 ± 3.8 years, range 18-31, p=0.006) and had a significantly higher body mass index (BMI) than the NG group (26.2 ± 3.9 vs. 20.7 ± 1.2 kg/m2; p=0.008).
Conclusion: The pilot study data indicate that hypogonadism in male SCD-SS patients is more common than reported previously and it likely results from hypothalamic and/or pituitary dysfunction. Hypogonadism seems to occur in older patients with more severe anemia. This indicates that development of hypogonadism is related to the severity and the duration of SCD. Since serum ferritin, an indirect marker for total body iron stores, was not different between the 2 groups, iron overload is not likely the cause of the gonadal dysfunction. However, such a conclusion should be tempered by the fact that cross-sectional ferritin levels do not reflect past history iron overload from repeated transfusions and does not exclude iron deposits in the hypothalamus and/or the pituitary. The higher BMI of the HG group could be a consequence of hypogonadism. Identifying and treating hypogonadism in SCD may improve quality of life and prevent morbidity from potential sequelae of testosterone deficiency.

Nothing to Disclose: PK, JWC, PS, ABA-S

P2-464

Diagnosis of Hypogonadism in Obese Males: More Complex Than We Thought?.

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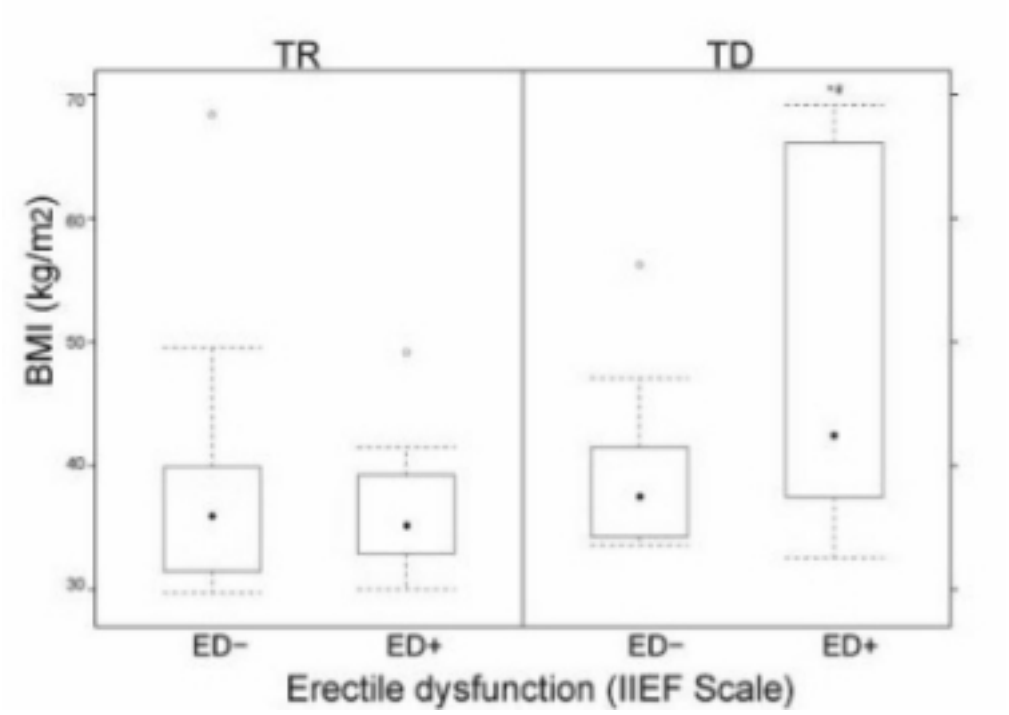
Obese men have “low normal” testosterone. The precision issues of immunoassay (IA) at low concentrations may also extend to this population. This study aims to (a) investigate the accuracy of an automated IA platform in diagnosing low testosterone concentrations in a group of adult obese men using isotope-dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) as a reference and (b) to examine the interaction between obesity, total testosterone concentrations (TT) and the presence of clinical symptoms.

We collected 324 early morning serum samples from 99 obese healthy men (mean BMI 37.5$\pm$0.75kg/m^2; mean TT 15.4 $\pm$0.31 (IA) vs 12.3$\pm$0.22nmol/L (LC-MS/MS)). Despite applying recognised reference ranges, IA demonstrated inadequate sensitivity (81.0%) for detecting low testosterone in obese healthy men when compared to LC-MS/MS. Men were significantly more obese only if symptoms and low testosterone were both present ($p<0.001$). In testosterone replete subjects, obesity did not contribute significantly to symptoms. Obesity also did not influence testosterone concentrations in the absence of symptoms. 62.5% of those who had testosterone deficiency were asymptomatic and this group was less obese. Similar results were found between symptoms of androgen deficiency, testosterone concentration and obesity.

Anthropometric Characteristics

		Testosterone Deficiency			
		Replete (TR)		Deficient (TD)	
Symptoms of	Absent	ED-/TR (n=52)		ED-/TD (n=10)	
Erectile Dysfunction	(ED-)	BMI	36.6(±0.93)	BMI	39.7(±2.28)
(IIEF)		Weight	115.4(±2.96)	Weight	122.3(±8.12)
		WC	122.1(±2.20)	WC	130.4(±5.50)
	Present	ED+/TR (n=26)		ED+/TD (n=6)	
	(ED+)	BMI	35.9(±0.85)	BMI*	48.4(±6.34)
		Weight	111.9(±2.86)	Weight*	139.5(±17.24)
		WC	121.8(±2.23)	WC*	143.1(±10.32)

Participants categorized into groups by presence/absence of symptoms of erectile dysfunction (ED+/ED-) and testosterone deficiency (TR/TD). Results in mean(±SEM). *significantly different from ED-/TR ($p<0.001$) and ED+/TR ($p<0.01$).



The lack of correspondence between symptoms and testosterone concentration suggest that further research to improve the definition of hypogonadism in obese men is needed.

Sources of Research Support: National Health and Medical Research Council Medical Postgraduate Research Scholarship, Princess Alexandra Hospital Research Foundation Grant.

Disclosures: PYL: Research Funding, Bayer, Inc.
Nothing to Disclose: CRO, MEF, PJT, JPG, PJB, LKP, TMO-S, JBP

P2-465

Evaluation of Total and Bioavailable Testosterone Levels in Patients with Different Metabolic Disorders.

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Patients with type 2 diabetes(DM2) have lower testosterone(T) levels and higher prevalence of hypogonadism. There is an association between insulin resistance(IR), obesity, metabolic syndrome(MS) and low T levels.
Aims: 1)evaluate total(TT) and bioavailable T(BT) levels in patients with DM2 and subjects without DM2; 2)evaluate TT and BT levels in patients with DM2, MS, IR and normal subjects and 3)assess levels of TT and BT according to DM2 metabolic control.
Methods: We included 67 patients with DM2 and 92 non-diabetic subjects. Physical exam: weight, height, body mass index(BMI)(kg/m^2) and waist circumference(W)(cm). In patients without DM2 oral glucose tolerance test was performed. Lab: TT, BT, glucose, insulin, glycated hemoglobin(A1C), HDL cholesterol and triglycerides. IR was assessed with HOMA(IR=HOMA>3). SM was defined according to ATPIII criteria. Patients in the non DM2 group included: 44 with MS, 24 with IR and 24 normal.
Results: There were no differences between patients with DM2 and subjects without DM2 in age, BMI, W, and TT. Patients with DM2 had lower levels of BT than subjects without DM2: 1.47±0.5 and 1.83±0.6 ng/mL,p=0.004. Normal subjects had higher values of TT and BT compared with patients with DM2, MS and IR. Patients with DM2 showed lower levels of BT compared with subjects with MS and IR(Table).

TT and BT values in patients with DM2, MS, IR and normal.

	DM2(n:67)	MS(n:44)	IR(n:24)	Normal(n:24)
TT(ng/mL)	4.2±1.1(*)	4.1±1.2(&)	4.2±0.9(#)	5.7±2.1(*,$,&,#)
BT(ng/mL)	1.47±0.5(+,**,^)	1.75±0.6(+,++)	1.76±0.5(**,%)	2.09±0.6(^,++,%)

(*)p=0.004;(&)p=0.002;(#)p=0.004;(+)p=0.01;(**)p=0.01;(^)p<0.0001;(++)p=0.04;(%)p=0.02.

We found a negative correlation between: TT and W: r:-0.39,p<0.0001; TT and BMI: r:-0.40,p<0.0001; BT and age: r:-0.32,p<0.0001; BT and W: r:-0.39,p<0.0001; BT and BMI: r:-0.29,p=0.0001. In DM2 patients there was no correlation between TT or BT with A1C or glucose. DM2 patients with good metabolic control(A1C≤7%)(n:41) had higher levels of BT compared with patiens with A1C>7%(n:26): 1.56±0.5 and 1.31±0.4 ng/mL,p=0.04. There was no difference in TT levels. Conclusions: Patients with DM2 have lower levels of BT, and BT values are higher with a better metabolic control. Normal subjects have higher levels of TT and BT compared to patients with IR, MS and DM2. We found a negative correlation between TT and BT with BMI and W, suggesting that obesity and IR play a role in male gonadal axis regulation.

Nothing to Disclose: PK, PRC, SMS, AA, AK, MB, LL

P2-466

Prevalence and Characterization of Hypogonadism among Men with Human Immunodeficiency Virus Infection: Preliminary Results.

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Introduction: Among various comorbidities of Human Immunodeficiency Virus-1 (HIV-1) infection male hypogonadism is very frequent with a prevalence of 19% in patients treated with highly active anti-retroviral therapy (HAART). Literature data are lacking and achieved by studies with less than 300 subjects each.
Aim of the study: Prevalence and clinical characterization of hypogonadism among a large number of men with Human Immunodeficiency Virus Infection.
Methods: Endocrinological examination and hormonal screening including serum total testosterone, LH and FSH assays have been performed in 950 outpatients aged 20-69 years (mean age 45.5 years) at the Metabolic Clinic of Infectious and Tropical Disease between 2005 and 2009.
Results: Mean serum total testosterone was 470.9±205.5 ng/dl. Considering Endocrine Society threshold for hypogonadism diagnosis, 15.7% of our patients was hypogonadic (T<300ng/dl); reffering to LH levels 8% of them was hypogonadotropic, 77.2% was normogonadotropic and 14.8% was hypergonadotropic. According to threshold proposed by International Society for the Study of the Aging Male (ISSAM) 23.7% of subjects results hypogonadic (T<346ng/dl) of which 5.8% was hypogonadotropic, 80% normogonadotropic and 14.2% hypergonadotropic.

Table 1

	Endocrine Society (T<300ng/dl)	ISSAM (T<346ng/dl)
Percentage of hypogonadism (n hypogonadic/n total)	15.7% (149/950)	23.7% (225/950)
LH<1.4 mUL/ml	8% (12/149)	5.8% (13/225)
1.4<LH<8.9 mUl/ml	77.2% (115/149)	80% (180/225)
LH>8.9 mUl/ml	14.8 % (22/149)	14.2% (32/225)

Conclusions: Our results demonstrate a prevalence of hypogonadism in HIV patients comparable to older healthy subjects (19.3% of hypogonadism in patients with mean age 58.7 years; Schneider 2009). This study, which is conduced on an elevated number of HIV patients, shows that the prevalence of hypogonadism is higher than in general population. Normogonadotropism predominance in subjects with hypotestosteronemia suggests also a possible involvement of a pituitary disfunction in the development of hypogonadism.

Nothing to Disclose: VR, DS, GB, LZ, CD, GO, CC, GG

P2-467

The Role of Adipose Tissue Hypercortisolemia in the Development of the Metabolic Syndrome in Severely Obese Patients.

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Introduction: Central obesity is a main component of the Metabolic Syndrome (MetS). There are many shared features between MetS and Cushing's syndrome. Circulating cortisol levels do not differ between obese and normal weight subjects. However, extra- adrenal cortisol production via the enzyme 11β-Hydroxysteroid Dehydrogenase Type 1 (11*β*-HSD1), which converts cortisone to cortisol, can result in high local cortisol levels. Data regarding the role of adipose 11*β*-HSD1 in obesity and metabolic disorders are conflicting.
Aim: Our aim was to compare the expression of the enzyme 11*β*-HSD1 and the Glucocorticoid Receptors α and β (GRα, GRβ) in visceral (VAT) and subcutaneous adipose tissue (SAT) in healthy lean and severely obese patients with or without Mets.
Patients and Methods: We studied 28 women. Of these, 9 normal weight metabolically healthy underwent hysterectomy (controls) and 19 severely obese underwent bariatric surgery, 8 without Mets (OM-) and 11 with Mets (OM+). None of the patients received any medication interfering with 11*β*-HSD1 regulation. All patients had a complete clinical, biochemical and endocrine work-out before surgery. VAT and SAT biopsies were obtained during surgery and 11*β*-HSD1, GRα and GRβ expression were evaluated with quantitative RT-PCR.
Results: Baseline cortisol values did not differ among groups. The VAT expression of 11*β*-HSD1 was marginally different among three groups (p=0.05, One-Way ANOVA; controls: 45,2 ±54,4, OM(-): 107,2 ±77,7, OM(+): 62,9 ±24,4). Post-hoc analysis (LSD) showed that the expression was higher in OM(-) vs controls ($p<0.05$) and tended to be higher than OM (+)(p=0.06), while the expression did not differ between controls and OM+. The 11β-HSD1 expression did not differ between SAT and VAT in OM (-) while it was marginally higher in SAT in OM (+) (independent t-test, SAT: 86,5±29,9 vs VAT: 62,9 ±24,4, p=0.06). The GRβ was not detected in VAT or SAT in all groups. GRα expression in VAT and SAT did not differ among groups.
Conclusions: Was not observed high VAT expression of 11β-HSD1 or GRα in OM (+), so it seems that VAT hypercortisolemia is not related with the Mets development in severe obesity. In contrast, the down regulation of 11β-HSD1 may be a count regulating mechanism protecting from the metabolic consequences of severe obesity.

Nothing to Disclose: MM, VK, AA, MN, AT, VK, FK, AGV, GV

P2-468

Morphological and Functional Features of Human Omental and Subcutaneous Adipose-Derived Stem Cells Isolated from Obese Patients.

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In the last years, the relevance and the interest in adipose tissues have greatly grown due to the knowledge of the presence of human Adipose-derived Stem Cells (hASC), which has been shown to differentiate into several cell lineages. The aim of this study is to characterize adipose progenitor cells subpopulations isolated from subcutaneous or visceral fat in normal and severe obese patients. The different state in the two kinds of patients could, indeed, influence the hASC phenotype and thus their proliferation capacity and differentiation potential.
The surgeons performed the harvesting of adipose tissue from subcutaneous and visceral areas, both in 10 normal and 10 obese (BMI> 35 Kg/m^2) subjects, who already needed a surgical intervention for different reasons. The fat was collected and immediately processed to obtain the progenitor cells, as previously described (L De Girolamo et al., 2007).
The experiments have been performed at 4th passage when the cells appeared to form an homogenous population with similar size and granularity.
The proliferation rate was quite similar in hASC isolated from subcutaneous tissues of normal and obese patients with a doubling time of 118 ± 61 and 96 ± 7 hours, respectively. However, hASC isolated from visceral tissue of obese patients showed a very lower rate of proliferation (doubling time of 227 ± 71 hours). Colony formation of the hASC population was determined by Fibroblast-Colony Forming Unit (CFU-F) assay. The clonogenic potential of hASC obtained from subcutaneous normal and obese patients was variable but higher than that showed by the hASC isolated from omental tissues. The immunophenotype of hASC has been analyzed by cytofluorimetry, evaluating the expression of the surface markers CD13, CD29, CD44, CD54, CD90 e CD105, CD133, CD14, CD34, CD45, CD71 e CD166. The results showed that the phenotypic profile of hASC was similar in the different tissues.
In conclusions the hASC isolated from normal and obese subcutaneous and visceral tissues showed different biological behaviours. The gene expression profile of the hASC isolated from visceral and subcutaneous tissues of normal and obese subjects will be analyzed, as well as their potential to differentiate into osteogenic, chondrogenic and adipogenic lineages. Defining the molecular and cellular components of obesity offers the foundation for identifying appropriate targets to ameliorate the management of the obesity.

L De Girolamo et al., J Tissue Eng Regen Med 2007; 1: 154-157.

Sources of Research Support: In part supported by the Italian Ministry of Health, RF 07-44.

Nothing to Disclose: LS, FC, LDG, DS, GFS, SM, LP, LR, CL, ATB, EP

P2-469

Evaluation of Lipocalin-Type Prostaglandin D_2 Synthase Expression in Human Adipocytes and Its Influence on Obesity.

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OBJECTIVE: To observe the correlation of lipocalin-type prostaglandin D_2 synthase (L-PGDS) expression in human adipocytes with body weight. L-PGDS is an enzyme that has been shown to have a role in vascular disease. Its metabolic role, although not well defined, may be related to the downstream formation of PGJ_2, a natural peroxisome proliferator activating receptor agonist (PPAR), from PGD_2. We have previously demonstrated that L-PGDS knockout mice exhibit adipocyte hypertrophy and insulin resistance, even when fed a low fat diet (Ragolia *et al*. 2005), suggesting that L-PGDS may serve as a protective mechanism against obesity and diabetes. METHODS: This is a descriptive study of six obese (BMI>40) and three normal weight (BMI < 25) individuals who underwent elective abdominal surgery. Three of the obese subjects were being treated for diabetes. Subjects who were acutely ill or on thiazolinedione therapy were excluded. We compared mRNA expression of L-PGDS in omental adipose tissue of normal weight and obese individuals using RT-PCR. RESULTS: A comparison between normal weight and obese subjects revealed a 3.4-fold increase in L-PGDS expression in normal weight subjects, while normal weight and obese subjects with diabetes revealed a 5.7-fold increase (Figure 1). Interestingly, obese versus obese diabetic subjects revealed 2.3-fold increase in L-PGDS expression (Figure 2). CONCLUSION: L-PGDS expression may provide a protective mechanism against obesity and *type 2* diabetes, however further studies are necessary.

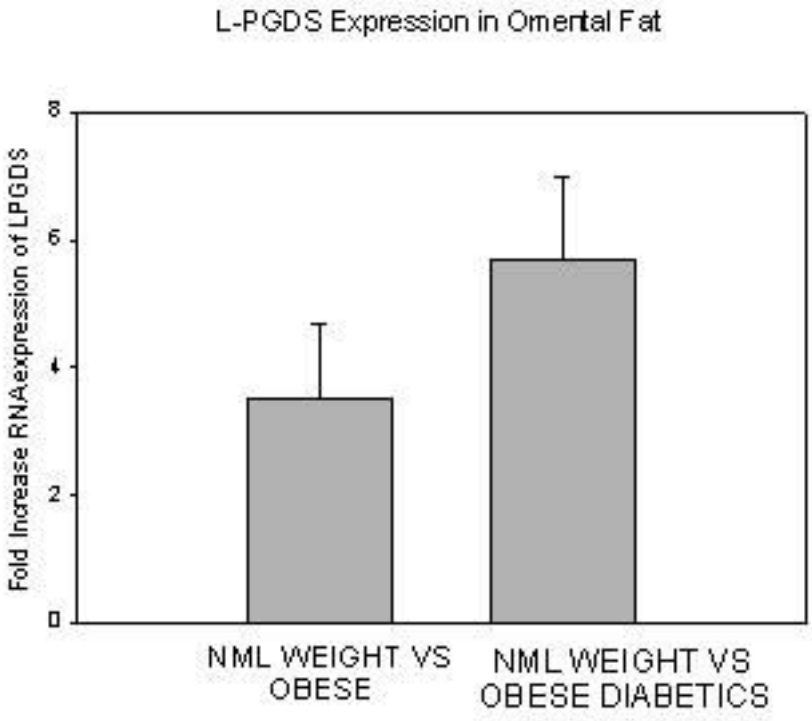

Figure 1

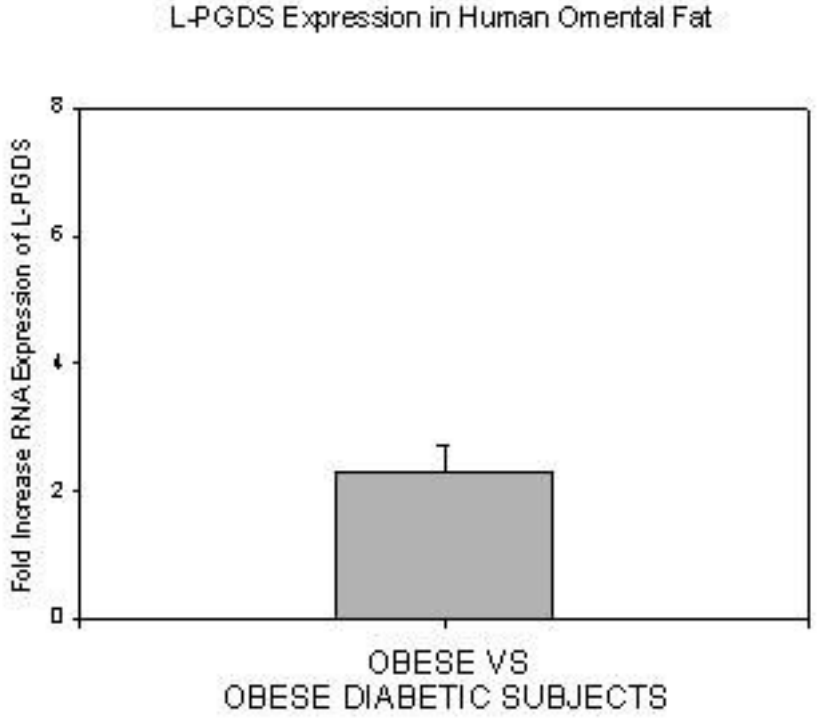

Figure 2

(1) Ragolia, L et al., Accelerated Glucose Intolerance, Nephropathy, and Atherosclerosis in Prostaglandin D2 Synthase Knockout Mice. J. Biol. Chem. 280(33), Aug 19, 2005: 29946-29955.

Nothing to Disclose: RGL, TP, LES, CB, LR, VA

P2-470

Human Adipocyte Differentiation Upregulates and Antagonizes Androgen Receptor.

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The characterization of genes associated with human adipogenesis is fundamental to understanding the pathogenesis of obesity. To better appreciate the genetic program controlling human adipogenesis, we compared gene expression profiles between terminally differentiated 3T3L1 and human adipocytes. As a result, we identified 1467 genes specifically associated with the human adipogenome. Pathway analysis showed that these genes were largely associated with TGF-beta signaling, while 817 shared human and mouse genes were comprised of cholesterol, lipid, and fatty acid synthesis. Surprisingly, a gene unique to the human dataset was the androgen receptor (AR). Validation of AR expression during human adipogenesis by qPCR showed that AR is upregulated 10-fold early (48h) with a 12-fold plateau after 14d of hormonal induction. We additionally bridged the genomic data with a single-cell, automated image-based approach with lipid accumulation as the central variable. Consistent with increased AR transcripts, we measured a 4-fold induction of AR protein, with no cytoplasmic distribution, after 96h of differentiation. Treatment with dihydrotestosterone (DHT) inhibited human, but not 3T3L1, adipocyte maturation. Further, DHT promoted receptor stabilization, decreased PPARγ nuclear accumulation (-40%), and downregulated CEBPA, FASN, ADFP, and PPARγ2 transcripts. We then demonstrated the effects of differentiation cocktail (MIX) using a stable HeLa cell line expressing GFP-AR and a fluorescent reporter driven by the AR-responsive promoter ARR2PB. Incubation with isobutylmethylxanthine, insulin, rosiglitazone, or dexamethasone, individually, showed dose dependent increases in GFP-AR translocation without ARR2PB induction, analogous to treatment with the AR antagonist, o-hydroxyflutamide (OHF). The effects of MIX on AR conformation were evaluated using a stable HeLa cell line expressing AR simultaneously fused to cyan and yellow fluorescent proteins at the amino- and carboxy-termini of AR, respectively, allowing FRET detection of intramolecular interactions. While DHT increased CFP-YFP FRET, MIX promoted a response similar to OHF, indicating an antagonist conformation, and consistent with the experiments performed in the GFP-AR/ARR2PB cell line. These results demonstrate a novel functional role of AR in human adipocytes whereby AR maintains an antagonist conformation to allow differentiation and expression of adipogenic target genes.

Nothing to Disclose: SMH, BH, SAO, MJB, DSL, NJM, BMB, MAM

P2-471

Fatty Acid Composition of Abdominal Adipose Tissues in Women with Visceral Obesity.

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Adipose tissue fatty acid composition, specifically the content in stearic acid (18:0), has been shown to be altered in obesity and insulin resistance. Reduced subcutaneous adipose tissue 18:0 content has also been associated with the presence of large adipocytes and down-regulated lipogenesis in this depot. We tested the hypothesis that visceral adipose tissue 18:0 content would be altered in women with visceral obesity. Omental and subcutaneous fat tissues were obtained from women undergoing gynecological surgery. We selected two subgroups of 10 women with either small or large omental adipocytes which were individually matched for age, total body fat mass and subcutaneous adipocyte size. Adipose tissue total lipids were extracted, fatty acids were methylated and the fatty acid profile of each sample was determined by capillary gas chromatography. Body composition and fat distribution were measured by DEXA and computed tomography respectively. Adipocyte size was measured in adipocyte suspensions from collagenase-digested tissues. Dietary fatty acids were determined using a food frequency questionnaire. Fasting blood lipids and insulin were also measured. By design, women from the two subgroups had similar values for age, total and subcutaneous adiposity. However, visceral adipose tissue area and omental adipocyte size were higher for women with visceral obesity ($p<0.05$ for both). Both groups had a similar dietary intake of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Fatty acid composition of fat in both groups was also similar for SFA, MUFA and PUFA content. However, the content of 18:0 was significantly lower in omental fat of women with visceral obesity and large omental adipocytes (2.37 ± 0.45 vs. 2.75 ± 0.30 mg/100g adipose tissue respectively, $p\leq0.05$). The 18:0 content of omental fat was negatively related to visceral adipose tissue area ($r=-0.44$, $p=0.05$) and serum triglyceride levels ($r=-0.56$, $p<0.05$). The 18:0 content of subcutaneous fat was negatively correlated with total body fat mass ($r=-0.48$, $p<0.05$) and fasting insulin ($r=-0.73$, $p<0.005$). The correlations obtained with the of 18:1-to-18:0 ratio (desaturation index) and the 18:0-to-16:0 ratio (elongation index) reflected those obtained with 18:0 alone. In conclusion, 18:0 content is reduced in omental adipose tissue of women with visceral obesity and large omental adipocytes. This alteration was independent of dietary fat intake.

Nothing to Disclose: MC-J, AM, DM, AV, SN, MPF, CM, PJ, AT

P2-472

A Novel Mutation in the Leptin Receptor Gene Leading to Extreme Infantile Obesity and Hyperphagia.

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Background: Early onset infant obesity is most commonly a multifactorial condition, although it can be part of a clinical syndrome, or rarely the result of a monogenic mutation. Leptin receptor (*LEPR*) mutations are infrequent, but important to diagnose for the purpose of genetic counseling and appropriate follow up of these patients with regards to variable immunodeficiency, short stature and hypogonadism.
Clinical Case: An 11 month old boy presented with hyperphagia and early onset weight gain. He was born at term with a birth weight of 3.81 kg and had an uneventful neonatal course. He demanded frequent feedings (hourly); at 4.5 months his weight was 13.8 kg (+6 Weight SDS) and his intake comprised 2100 ml of formula daily (1400 Kcal/d, Age appropriate energy need is 650 Kcal/d). The patient was the first child of healthy, non-obese, consanguineous parents of Turkish descent. On physical examination, his weight was 18 kg (+ 4.9 Weight SDS), and his length was 74cm (-0.3 Height SDS). No dysmorphism was noted, and the examination was unremarkable aside from obesity. Endocrine testing (TFT's, cortisol, ACTH, IGF1 and insulin levels) were all within the normal range. Additional testing revealed an elevated Leptin level of 86.6 ng/ml (1-35). Sequencing of the Leptin receptor revealed 2 homozygous missense mutations in the *LEPR* gene (Trp646Cys, Pro316Thr), not reported previously. These mutations were located in the fibronectin 3 domain consistent with previously described mutations associated with complete or partial loss of function of the receptor (1). Both parents were heterozygote for both mutations.
Conclusion: This case highlights a novel *LEPR* mutation responsible for extreme, early onset obesity and hyperphagia in the setting of a significant family history of early onset obesity and parental consanguinity in a high risk ethnic group. These characteristics, rather than the elevated leptin level, prompted genetic testing which confirmed this rare genotype. The insertion of a new Cysteine residue likely results in drastic changes of the protein structure and resultant impaired function.

(1) Farooqi et al., NEJM 2007;18;356(3):237-47.

Nothing to Disclose: YY, FHM

P2-473

Cellular Characterisation of Human Brown Adipose Tissue Identified by Positron Emission Tomography.

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Brown adipose tissue (BAT) is a key regulator of energy homeostasis in animals. Positron emission tomography (PET)-CT scanning using 18F-deoxyglucose (FDG) to localise tissue of high metabolic activity has challenged the belief that BAT in humans is lost during infancy. Up to 20% of adults show avid FDG uptake in adipose tissue in the neck, suggesting the presence of BAT (1). However, little is known about the nature and characteristics of putative BAT in adult humans.
The aim of our study is to characterise the histological/molecular properties of fat in the neck in relation to FDG uptake on PET-CT.
Ten patients who had pre-operative PET-CT for staging of head & neck malignancy were recruited. In patients with positive PET-CT not attributable to malignancy, biopsies were obtained intra-operatively from the supraclavicular fossa, within fat with high FDG uptake (SUV>4), as delineated by PET-CT. In those with negative PET-CT, fat biopsies were obtained from an identical region in the neck. Subcutaneous (SC) fat was obtained from all patients as negative control. Fat samples were processed for histological/molecular analyses. BAT was defined by the presence of multilobulated lipid droplets, UCP1 immunostaining and mRNA for UCP1, NDUFS3 & β3-adrenoceptor (AR), which are characteristic of BAT. Transcripts were quantified by PCR and expressed as multiples over SC fat.
PET-CT was positive in 3 and negative in 7 patients. By histology, PET positive fat harboured multilobulated lipid droplets and stained strongly for UCP1. SC fat did not show any cells with multilobulated droplets or with UCP1 staining. By contrast, PET negative fat demonstrated a predominance of cells with unilobulated lipid droplets, with scattered cells containing multilobulated lipid droplets and variable UCP1 staining. Transcripts for UCP1, NDUFS3 & β3-AR were present in PET negative fat, but abundance was significantly lower than in PET positive fat:

Gene expression in fat biopsies

	UCP1	NDUFS3	ß3-AR
PET positive (n=3)	7.8x10^4	1.7x10^5	120
PET negative (n=7)	75	125	3

Expression levels were normalized to that of ß-actin and are expressed as multiples over SC fat. $p<0.001$ for all (t-tests)

In summary, high FDG uptake on PET-CT corresponds to high abundance of fat with histological and molecular characteristics of BAT. Absent uptake however does not exclude the presence of brown adipocytes in the neck. Supraclavicular fat, regardless of FDG uptake, is distinct from subcutaneous fat.

(1) Lee P, Ho KK, Fulham MJ. N Eng J of Med 2009; 361: 418

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Nothing to Disclose: PL, JTZ, RB, GG, JF, JRG, KKYH

P2-474

Glucocorticoid and Insulin Regulation of Lipogenesis in Human Adipose Tissue.

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Patients with glucocorticoid (GC) excess develop a classic phenotype characterized by central obesity and insulin resistance, however, the impact of GCs upon the processes that regulate lipid accumulation has not been fully explored. We hypothesize that intracellular generation of cortisol from cortisone by 11b-hydroxysteroid dehydrogenase type 1 (11bHSD1) is an important regulator of lipid metabolism.
In adipocytes, acetyl-CoA carboxylase 1 and 2 (ACC1/2) convert acetyl-CoA to malonyl-CoA. ACC1 predominates and is the rate-limiting step in lipogenesis. ACC2 is localized to the mitochondrial membrane, and the malonyl-CoA generated inhibits β-oxidation. Using a human subcutaneous cell line, Chub-S7, we characterized the expression of ACC1/2 and the regulation of lipogenesis (1-[^{14}C]acetate incorporation) and β-oxidation ([^{3}H]-palmitate oxidation) across differentiation and determined the influence of GCs and insulin.
Across differentiation ACC1 and ACC2 mRNA expression increased (ACC1, 2.3-fold, $p<0.05$; ACC2, 42.2-fold, $p<0.05$), as did total ACC protein (3.2-fold, $p<0.05$). Basal and insulin stimulated ACC1 activity also increased (Undifferentiated [dpm] 412±44 vs. 662±93 [5nM ins], $p<0.05$, Differentiated 5351±304 vs. 11250±880 [5nM ins], $p<0.05$) whereas β-oxidation decreased (13.6-fold, $p<0.05$). In differentiated cells Dexamethasone (Dex) [0.5uM] increased ACC2 mRNA expression (2.8-fold, $p<0.01$) but not ACC1, total ACC protein increased (3.0-fold, $p<0.05$). Dex decreased basal ACC activity in a dose dependent manner (76.8±7.8% [5nM], 60.3±3.2% [500nM], $p<0.05$), consistent with this, inactivating phosphorylation of ACC1/2 increased (2.4 fold, $p<0.05$). However, low dose Dex enhanced insulin stimulated lipid accumulation (33.9±10.1% [5nM ins] vs. 106±25.5% [5nM ins, Dex 5nM], $p<0.05$). Dex treatment had no effect upon β-oxidation. Cortisone decreased basal ACC activity and this was recovered with the selective 11bHSD1 inhibitor PF877423 (77.4±1.6% [cortisone 500nM], 144.2±18.6% [PF877423 + cortisone], vs. PF877423).
We have described a novel interaction between insulin and GCs in the regulation of lipid accumulation within human adipocytes. These data also demonstrate the importance of pre-receptor GC metabolism in the regulation of adipocyte lipid metabolism.

Sources of Research Support: Wellcome Trust.

Nothing to Disclose: LLG, SAM, IJB, PMS, JWT

P2-475

Restoration of Glycemic Control Following Bariatric Surgery (BS) Is Associated with Marked Reduction in Endothelial, Monocyte and Platelet Microparticle Concentration.

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Microparticles are membrane fragments that bud off of leukocytes (CD14), platelets (CD41) and vascular endothelial cells (CD 144, CD 105) during cellular activation and are key to the inflammatory process associated with micro/macro vascular complications related to diabetes (DM) and obesity. Although weight loss reduces inflammation, the effects of bariatric surgery (BS) on changes in microparticle concentration and composition are not known. We studied 14 obese subjects with DM (age 52 ± 13 y, 7M/7F, BMI 47.3 ± 9 kg/m2, fasting glucose 141 ± 43 mg/dl, HbA1c 7.2 ± 1%) 2 weeks before and 1, 6, 12 months (m) following bariatric surgery. Blood was drawn for biochemical and microparticle concentration and composition determination at each visit. Quantitative flow cytometry with isotype controls was performed for plasma expression of annexin, CD14, CD41, CD105 and CD144. Microparticles concentration before and after surgery were compared using paired t-test. Correlation analysis was done to determine the relationship of changes in microparticle concentration to changes in clinical indices. One month following BS, BMI reduced by 5 ± 1 kg/m^2 ($P < 0.01$)**.** Fasting glucose (pre:138 ± 43 vs. post: 103 ± 21 mg/dl, $P < 0.01$) and HbA1c (7.2 ± 1 % vs. 6.2 ± 0.6 %, $P < 0.01$) reduced markedly as did levels of CD14 (3916 ± 3282 vs. 2355 ± 2046 mP/ml, $P < 0.05$)and CD41 (1620613 ± 2436835 vs. 267010 ± 259628 mP/ml $P < 0.05$). Twelve months following BS, BMI reduced by 11 ± 5 kg/m^2 ($P < 0.01$), fasting glucose decreased to 93 ± 19 mg/dl and Hba1c decreased to 5.7 ± 0.5 % ($P < 0.01$). CD14 (pre: 3916 ± 3282 vs. post: 1462 ± 1342 mp/ml), CD41 (1620613 ± 2436835 mp/ml vs. 511672 ± 166794 mp/ml) and CD144 (257987 ± 282744 mp/ml vs. 75130 ± 59906 mp/ml) were all found to reduce significantly reduced ($P < 0.05$). The reduction in CD41 ($R = 0.49$, $P <0.05$) and CD 14 ($R = 0.49$, $P < 0.05$) microparticles one month after BS strongly associated with the reduction in HgbA1c. The reduction in CD 14 ($R = 0.47$, $P < 0.05$) 12 months following BS correlated linearly with the reduction in BMI. These data suggest that weight loss and restoration of euglycemia following bariatric surgery is associated with reduction of platelet, monocyte and endothelial microparticles. Reduced microparticles may be a mechanism by which bariatric surgery improves inflammation associated with DM and obesity.

Sources of Research Support: NIH/NCRR.

Nothing to Disclose: VC, MK, JK, PS, KM, SRK

P2-476
SAA Lipoprotein Distribution in Obese Humans.

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Background: Serum amyloid A (SAA) is a major acute phase protein that increases 100-1000 fold during acute inflammation. In obesity, metabolic syndrome, or diabetes we and others have demonstrated a chronic, modest elevation in SAA concentrations. In lean, healthy individuals SAA is carried in the plasma primarily on HDL particles. In murine models of obesity there is an increase in plasma SAA levels along with redistribution of SAA from HDL to apo-B containing lipoprotein particles, namely LDL and VLDL. It has been proposed that in obesity this increase in SAA levels and its association with proatherogenic lipoprotein particles contributes to atherosclerotic lesion formation. The goal of this study was to determine if obesity is associated with similar SAA lipoprotein redistribution in humans.
Methods: 10 obese individuals were recruited from a weight loss clinic. Patients with signs or symptoms of acute inflammation, history of diabetes mellitus and other chronic inflammatory diseases were excluded. Subjects taking medications known to affect SAA levels, such as statins, thiazolidinediones and steroids were also excluded. Plasma was collected before initiating weight loss and following at least 10% weight loss after 12 weeks of diet and exercise. SAA content was measured in total plasma before and after weight loss using an ELISA kit for human SAA. Plasma was then separated using fast protein liquid chromatography. SAA content was measured in individual fractions using the ELISA kit and western blotting to determine the SAA lipoprotein distribution.
Results: Baseline BMIs were 42.8± 7.7 kg/m2 and decreased to 36.1± 6.8 kg/m2 after 12 weeks. As expected, the total plasma SAA levels were elevated in the pre weight loss samples (63.8 ± 7.4 mg/L) and decreased with weight loss (43.8 ± 7.2 mg/L, p=0.03). However, unlike mice, the majority of the SAA was in the HDL fractions, with negligible SAA detected in either the VLDL or LDL fractions.
Conclusion: Total plasma SAA levels are increased in obesity and weight loss can result in reduction of these levels. However, in human subjects, as opposed to mice, obesity does not cause redistribution of SAA to apo-B containing lipoprotein particles. Thus, mice may not be a good model for studies of obesity associated complications.

Nothing to Disclose: AB, AJ, LRT

P2-477

A New Link between Skeleton, Obesity and Insulin Resistance: Relationships between Osteocalcin, Leptin, and Insulin Resistance in Obese Children before and after Weight Loss.

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Background: The skeleton is regarded recently as an endocrine organ that affects energy metabolism. However, there are very limited data available concerning the relationships between the osteoblast derived hormone osteocalcin, weight status, adiponectin, and leptin in obese humans, especially in children.
Methods: We analyzed osteocalcin, adiponectin, leptin, and insulin resistance index HOMA in 60 obese (mean age 10.9 ±0.3 years, 53% female, mean BMI 28.3 ±0.5 kg/m²) and 19 age- and gender matched normal-weight children. Furthermore, these parameters were determined in the 60 obese children after participating in an outpatient one-year lifestyle intervention based on exercise, behavior and nutrition therapy.
Results: The 60 obese children had significantly lower osteocalcin levels (26.8±0.8ng/ml) than the 19 normal-weight controls (32.2±2.3ng/ml). Boys (29.9±1.1ng/ml) demonstrated significantly (p=0.046) higher osteocalcin levels as compared to girls (26.4±1.2ng/ml). In stepwise multiple linear regression analysis adjusted for age, gender, and pubertal stage, osteocalcin was significantly negatively related to leptin (beta-coefficient: -0.14 ±0.06, p=0.037) and HOMA (beta-coefficient: -0.78 ±0.38, p=0.041), but not to adiponectin. Changes of osteocalcin in the course of 1 year correlated negatively significantly to changes of insulin resistance index HOMA (r=-0.25), SDS-BMI (r=-0.33), and leptin (r=-0.50). Substantial weight loss in 29 obese children led to a significant increase of osteocalcin (+2.8 ng/ml) and adiponectin (+0.9 µg/ml), as well as a significant decrease of leptin (-10.5 ng/ml) and HOMA (-1.2). In the 31 obese children without substantial weight loss, HOMA, osteocalcin, adiponectin, and leptin levels did not change significantly in the course of 1 year.
Conclusions: Osteocalcin levels were lower in obese children and were related to insulin resistance and leptin both in cross-sectional and longitudinal analyses. Therefore, osteocalcin might be a new promising link between obesity and insulin resistance.

Nothing to Disclose: TR, CLR

P2-478

Diet-Induced Obesity Worsens Alzheimer's Pathology in a Mouse Model of Alzheimer's Disease: Investigation of Interactions between Gender and Metabolic Syndrome.

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Multiple factors of metabolic syndrome have been implicated in the pathogenesis of Alzheimer's disease (AD), including abdominal obesity, insulin resistance, and dislipidemia. A high fat diet is commonly used as an experimental model of obesity and metabolic syndrome; and has been previously demonstrated to accelerate cognitive decline and AD neuropathology in animal models. However, gender interacts with the metabolic outcomes of high fat diet and, therefore, may also alter neuropathological consequences of dietary manipulations. To determine the effect of sex on metabolic and AD-related neuropathological outcomes of a high fat diet in 3xTgAD mice, 3 month-old male and female APP/PS1/Tau triple transgenic mice (3xTgAD) were fed either regular (Rodent Diet 8604; Harlan Teklad) or high fat diets (TD06414; Harlan Teklad) for 4 months. Obesity was observed in all high fat fed mice, however sex-specific metabolic effects were observed on measures of abdominal adiposity and hyperinsulinemia (fasting blood levels of insulin and glucose). Although the relative accumulation of abdominal adiposity was greater in female than male mice, significant hyperinsulinemia was observed only in males. Interestingly, despite the different metabolic outcomes of the high fat diet, the neuropathological consequences were similar - both male and female high fat fed mice exhibited similarly accelerated memory impairment and hippocampal accumulation of the neurotoxic beta amyloid peptide. Because diet-induced obesity worsened AD-like pathology in both sexes, these data support a role of obesity-related factors in promoting AD pathogenesis. However the absence of significant changes in fasting glucose and insulin levels in obese females, despite the worsened AD pathology, questions the role of hyperinsulinemic factors in the promotion of AD pathogensis in our model.

Sources of Research Support: American-Australian Association Neurological Fellowship Awarded to AMB.

Nothing to Disclose: AMB, ERR, MAB, RE, CJP

P2-479

Changes in ERp44 and Gstk1 Chaperone Protein Expression Are Associated with Reductions in Ad Secretion in Human Visceral Adipose Tissue.

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Adiponectin levels are reduced in obesity and correlate most strongly with visceral adipose tissue (VAT) mass. Using cultured human AT explants we have previously shown that adiponectin secretion is impaired in VAT. Recently adiponectin maturation and secretion in rodent cell lines has been shown to depend on thiol-mediated retention in which adiponectin forms a mixed disulfide bond to a resident endoplasmic reticulum (ER) protein, ERp44. Ero1-Lα, an oxidoreductase in the ER, is involved in the release of adiponectin from ERp44. GST-K1, another oxidoreductase, promotes formation of biologically active high molecular weight (HMW) adiponectin. We therefore postulated that reductions in adiponectin secretion in human VAT are related to changes in the expression or function of ER chaperone proteins involved in thiol-mediated retention of adiponectin. Paired SAT and VAT biopsy samples were obtained from 10 obese, weight stable, subjects undergoing Roux-en-Y surgical weight reduction. Serum was analyzed for total and HMW adiponectin, AT histology was assessed for cell size. Analysis of AT mRNA and fat cell protein expression of adiponectin and ER chaperone proteins and AT conditioned media was performed following 24hr of explant culture. Reduced VAT mRNA expression of ERp44 (P = 0.0037), Gstk1 (P= 0.0004) and adiponectin (P=0.027) was found. Compared to SAT, these changes were associated with significantly less adiponectin secreted into the media by VAT. Despite these changes, no differences between depots were detected in adiponectin or chaperone protein expression. These findings suggest that depot differences in the cellular expression or function of ER chaperone proteins may underlie changes in insulin sensitivity observed in visceral obesity and implicate assembly and secretion of adiponectin as key regulatory events in adiponectin levels.

Disclosures: RRH: Speaker Bureau Member, Novo Nordisk; Medical Advisory Board Member, Novo Nordisk.
Nothing to Disclose: SAP, TPC, LKM, ACW

P2-480

Increased Storage Capacity of Adipose Tissue during Critical Illness.

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Critically ill patients requiring prolonged intensive care are characterized by a hypercatabolic response with wasting of the lean body mass, but preservation of adipose tissue. Several large observational studies now suggest that patients who are overweight or mildly obese have a lower mortality rate in the ICU than lean individuals. Although this may point to a protective role for this organ system, adipose tissue has hitherto not been thoroughly investigated in critical illness. In view of the hyperglycemic and hypertriglyceridemic response to critical illness, which may exert toxic effects, we hypothesized that adipose tissue could respond protectively by increasing its storage properties. We therefore studied the capacity of the subcutaneous and omental adipose tissue to take up and release lipids, by investigating biopsies of 61 patients with prolonged and lethal critical illness as compared with biopsies of 22 non-critically ill patients undergoing elective surgery.
Lipoprotein lipase is responsible for hydrolysis and uptake of circulating triglycerides. On the other hand, activated hormone sensitive lipase (HSL) is the rate limiting enzyme in the hydrolysis and release of stored adipose triglycerides back into the bloodstream. In both omental and subcutaneous adipose tissue homogenates of critically ill patients, levels of activated (Ser563 phosphorylated) HSL (pHSL) remained comparable to healthy controls. In contrast, protein levels of LPL were several fold increased in both omental and subcutaneous adipose tissue homogenates of critically ill patients. Total lipase activity was also several fold increased in omental and subcutaneous adipose tissue homogenates of critically ill patients.
Taking into account the known association between high triglyceride levels and mortality of critical illness, the observed alterations may represent an adaptive and protective response. Some excess adipose tissue, which has not evoked chronic severe co-morbidity, may thus be an advantage during critical illness as it becomes a storage depot for possibly toxic metabolites.

Nothing to Disclose: LL, SVP, GH, GVdB

P2-481

Characterisation of Serum and Glucocorticoid-Induced Kinase 1 in Adipose Tissue.

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Serum and glucocorticoid-induced kinase 1 (Sgk1) is an early transcriptional target of glucocorticoids with antiapoptotic effects. Sgk1 becomes active after phosphorylation by growth factors such as insulin and IGF-1 through the PI3 kinase pathway. Activated Sgk1 increases the cellular uptake of glucose by increasing the membrane abundance of glucose transporters GLUT1 and GLUT4. Here we present data on the expression and regulation of Sgk1 in adipose tissue using three different models: human obesity, diet-induced murine obesity and adipogenesis in 3T3-L1 cells.
Sgk1 gene expression was increased in both omental and subcutaneous adipose tissue of 20 morbidly obese patients when compared to 20 age- and sex-matched control subjects. Adipose tissue fractionation revealed a predominant expression of Sgk1 in adipose tissue macrophages and a very low expression in adipocytes. Similarly, high-fat diet-induced obesity in mice was associated with a significant upregulation of Sgk1 in adipose tissue, and predominantly in the stromal-vascular tissue. Sgk1 transcription in 3T3-L1 preadipocytes was strongly induced by dexamethasone and its phosphorylation was enhanced by insulin. Nevertheless, induction of adipogenesis in 3T3-L1 cells led to a progressive reduction in Sgk1 mRNA and protein levels. During adipogenesis, Sgk1 mRNA levels correlated negatively with the intracellular fat content and with the increase in PPARgamma mRNA. Overexpression of Sgk1 in 3T3-L1 adipocytes did not influence PPARgamma levels, while the addition of rosiglitazone decreased Sgk1 transcription.
In summary we conclude that Sgk1 expression is increased in adipose tissue macrophages in both human and murine obesity, but downregulated in adipocytes during adipogenesis and after PPARgamma activation. The reduced Sgk1 levels in adipocytes may be linked to increased insulin resistance, as Sgk1 increases glucose uptake via upregulating the membrane abundance of glucose transporters. In parallel, the increased Sgk1 expression in macrophages points towards a potential role in adipose tissue inflammation.

Nothing to Disclose: GV, FK, MHR, GP, MZ, TMS, AL

P2-482

The Effect of Fructose upon Human Subcutaneous and Visceral Adipocytes.

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Background; Within the last 10 years, in the USA there has been a switch in food sweeteners from sucrose to high fructose corn syrup, mirroring the rapid increase in obesity. This has resulted in a fructose intake over five times that of our ancestors, therefore extra-hepatic tissues such as fat which have had little exposure to fructose throughout evolution, may now be exposed to significantly raised levels. Our aim was to investigate whether exposure to fructose alters the function of human adipocytes.
Materials and methods; Preadipocytes cultured from subcutaneous and visceral origin were differentiated for 14 days in media containing low/physiologically normal glucose (5mM), high glucose (25mM) or high fructose (20mM). Alternatively, cells were differentiated to maturity in low glucose media, followed by a 48 hour exposure to low glucose, high glucose or fructose. Cell differentiation was assessed by assaying GPDH activity and staining lipid droplets with Oil red O. 2-Deoxyglucose uptake assays were performed with or without insulin (15 mins, 100nM). The glucose transporters GLUT1 and GLUT4 and adipocyte fatty acid binding protein (AFABP) were assessed by western blotting.
Results; Visceral adipocytes differentiated in fructose showed an increase in Oil red O staining, which suggests an increase in differentiation. This observation was verified by a significant 4 fold increase in GPDH activity and a 2 fold increase in AFABP abundance. However, adipocytes from both fat depots differentiated with fructose showed a significant decrease in insulin-stimulated glucose uptake, which was not due to changes in total GLUT4 abundance. Interestingly, when mature adipocytes were exposed to fructose, insulin-stimulated glucose uptake showed a significant 2 fold increase in the subcutaneous cells and a significant 1.5 fold increase in the visceral cells, again with no change in total GLUT4, however, total GLUT1 abundance decreased in adipocytes, which corresponded to a decrease in basal glucose uptake.
Conclusions; Although mature adipocytes showed an increase in insulin sensitivity following a short exposure to fructose, differentiating pre-adipocytes from both depots in fructose appeared to have a detrimental effect on insulin sensitivity. Fructose also increased differentiation of visceral adipocytes, indicating a specific effect on this depot which is known to be more closely associated with the health risks of obesity and the metabolic syndrome.

Sources of Research Support: Diabetes UK Grant 06/0003326.

Nothing to Disclose: GC, JMPH, JPHH-S, GIW, EJF

P2-483

Acute Exposure to Phosphodiesterase Type-5 Inhibitors Up-Regulates Aromatase Expression in Human Adipocytes In Vitro.

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It has been reported that in men with erectile dysfunction prolonged tadalafil administration is associated with increased testosterone/estradiol (E_2) ratio mainly related to reduction of E_2 levels. Adipose tissue is the main site of androgen aromatization and subsequent estrogen production in men. We evaluated the presence of specific phosphodiesterase type-5 (PDE5) isoenzyme in primary human adipocytes and investigated whether different PDE5 inhibitors (PDE5-i) could directly modulate aromatase (ARO) expression in differentiated human adipocytes in vitro. Purified primary human visceral pre-adipocytes were differentiated *in vitro* and then exposed to tadalafil or sildenafil (1 μM) for different intervals of time (6-24-96-168 hrs). ARO mRNA expression was quantified by Real Time RT-PCR. Testosterone (T) and E_2) concentrations were measured by RIA in supernatants at all time-points studied. Differentiated adipocytes were found to express detectable levels of PDE5 transcripts. Acute exposure (6 hrs) to PDE5 inhibitors tadalafil and sildenafil increased ARO mRNA expression by 4.7- ($p<0.001$) and 2.8-fold , respectively. Such effect was lost after 24 and 96 hrs, and was mimicked by a PDE-resistant cyclic-GMP analogue (e.g., 8-bromo-cGMP); differently, the PDE3B specific inhibitor milrinone (1 μM), displayed no effect, at all studied time points. In line with mRNA studies, 24 and 96 hrs exposure to PDE5-i caused a significant increase in E_2 concentrations in the supernatant (1.7 and 2 fold, respectively; $p<0.001$), with a parallel reduction of T (15 and 30%, respectively; $p<0.001$). As expected, milrinone exposure did not affect T and E_2 concentrations.
We demonstrate that PDE5 is expressed in human visceral adipocytes and that acute exposure to PDE5-i selectively stimulates ARO expression; such effect is related to a specific PDE5 blockade, and not to other PDE isoforms. We speculate that modulation of ARO activity by PDE5-i could be one leading mechanism responsible for the beneficial endothelial and prostatic effects observed after acute exposure to these drugs.

Nothing to Disclose: MC, AA, EG, GS, AL, GMCR, AF, SM, AA

P2-484

Apoptosis of Fat Cells Is Linked to Macrophage Infiltration in Human Adipose Tissue.

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Introduction: Obesity is associated with macrophage accumulation and inflammation in adipose tissue leading to insulin resistance. Macrophages are responsible for removing dead cell material in chronic inflammation. We hypothesize that apoptosis of fat cells occurs and that apoptosis is involved in the inflammatory process of adipose tissue.
Methods: Paired samples of human subcutaneous and visceral adipose tissue were subjected to immunohistochemical analysis. Human SGBS preadipocytes and adipocytes were incubated with macrophage-conditioned medium. Apoptosis was determined by flow cytometry. Insulin signaling was investigated by western blot. De novo lipogenesis was determined by incorporation of 14C-Glucose into cellular lipids.
Results: Apoptotic adipocytes in human adipose tissue were detected by staining the active fragment of caspase-3. Dead adipocytes were surrounded by macrophages in crown-like structures. Percentage of macrophages correlated positively with percentage of apoptotic adipocytes.
Factors secreted from macrophages induced apoptosis in preadipocytes as well as in mature, lipid-laden adipocytes in a time- and dose-dependant manner. Incubation with macrophage-conditioned medium induced insulin resistance in adipocytes as shown by reduced Akt phosphorylation and inhibition of insulin-stimulated de novo lipogenesis, suggesting a link between insulin resistance and apoptosis sensitivity. Accordingly, pharmacological inhibition of insulin signaling pathways sensitized fat cells to apoptosis induction by macrophage factors.
Conclusion: Apoptosis of adipocytes is linked to macrophage infiltration in human adipose tissue. In vitro, macrophage-secreted factors provoke insulin resistance and induce apoptosis of adipocytes.
Our study suggests a key pathogenic role for adipocyte apoptosis in the recruitment of macrophages to adipose tissue and the maintenance of adipose tissue inflammation.

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Nothing to Disclose: PF-P, MK, MB, PM, K-MD, MW

P2-485

Whole Blood Gene Expression Changes Following Bariatric Surgery Are Related to Recovery of Type 2 Diabetes.

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To investigate the effects of bariatric surgery on gene expression profile changes in whole blood in obese subjects with type 2 diabetes (T2DM) in a pilot study setting. Whole blood from eleven obese subjects with T2DM (age 50.5±12 y, 5F/6M, BMI 47±11 kg/m2), was collected in PAXgene tubes 2 weeks prior to and 6-12 months after bariatric surgery. Total RNA was isolated, amplified, labeled and hybridized to Illumina gene expression microarrays. Validation of data with real time quantitative PCR and protein expression of selected transcripts was performed. Biological pathways were identified using Ingenuity Pathway Analysis and DAVID gene ontology software. Bariatric surgery resulted in significant reduction of BMI (37±10 vs. 47±11 kg/m2, P < 0.01), fpg (98 ± 26 vs. 170 ± 54 mg/dl, P < 0.01) and normalization of HbA_{1c} levels (5.9 ± 0.6 vs. 7.9± 1.5%, P < 0.01). The expression levels of 204 blood transciptomes, representing 200 unique genes, were significantly altered after bariatric surgery. Among the significantly regulated genes were gamma glutamyltransferase 1 (GGT1;-10% change, p=6.35E-05), cathelicidin antimicrobial peptide (CAMP -41% change, p=1.76E-04), alpha defensins (DEFA1, -45% change, p = 1.65E-03), lipocalin 2 (LCN2 -46%, p = 4.37E-03), TP53, ZNF684, GPR50, PDSS1, OLR1, CNTNAP5, DHCR24, HHAT and SARDH, which have been previously implicated in lipid metabolism and acute inflammation. Quantitative PCR showed >50% reduction of DEFA1, CAMP, CEACAM (carcinoembryonic antigen-related cell adhesion molecule) and ADIPOR1 (adiponectin receptor 1). The changes in expression of seven transcripts, WDR35, FLF45244, DHCR24, TIGD7, TOPBP1, TSHZ1, and FAM8A1 were strongly correlated with the changes in body weight, fasting plasma glucose and HbA_{1c} content (p < 0.05). Serum protein levels of ALT, VLDL and GGT activity reduced by >50% following surgery (P< 0.01). These preliminary data suggest that whole blood expression levels of specific transcripts that associate with inflammation and lipid metabolism are significantly altered by bariatric surgery and may be useful in the future for identifying biomarkers associated with susceptibility for T2DM and/or therapeutic response.

Sources of Research Support: NIH/NCRR.

Nothing to Disclose: SRK, SB, DS, PS, JS

P2-486

Adipose Expression of Stra6 Gene: Correlation with Insulin Sensitivity.

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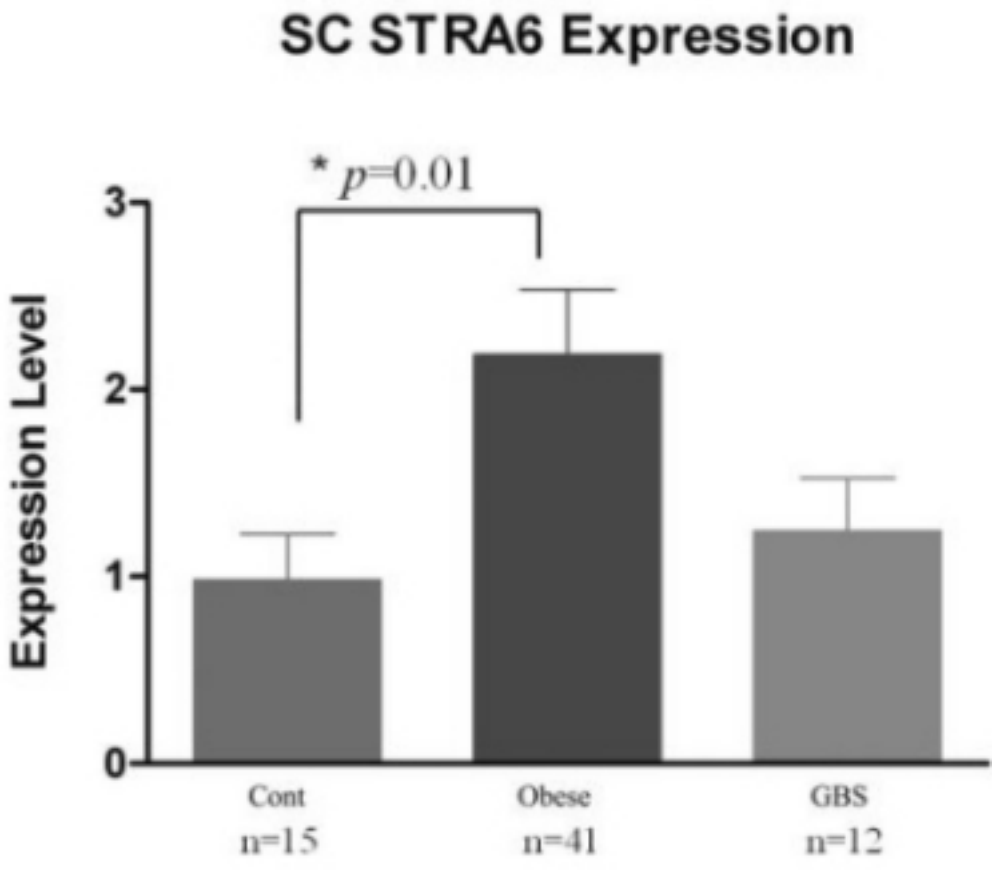
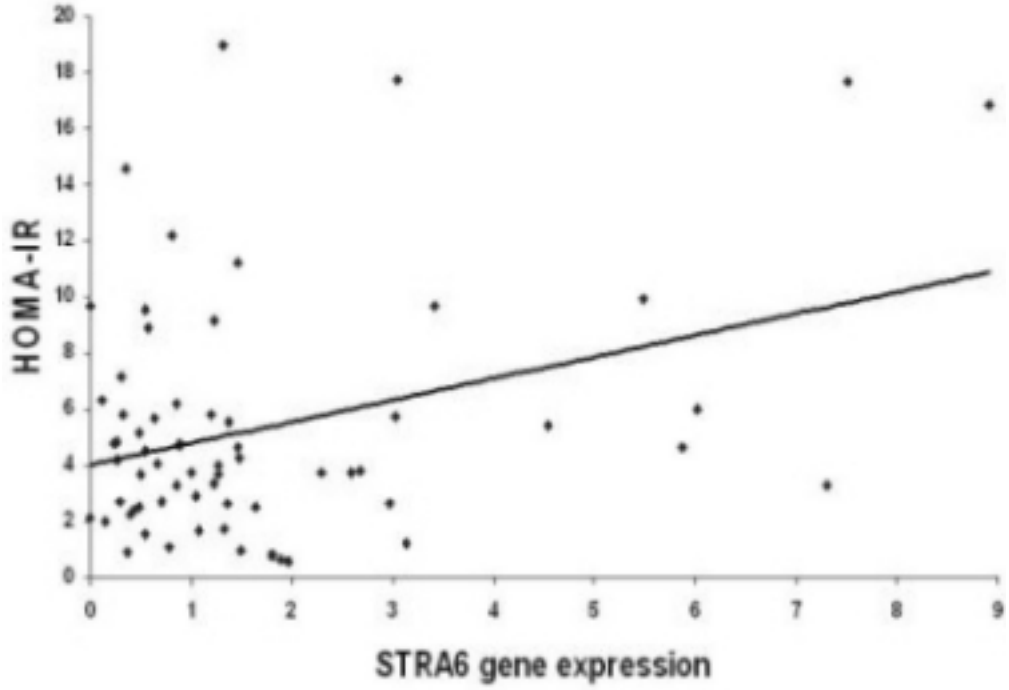
Introduction: Stra6 (*St*imulated by *r*etinoic *a*cid gene) is a recently identified high-affinity cell-surface receptor for RBP (Retinol binding protein) 4. RBP4 induces insulin resistance by impairing skeletal muscle insulin signaling and inducing liver gluconeogenesis. However, Stra6 is not expressed in skeletal muscles and has a low expression in liver. Stra6 expression in adipose tissue and its relationship with insulin resistance have never been evaluated. **Objective:** To evaluate Stra6 gene expression in paired visceral omental (OM) and subcutaneous (SC) adipose tissue, and determine the relationship with insulin sensitivity. **Methods:** SC and OM fat biopsies and fasting serum, were obtained from: 1) subjects with class II and III obesity undergoing gastric bypass surgery (GBS) (n=41); 2) subjects one year status post GBS undergoing elective surgery (n=12); 3) non-obese subjects (n=16) undergoing elective surgery. Obese and normal lean subjects were age and gender matched to the post-GBS group. Extracted total RNA was subjected to quantitative RT-PCR for adiponectin, and Stra6 gene expression. Sera were assayed for insulin, glucose, and high-sensitivity C-reactive protein. HOMA-IR was determined. **Results:** In obese subjects, Stra6 expression in the SC was significantly increased compared to lean control subjects (p=0.01) (Fig.1). Stra6 expression levels in OM were similar among groups. Stra6 expression in SC correlated with HOMA-IR (R=0.34, p=0.005) (Fig 2) and BMI (R=0.27, p=0.02). OM Stra6 expression levels negatively correlated to adiponectin expression (R=-0.26, p=0.04). **Conclusion:** Stra6 gene is expressed in human fat tissue, and expression correlates with insulin sensitivity. Considering the very high binding affinity of RBP4 for STRA6, this raises the possibility that STRA6 may have a role in mediating insulin resistance.





Sources of Research Support: NIH K23DK075907 to A.T.

Nothing to Disclose: JGC, AS, ZP, AT

P2-487

Dunnigan Type Lipodystrophy – Altered Adipocyte Distribution in Patients & Altered Adipocyte Differentiation in Cell Models.

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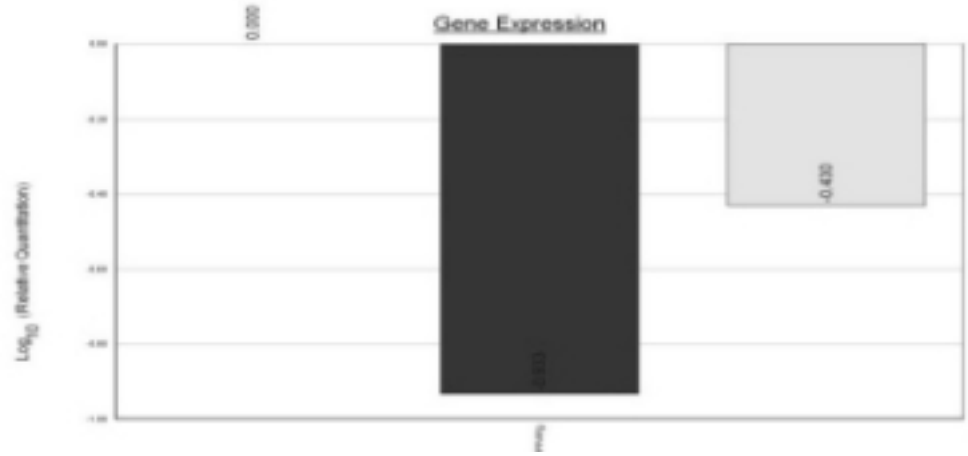
Mutations in the LMNA gene encoding A-type lamins cause several diseases, including Dunnigan-type familial partial lipodystrophy (FPLD).
Two adolescent subjects with a severe phenotype of FPLD due to the R482W LMNA gene mutation were treated with metformin for 14 months. Due to continued amenorrhoea, a trial of rosiglitazone was instigated. Rosiglitazone improved Insulin Resistance scores and increased adiponectin in patients with FPLD while redistributing adipose tissue (Table 1).

Table 1. Biochemical and radiological results.

	Patient A	Patient B
OGIS(ml/min/m2) Baseline	159	195
OGIS 14/12 Rx Metformin	437	341
OGIS 12/12 Rx Rosiglitazone	564	381
Neck adipose pad pre-rosiglitazone	462.9 cm3/ 416g	782.0 cm3/ 703g
Neck adipose pad 12/12 Rx rosiglitazone	973.4 cm3/ 876g	1155.2 cm3/ 1039g
Hepatic Fat Fraction Baseline (%)	24.7	26.5
Hepatic Fat Fraction 12/12 Rosiglitazone	10.3	2.9
Adiponectin(ug/ml)- Baseline	1.7	2.3
Adiponectin-12/12 Rosiglitazone	3.5	6.4

We used two cell lines to investigate the pathophysiology of FPLD. Over-expression of R482W mutated LMNA in 3T3-L1 cells reduced PPARγ expression on Day 2 of adipocyte differentiation when compared with those over-expressing wild-type and controls. We then induced adipocyte differentiation in LMNA gene knockout MEF's that had been tranfected with pcDNA3 plasmid containing wild type LMNA and R482W mutated LMNA. RT-PCR of these cells again showed a reduction of PPARγ expression on Day 2 of adipocyte differentiation in cells transfected with mutant LMNA as opposed to those transfected with wild type LMNA and controls (Figure1).



Lamin A has previously been described to inhibit adipocyte differentiation.Annual turnover of adipocyts in humans has recently been estimated to be as high as 10%(1). This study shows alterations in adipocyte differentiation of animal cell models as a result of over-expressing the R482W mutant as compared to over-expressing wild type LMNA. Site-specific adipocyte expression of mutant LMNA may underlie the phenotypic changes seen in FPLD - populations of adipocytes expressing more R482W mutated LMNA may have impaired ability to re-generate.

(1)Spalding et al., Nature 2008; 453(7196):783-7.

Nothing to Disclose: JOR, CMO, DB, VC, RO, TM, DJO

P2-488

Human AT Histologic Indices and Relation to Adiponectin.

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At the cellular level, as body weight increases, adipocytes respond by storing fat, thus growing in size. This reflection of whole body adiposity in individual cells has implications on the degree of insulin sensitivity of adipose tissue, with larger cells showing decreased insulin sensitivity relative to smaller cells. To assess this relationship, measurement of adipocyte size must be obtained. This measurement is technically difficult and is generally accomplished by one of three prevailing methods, namely histologic processing and optical analysis, sizing by the Coulter particle counter, and microscopy of osmium-fixed adipocytes. Histologic analysis is traditionally viewed as the preferred method due to the fact that cells are analyzed after only minimal processing. We postulated that with the appropriate size specifications, the Coulter particle counter method would relate well to measurements obtained from the histologic samples and both measures would relate to insulin sensitivity as reflected by circulating levels of total and high molecular weight (HMW) adiponectin. Serum and paired subcutaneous (S) adipose tissue (AT) and visceral (V)AT biopsy samples were obtained from 10 obese (BMI 45.9 ± 6.5) weight stable subjects undergoing Roux-en-Y surgical weight reduction. Biopsy samples were analyzed by each of the three methods. Only histologic measures of fat cell size correlated significantly with serum adiponectin levels. SAT fat cell diameter was inversely related to both total adiponectin (P=0.04) and HMW adiponectin (P = 0.05) and VAT fat cell diameter was inversely and significantly correlated with serum HMW Ad (P=0.01). VAT diameter showed a tendency to correlate with SA (P= 0.1). Neither the Coulter method nor osmium-fixed adipocyte microscopy related significantly to total or HMW adiponectin levels. Further study is needed to determine the specifications necessary for successful analysis using the remaining the coulter and osmium fixation methods. We conclude that of the three adipocyte sizing techniques, only histologic measures provide physiologically meaningful data on the relationship between adipocyte size and adiponectin, an indicator of insulin sensitivity.

Disclosures: RRH: Speaker Bureau Member, Novo Nordisk; Medical Advisory Board Member, Novo Nordisk.
Nothing to Disclose: LKM, TPC, WCW, SPP

P2-489

Influence of the Fat-Mass and Obesity-Associated Gene (*FTO)*, Uncoupling Protein *(UCP)1*, *UCP2* and *UCP3* Gene Polymorphisms with Extreme Obesity, Cardiovascular Risk Factors and Its Biogeographical Genomic Ancestry in Brazilian Population.

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Obesity has become the most common human disorder conveying a serious threat to health and quality of life through its association with diabetes, hypertension and cardiovascular diseases. Recently, several candidate genes were investigated to determine the genetic factors implicated in the pathogenesis of obesity, related metabolic disorders and diabetes. Variations in fat-mass and obesity-associated gene (*FTO*) are associated with obesity phenotype in many Caucasian populations. In addition, uncoupling protein *(UCP)-1, UCP-2, UCP-3*, which play a major role in thermogenesis, have been also associated with an increased risk of obesity. The association of these genes polymorphisms with the obesity phenotype and its biogeographical genomic ancestry in the Brazilian population has not been established.
Objective: Our aim was to examine the relationship between *FTO, UCP-1, UCP-2* and *UCP-3* genotypes with body mass index (BMI), cardiovascular risk factors and biogeographical genomic ancestry in a Brazilian population.
Methods: We genotyped single nucleotide polymorphism (SNPs) in the *FTO, UCP-1, UCP-2, UCP-3* genes from severely obese subjects [n=122, (BMI ≥ 35kg/m^2) and n=43, normal-weight control subjects (BMI <25kg/m^2)]. Genotyping of *FTO, UCP-1, UCP-2* and *UCP-3* gene polymorphisms were performed through allelic discrimination using real-time PCR. Each sample was independently typed for 40 biallelic short insertion/deletion polymorphism (indels). We used the software *Structure* to estimate the proportion of European, African and Amerindian biogeographical ancestry of each group. Height, weight, waist, blood pressure, glucose and serum lipids were also measured.
Results: We already analyzed severely obese subjects and normal-weight control subjects. Higher systolic blood pressure, diastolic blood pressure, waist, triglycerides, low density lipoprotein (LDL), total cholesterol and blood glucose levels were observed in obese than non-obese ($P < 0.01$). The distribution of European, African and Amerindian biogeographical ancestry of each group were not significantly different between the obese and the non-obese groups (87%, 9% and 6% respectively). The *FTO* SNPs was not found to be associated with BMI and severe obese in our sample.
Conclusion: Our data do not support the hypotheses *FTO* common variants are major contributors to obesity in a Brazilian population.

Nothing to Disclose: AVR, LB-R, BA, LC-V, MS, LDM

P2-490

Dietary Resistant Starch Increases Colonic Fermentation and Proglucagon and PYY Gene Expression in Healthy Aged Mice.

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Background: Resistant starch (RS) is a dietary fermentable fiber that resists digestion in the small intestine and is fermented in the large intestine by gut bacteria. Health benefits of dietary RS, such as improving insulin sensitivity and decreasing body fat, have been reported. GLP-1 and PYY are anti-diabetic/obesity hormones secreted in the proximal large intestine. We recently reported that young adult rodents fed RS exhibited increased bioactive, circulating GLP-1 and PYY, and higher mRNA expression of proglucagon (the gene that encodes GLP-1) and PYY in the large intestine, where colonic fermentation of RS occurs. We also found that RS-mediated increased insulin sensitivity and decreased body fat did not occur if colonic fermentation of RS was eliminated. The aforementioned effects of dietary RS have not been reported in aged mice.
Objective: To determine if healthy, aged mice can ferment dietary RS and increase colonic gene expression of proglucagon and PYY.
Method: Male C57BL/6J mice (20 month old) were divided into three dietary treatment groups based on their body weights for a 10 week study: 1) control, 2) 18% RS, and 3) 36% RS. Because fermentation of RS is accompanied by an enlarged cecum, at the end of the study, the full gastrointestinal (GI) tracts were removed and full and empty cecum weights were recorded. Cecal epithelial cells were collected for measurement of proglucagon and PYY mRNA expression by quantitative RT-PCR.
Results: As shown below, dietary RS increased full GI weight, full and empty cecum weights and proglucagon mRNA in a dose dependent manner. PYY expression increased only with 36% RS.

Intestinal weight (mg), Proglucagon and PYY mRNA (Fold changes)

Group	Full GI	Full Cecum	Empty Cecum	Proglucagon	PYY
Control	2.22 ±0.09 a	0.27±0.02 a	0.069±0.003 a	1.00±0.08 a	1.00±0.13 a
18% RS	2.94±0.14 b	0.53±0.04 b	0.112±0.006 b	1.90±0.15 b	1.46±0.26 a
36% RS	3.86±0.15 c	1.03±0.05 c	0.151±0.011 c	2.78±0.32 c	2.93±0.27 b

Data are mean±SE (n=13-15). Values that do not share a common letter differ by P<0.01.

Conclusion: These data suggest that in healthy aged male mice, as in their younger counterparts, dietary RS increases colonic fermentation, with subsequent increases in proglucagon and PYY gene expression.

Nothing to Disclose: JZ, MJK, JK, SOF-K, PJP, RTT, AMR, LS, HZ, RJM, MRB

P2-491

Pancreatic Acinar-Specific Overexpression of Reg2 Gene Offered No Protection Against Either Experimental Diabetes or Pancreatitis in Mice.

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Reg proteins are normally expressed in pancreatic acinar cells, the level of several members was significantly induced upon damage to the endocrine or exocrine pancreas. Among them, Reg1 and INGAP have been established to promote the growth or regeneration of the endocrine islet cells. Recent reports suggested that Reg2 was an autoantigen normally expressed in islet ß-cells (1); its overexpression in vitro offered protection to insulinoma cells; Reg3α stimulated proliferation of insulinoma cells; and acinar-specific overexpression of INGAP increased ß-cell mass and protected the animals from streptozotocin-induced diabetes (2). Moreover, Reg2 gene expression was induced during pancreatitis and thought to be protective to acinar cells. We hypothesized that Reg2 is a secreted protein promoting the growth, survival and/or regeneration of pancreatic endocrine and exocrine cells. In order to test its effectiveness, we developed an acinar cell-specific overexpression of Reg2 gene using elastase-1 promoter (Ela-Reg2). Using Western blot and immunohistochemistry, Reg2 was hardly detectable in normal wild-type pancreas; Ela-Reg2 mice exhibited increased Reg2 level in the pancreas of multiple founder lines. Compared to wild-type littermates, Ela-Reg2 mice were normal in growth, blood glucose and insulin levels and glucose tolerance; pancreatic histology revealed no obvious change in either the endocrine or exocrine tissues. To test its possible role in protecting the pancreas, we challenged the mice with experimental diabetes and pancreatitis. Thus, acinar-specific overexpression of Reg2 gene offered no protection against streptozotocin-induced ß-cell damage and diabetes, in hyperglycemia and weight loss, and exhibited no advantage in restoring glucose homeostasis and islet function within 3 months. Moreover, Reg2 overexpression did not protect acinar cells against caerulein-induced acute pancreatitis based on serum amylase level and pancreatic histochemistry. In contrast to INGAP or Reg3ß, exocrine overexpression of Reg2 offered no protection either to the endocrine or exocrine pancreas, indicating clear subtype specificities within the family of Reg proteins.

(1) Gurr W et al., Diabetes 2007; 56:34
(2) Taylor-Fishwick DA et al., J Endocrinol 2006; 190:729

Sources of Research Support: CIHR Grants MOP-84389, CCI-85675; Canadian Diabetes Association grant IG-1-07-2305-JL; National Natural Science Foundation of China grant 30700382; senior Chercheurs-boursier of Fonds de la recherché en santé Quebec.

Nothing to Disclose: BL, XW, J-LL

P2-492

Atypical Antipsychotics Influence Intestinal Hormones That Enhance Insulin Secretion in Rats.

S Han[1], EW Englander PhD[1] and GH Greeley PhD[1].

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Long-term treatment of psychiatric patients with atypical antipsychotic drugs is associated with development of obesity, impaired glucose tolerance, and insulin resistance. The extent to which atypical antipsychotics acutely alter glucose and insulin secretion independent of obesity is not known. The aim of these experiments was to define the effects of acute clozapine treatment (20 mg/kgBW/day, 4 days) on glucose disposal and insulin secretion in response to either an oral or intraperitoneal glucose tolerance test in Sprague-Dawley rats. Whether clozapine treatment alters secretion of glucagon-like peptide-1 [GLP-1] was examined. Additionally, the effects of clozapine, risperidone and ziprasidone (10^{-4}-10^{-6} M) on insulin secretion in response to glucose or glucose + GLP-1 stimulation were examined in vitro using cultured β-cells. **Results.** Acute clozapine treatment in vivo impaired glucose disposal and glucose-induced insulin secretion significantly ($p<0.05$). Oral glucose-induced GLP-1 secretion was decreased significantly by clozapine treatment. Acute exposure of β-cells to clozapine, risperidone or ziprasidone reduced glucose- and GLP-1 + glucose-induced insulin secretion ($p<0.05$). **Conclusions and Discussion**. The present findings show that acute clozapine treatment in vivo impairs glucose and insulin homeostasis independent of obesity. Furthermore, in vivo and in vitro experiments indicate that clozapine, risperidone, and ziprasidone act directly on the β-cell to impair glucose- and glucose + GLP-1-induced insulin secretion. In addition, clozapine exposure reduces oral nutrient-induced GLP-1 secretion. Furthermore, a compromised GLP-1 secretion plus a reduced insulin releasing activity of GLP-1 may underlie the decreased insulin secretion in rats treated with clozapine. These findings imply that atypical antipsychotics impair glucose and insulin homeostasis independently of obesity in patients.

Nothing to Disclose: SH, EWE, GHG

P2-493

GLP-1 Compensates for Poor Proliferation of β-Cells Lacking Insulin Receptors.

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Insulin and insulin-like-growth-factor-1 (IGF-1) signaling pathways regulate pancreatic β-cell proliferation and function. Mouse models exhibiting impaired insulin/IGF-1 signaling in β-cells mimic features of human type 2 diabetes by manifesting age-dependent decrease in β-cell mass and poor nutrient-stimulated insulin secretion. The incretin hormone, glucagon-like peptide-1 7-36-amide (GLP-1), augments glucose-stimulated insulin secretion and has been harnessed for the treatment of type 2 diabetes. Although GLP-1 activates several proteins in the insulin/IGF-1 signaling pathway, its direct effects on β-cell proliferation in insulin resistant states are not fully understood. Therefore, in this report, we examined the effects of GLP-1 on proliferation of β-cells lacking insulin receptors (βIRKO) compared to controls. In control β-cells, acute GLP-1 stimulation (10 nM, 15 min) enhanced phosphorylation of Akt, both in the basal state and when insulin exocytosis is blocked by the Ca^{2+} channel blocker, nifedipine, suggesting that GLP-1-induced Akt phosphorylation is independent of insulin action. This conclusion was confirmed by the ability of GLP-1 to phosphorylate Akt in βIRKO cells. Meanwhile, chronic activation of GLP-1 signaling by Exendin-4 (Ex-4; 10 nM, 24 h) significantly increased phosphorylation of Akt and expression of cyclin A, D1, and E, in βIRKO but not in control β-cells. Ex-4 treatment also significantly increased cyclin D2 expression in controls, while, interestingly, cyclin D2 was virtually undetectable in βIRKO cells. Re-expression of insulin receptors in βIRKO cells attenuated the upregulation of cyclins by Ex-4. In control cells, Ex-4 treatment upregulated both insulin and IGF-1 receptor expression, while in βIRKO cells IGF-1 receptor expression was already enhanced in the basal state. These data suggest that GLP-1 upregulates cyclin A/D1/E to compensate for β-cells lacking insulin receptors and cyclin D2 expression, in part, via IGF-1 signaling. Consistent with these data, Ex-4 treatment upregulated cyclin D1 expression in islets from patients with type 2 diabetes. Taken together, these data suggest that GLP-1 treatment is able to compensate for impaired insulin signaling to maintain β-cell proliferation. These observations have therapeutic implications for the use of GLP-1 in improving β-cell mass and function in patients with type 2 diabetes who manifest insulin resistant β-cells.

Nothing to Disclose: DK, CWL, RNK

P2-494
Translational Research of Ghrelin and GLP-1.

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The molecular mechanisms of energy balance are coming to light by the recent robust progresses in the endocrinology and molecular biology. The gastrointestinal tract produces a large array of substances to regulate feeding. Ghrelin, a 28-amino acid peptide, is produced mainly by gastric endocrine cells and stimulates hunger centers in the hypothalamus. Peripherally administered ghrelin preferentially enhanced carbohydrate intake. At present, ghrelin is the only peptide to transmit hunger information from the periphery to the brain. We have clarified that the vagal afferent is the major pathway conveying signals of gut hormones to the brain. We presented therapeutic potential of ghrelin for the improvement of quality of life in consumptive patients with intractable pulmonary diseases. Ghrelin suppressed proinflammatory cytokines expression in leukocytes and this anti-inflammatory activity had a potency to suppress the development of cachexia induced by cancer progression. Ghrelin administration to acute lung injury model mice suppressed neutrophils accumulation in the lungs and extended their survival time. We also showed that ghrelin prevented and ameliorated experimental diabetic polyneuropathy induced by streptozotocin in mice. We extended clinical application of ghrelin to type 2 diabetic patients. Ghrelin also improved muscle atrophy in mice model of disused muscle atrophy. Ghrelin is highly likely to be used as a novel peptide drug for anorexia nervosa, cancer, diabetes mellitus, and chronically exhausted disorders. Glucagon-like peptide 1 (GLP-1) is a peptide produced in the endocrine L cells of the distal intestine. GLP-1[7-36]NH_2 is a major molecular form that stimulates insulin release, reduces food intake, and has a potential to promote β-cell regeneration. We have developed a device for intranasal application of GLP-1[7-36]NH_2 and completed a double-blind clinical trial of intranasal administration of GLP-1[7-36]NH_2 to 26 type II diabetic patients. Intranasal administration of GLP-1 increased its plasma level, stimulated postprandial insulin release, and suppressed glucagon release. This clinical study improved glucose metabolism in the patients. I will present translational research of ghrelin and GLP-1 for the treatment of endocrine disorders.

Nothing to Disclose: MN

P2-495

The Central Nervous System Effects of Glucagon-Like Peptide-1 on Peripheral Glucose Homeostasis.

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Glucagon-like peptide-1 (GLP-1) is a gut peptide that promotes glucose homeostasis through regulation of islet-cell hormone secretion, gastric emptying, and hepatic function. GLP-1 is also synthesized in the brain, and recent findings indicate that central GLP-1 plays a role in the regulation of peripheral glucose homeostasis through effects on insulin secretion, hepatic glucose production, and insulin sensitivity. To gain further insights into those processes, we investigated the effects of central GLP-1 receptor (GLP-1r) activation and antagonism on insulin and glucagon release before and during experimental hyperglycemia in rats.

Thirty male Long Evans rats were equipped with third cerebral ventricular (i.c.v.) cannulae and catheters in the carotid artery and jugular vein. After an overnight fast freely-moving animals had continuous i.c.v. infusion with GLP-1 (18 µg/hr), the GLP-1r antagonist Exendin 9-39 (Ex-9) (100 µg/hr), or saline for 125 min; all rats received 8 µl/hr of infusate. Sixty min after the start of i.c.v. infusion, blood glucose was raised to 230 mg/dl with a primed i.v. infusion of 25% glucose. For 65 min the glucose infusion rate was varied to maintain hyperglycemia (230 mg/dl). Arterial blood was sampled during the infusion period every 5-15 min for measurements of blood glucose and plasma levels of insulin, glucagon, and corticosterone.

Infusion of i.c.v. GLP-1 increased fasting blood glucose levels, compared to the saline control (105.5 ± 3.8 vs 91.5 ± 2.9 mg/dl), reduced the average glucose infusion rate required to maintain the glucose clamp, and significantly reduced plasma insulin levels compared to the saline group ($p < 0.05$ for each). GLP-1-treated rats also had significantly higher glucagon and corticosterone ($p < 0.05$ for each) levels than Ex-9- and saline-treated rats. Surprisingly, there was also a trend for i.c.v. Ex-9 to reduce insulin levels ($p = 0.1241$) and raise plasma glucagon ($p = 0.1009$).

These results show that central GLP-1r activation and GLP-1r blockade do not lead to opposing effects on glucose homeostasis in rats. In the doses used in this study GLP-1 activates the stress response, likely causing autonomic suppression of insulin secretion and stimulation of glucagon secretion. Central GLP-1r blockade using Ex-9 is also associated with lower insulin secretion and higher glucagon levels, supporting a role for brain GLP-1 in the regulation of islet function and peripheral glucose homeostasis.

Nothing to Disclose: LJ, TMG, MEI, DAS, RJS, DAD

P2-496

Intravenous Insulin Infusion Increases Regulatory T Cell Population and Suppresses CD14+/CD16+ Monocyte Population.

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We have previously shown that low dose intravenous insulin infusion suppresses inflammation and oxidative stress in circulating mononuclear cells (MNC).
We now hypothesized that insulin infusion induces an increase in the anti-inflammatory regulatory T cells (Treg) population and suppresses the pro-inflammatory CD16+/CD14+ monocytes and activated T cells population. Eight healthy obese subjects were infused with 3.5 U/h insulin, and glucose to maintain euglycemia, for 8 hr in one visit and with only 5% glucose or saline (100ml/h)in another two separate visits and blood samples collected. MNC fraction was separated and stained with specific antibodies for Treg (CD4+, CD25+ and Foxp3+), activated monocytes (CD14+/CD16+) and activated T cells (CD3+/CD146+) and cell count measured by flow cytometry. Following insulin infusion, plasma insulin concentrations increased by 6 folds (from 13.2 to 73.4 mU/ml) while glucose levels did not change. Insulin infusion induced a significant increase in the population of Tregs by 22±6% over the baseline ($P<0.05$) while suppressing the pro-inflammatory CD14+/CD16+ monocytes by 20±5% below the baseline ($P<0.05$) at 8 hr. There was no significant change in activated T cell population following insulin infusion. These changes were associated with significant fall in C- reactive protein (CRP) concentrations by 31±6% below the baseline (from 4.1±1.1 to 3.2±1.0mg/L, $P<0.05$) at 8 hr following insulin infusion. Glucose or saline infusions alone did not alter any of the cell populations. In conclusion, a low dose infusion of insulin results in a significant increase in Treg while decreasing activated monocytes population and CRP concentrations, a marker of systemic inflammation. The induction of Treg cells and the suppression of pro-inflammatory activated monocytes may contribute to the comprehensive anti-inflammatory effect of intravenously administered insulin.

Disclosures: PD: Speaker, Sanofi-Aventis, Lilly USA, LLC, GlaxoSmithKline, Amylin Pharmaceuticals; Research Funding, Sanofi-Aventis, GlaxoSmithKline, Amylin Pharmaceuticals.
Nothing to Disclose: LZ, HG, TL

P2-497

Potential Cross-Talk between Glucagon-Like Peptide-1 Receptor (GLP1R) and Receptor Tyrosine Kinase (RTK) in Pancreatic β Cells.

Eun Bee Cho PhD candidate[1], Mi Jin Moon PhD candidate[1], Jong-Ik Hwang PhD[1] and Jae Young Seong PhD[1].

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Glucagon-like peptide-1 (GLP-1) decreases postmeal glucose excursions by enhancing insulin secretion via activation of the GLP-1 receptor in b cell. In pancreatic b cells, many receptor tyrosine kinases (RTKs) as well as GLP1R are involved in insulin secretion and b cell proliferation. However, cross-talk between RTKs and GLP1R has been poorly understood. This study attempted to identify the RTK signaling pathway that regulates GLP1R activity. In INS-1 cells, rat pancreatic b cells, GLP1R activity was increased by RTK signaling inhibitors, PP2 (src inhibitor), genistein (RTK inhibitor) and LY294002 (PI3K inhibitor). In addition, pretreatment of b cells with AG1478, an EGFR inhibitor and HNMPA, an insulin receptor inhibitor significantly increased GLP1R activity. This result suggests potential cross-talk between GLP1R and RTKs in insulin secretion and proliferation of b cells.

Nothing to Disclose: EBC, MJM, J-IH, JYS

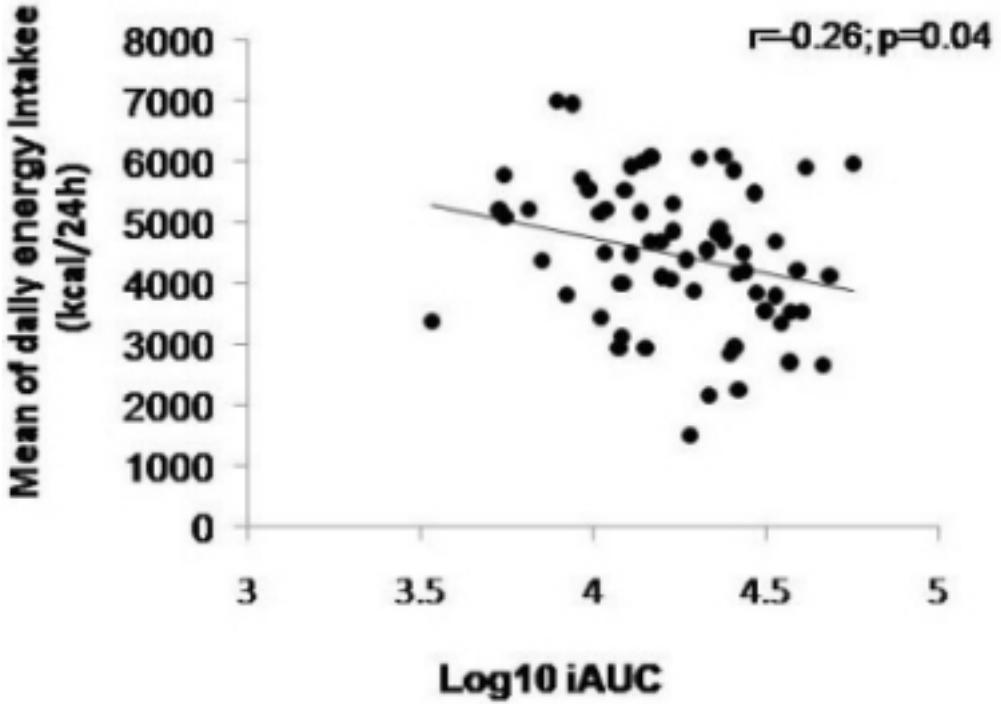
P2-498

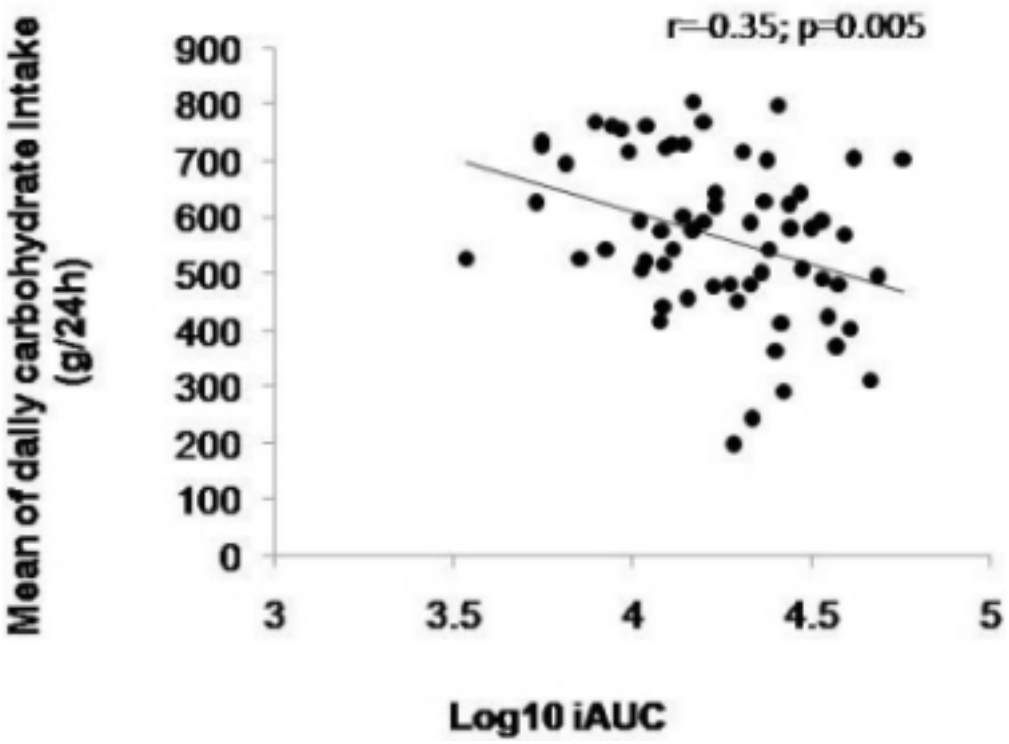
Higher Incremental Insulin Area under the Curve in Response to an Oral Glucose Tolerance Test Predicts Lower Food Intake.

Jianying He MD.,Ph.D[1], Susanne Votruba Ph.D.[1], Colleen Venti MS., RD[1] and Jonathan Krakoff MD.[1].

[1]Natl Inst of Hlth Phoenix, AZ.

Weight gain occurs when energy consumption exceeds energy expenditure. Thus understanding factors which may regulate food intake is crucial. Central administration of insulin has been shown to reduce food consumption in animals and lead to weight loss in men. However, the role of peripheral insulin in modulating energy intake is unclear. We investigated the association of peripheral plasma insulin concentrations in response to an oral glucose tolerance test with subsequent measures of ad libitum food intake in 67 Pima Indians with normal glucose regulation (fasting glucose < 100 mg/dl and two hour glucose < 140 mg/dl) (BMI= 34.2± 9.4 kg/m^2, 63% male). Ad libitum food intake was measured using a computerized vending machine system over 3 days. Plasma insulin incremental area under the curve (iAUC) did not differ by sex. iAUC was negatively associated with the mean of ad libitum daily energy intake (DEI) (r=-0.26, p=0.04), percent weight maintenance energy needs (%WMEN) (r=-0.38, p=0.002), and with carbohydrate intake (g/day) (r=-0.35, p=0.005). In models adjusted for age and sex, the association of iAUC with DEI was attenuated (p=0.06) but still strong with %WMEN and carbohydrate intake (p=0.005 and p=0.008). In conclusion, in Pima Indians with normal glucose regulation, higher plasma insulin iAUC in response to an OGTT predicted lower food intake and in particular lower carbohydrate consumption indicating a possible physiologic role for insulin as a negative feedback signal in the regulation of energy intake.





Woods SC et al., Nature 1979;282:503-5
Hallschmid M et al., Diabetes 2004;53:3024-9

Sources of Research Support: Intramural Research Program of the NIH, NIDDK.

Nothing to Disclose: JH, SV, CV, JK

P2-499

Detecting Incretin and Pancreatic Biomarkers on Multiple Assay Platforms Utilizing a Novel Sample Extraction Method.

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[1]Pfizer Inc Groton, CT and [2]Mercodia Inc Winston-Salem, NC.

Incretin and pancreatic hormone analysis is an important component in understanding post prandial glucose homeostasis. In rodents, this is often assessed by a glucose tolerance test with serial blood sampling. Major limitations to this model are the small blood volumes obtained and the low baseline (fasting) values for analytes of interest. To address this, we have developed a solid phase extraction (SPE) method that minimizes matrix effect and improves sensitivity on the subsequent analyte measurement. Samples and standards, ranging in volume of 50-150 ul, are subjected to the SPE extraction on an Oasis HLB 60 mg plate. Samples are then reconstituted in a standard buffer (Linco GLP-1 buffer) which does not interfere with any of the assays. Sample aliquots are then analyzed on multiple assay platforms (electro-chemiluminscent sandwich assays, bead immunoassays, and enzyme-linked immunoassays) using commercially available kits to detect active GLP-1, total amide GLP-1, total PYY, GIP, C-Peptide and insulin. The assays were qualified to show good inter and intra assay variability (< 20%). In positive control experiments, this assay showed significant increases in total amide GLP-1, GIP and C-Peptide for a 6-point AUC (-60 to 120 min) in rats treated with a GPR119 agonist prior to an OGTT. With this novel technology, we can now measure multiple analytes from a single, extracted small-volume sample with increased sensitivity at the lower end of detection.

AUC, Fold Change Vs Vehicle

Treatment	Gluc	Ins	C-Peptide	Total Amide GLP-1	GIP	PYY
GPR119 Agonist	-0.84	1.33	1.72*	2.58*	2.26*	1.12

Disclosures: CA: Employee, Mercodia Inc. CWJ: Employee, Mercodia. JLT: Employee, Pfizer, Inc.
Nothing to Disclose: DGP, DEM, JEE, JJ, GP

P2-500

Dynamic Association of Ghrelin with Insulin, Cortisol, and Free Fatty Acids in the Fed and Fasted State.

Leon S Farhy PhD[1], Ralf Nass MD[1], Jianhua Liu PhD[1], Bruce Gaylinn PhD[1], Michael L Johnson PhD[1,2], Mark L Heiman PhD[3] and Michael O Thorner MD[1].

[1]Univ of Virginia Hlth Syst Charlottesville, VA ; [2]Univ of Virginia Charlottesville, VA and [3]Eli Lilly and Co Indianapolis, IN.

Ghrelin is a gut peptide that regulates GH, appetite and metabolism. It increases before meals and at midnight, but decreases with long-term fasting. Insulin and cortisol suppress ghrelin secretion. Acylation of serine^3 with octanoate is essential for biological activity of ghrelin. We use a differential equation-based mathematical model of acyl-ghrelin release to test whether variations in circulating insulin, cortisol and FFA are associated with changes in acyl-ghrelin in the fed and fasted state. The construct describes ghrelin release as negatively associated with insulin, cortisol, and FFA and was tested on data from eight young men studied on a fed and long-term fasting admissions. Acyl-ghrelin, insulin and cortisol were measured every 10 min and FFA (C6, C8, C10, C12, C14, C16, C16:1, C18) every hour for 24 hours during a fed admission when 3 standard meals were served and for another 24 hours from 37.5 to 61.5 hours of fasting. Eight models differing only by the specific FFA were tested for their ability to explain the variation in ghrelin concentration: Tables 1 and 2.

Table 1. Percent variation in acyl-ghrelin concentration explained by models based on the FFA C6, C8 and C10

Model	C6	C8	C10
Variation explained (MEAN±SD)	29.50±16.23%	7.28±13.43%	1.76±3.45%

Table 2. Percent variation in acyl-ghrelin concentration explained by models based on LCFA.

Model	C12	C14	C16	C16:1	C18
Variation explained (MEAN±SD)	39.42±29.21%	44.12±26.81%	59.84±18.87%	59.25±20.29%	54.65±25.93%

The best explanation of the acyl-ghrelin dynamics was provided by the models incorporating long chain FFA C16 and C16:1, followed closely by C18. The models including FFA C14 and C12 explained less of the variation (NS). The MCFA C10 and C8 models explained a small percent of the variation, significantly lower ($p<0.05$) than the variation explained by the LCFA models. The C6 model explained some of the data variation performing better ($p=0.01$) than the C10 and C8 models, but worse ($p<0.05$) than the C16, C16:1, and C18 models. To study the significance of including C16:1 into the model the C16:1 model was compared to a model using only insulin and cortisol. The Akaike information criterion showed that including C16:1 resulted into a model improvement in 70% of the cases. This findings suggests a relationship between insulin, cortisol and long-chain FFA and ghrelin regulation both in the fed and fasting state.

Sources of Research Support: NIH grants DK076037; HD28934; AG032555; RR-00847; RR019991; DK064122.

Nothing to Disclose: LSF, RN, JL, BG, MLJ, MLH, MOT

P2-501

12-Hydroxyeicosatetraenoic Acid (12-HETE) Play an Important Role in Diabetic Cardiomyopathy.

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[1]Jikei Univ Sch of Med Tokyo, Japan.

Adverse effects of hyperglycemia on endothelial function, interstitial fibrosis and cardiomyocytes are known as pathogenesis of diabetic cardiomyopathy. But precise mechanism underlying diabetic cardiomyopathy is still unknown. On the other hand, it has been reported that 12-lipoxygenase (12-LO) which gene is one of the arachidonate cascade pathway plays a dominant role in the development of arteriosclerosis and the cell growth of cardiac fibloblast. Moreover, it has been also reported that 12-LO knockout mice were highly resistant to diabetes development compared with control mice. Therefore, 12-LO pathway may play a part in the diabetic cardiomyopathy. Then, we examined the role of 12-LO in cardiomyopathy by using the diabetic cardiomyopathy rat model. The diabetic cardiomyopathy rat was induced by a single percutaneous injection of streptozotocin. Echocardiogram revealed Fractional Shortning was decreased in the cardiomyopathy rat model model compared with that of control rats.When we extracted RNA from the heart and examined, not only natriuretic peptide (BNP), a marker of cardiac failure, but also mRNA level of 12-LO and its major metabolites 12-hydroperoxy-eicosateraenoic acid (12- HETE) were elevated in the heart of diabetic cardiomyopathy rat model compared with those of control rats. The in vitro study demonstrated that hyperglycemia stimulation induced 12-LO and 12-HETE expression which promoted cardimyocyte inflammation and apoptosis. We also detected higher plasma level of 12-HETE in the diabetic patients compaired to the subjects with normal glucose tolerance. Interestingly, in the diabetic patients, higher level of plasma 12-HETE and CRP were observed which left ventricular systolic function was impaired in echocardiography. These results together suggest that 12- HETE may play a part in the pathogenesis of diabetic cardiomyopathy.

Nothing to Disclose: MS, HS, KT, NT

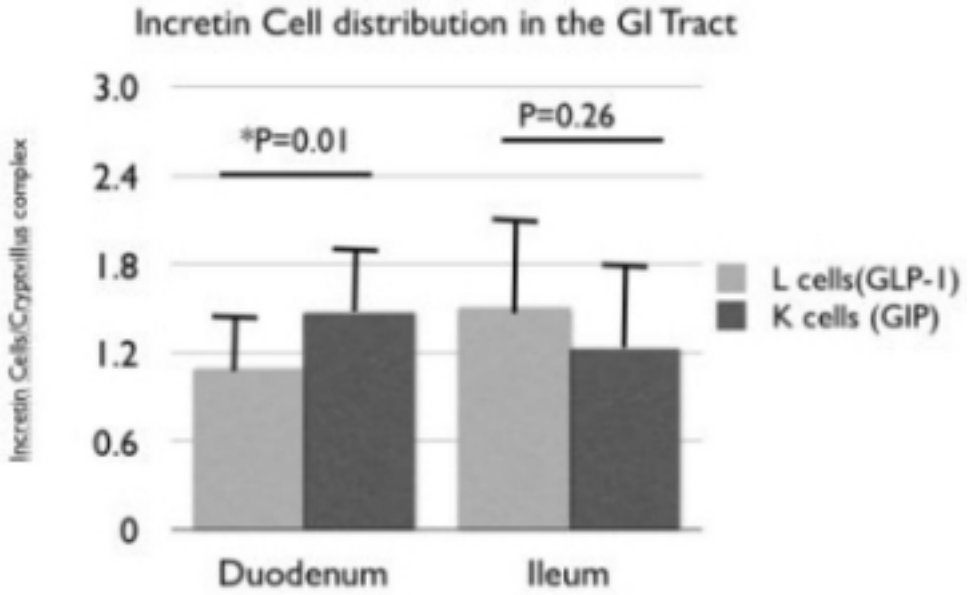
P2-502

New Map of the GLP-1 and GIP Producing Incretin Cells in the Non-Diabetic, Small Intestinal Tract of Humans.

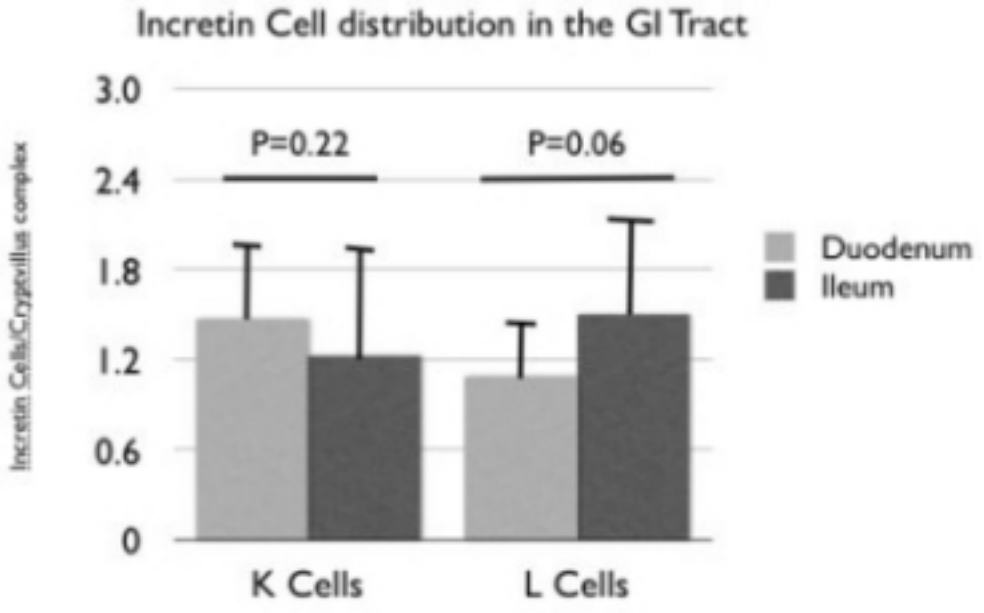
WA Lee DO[1], EO Beale MD[1], V Yu MD[2] and D Cortez MD[2].

[1]USC, Keck Sch of Med Los Angeles, CA and [2]USC Los Angeles, CA.

Background: The incretin system has gained wide popularity through its contribution to our understanding and management of type 2 diabetes. It has been widely accepted that GLP-l producing L cells resided mainly in the ileum and very little in the duodenum.(1) For GIP producing K cells, these were the opposite, with predominance in the duodenum. Our purpose was to re-evaluate the presense of L and K cells throughout the human small intestinal tract without diabetes.
Methods: Through a query of paraffin embeded intestinal specimens collected at the Los Angeles County Medical Center, we selected normal reported biopsies of patients without a known history of diabetes. 18 patient duodenal samples and 15 patient terminal ileum samples. Samples were immunostained for GLP-1(L cells) and GIP(K cells). Manual counting was reported by incretin Cells(L or K) per Crypt Villus Complex(CVC).
Results: 18 patient's duodenal samples. K/CVC was 1.47(+/-0.44). L/CVC was 1.09(+/-0.41). There was slightly more K cells than L cells(P=0.01), the difference of 0.38 cells/CVC.



15 patient's terminal ileum samples. K/CVC was 1.23(+/-0.63). L/CVC was 1.50(+/-0.71). There was no statistical difference between K and L cells in the K and L cells P=0.26. Comparison between the duodenum and ileum regions: No difference in the K/CVC (p=0.22). No difference in L/CVC p=0.06.



Conclusion: In mapping the incretin cells of non-diabetic humans, L cells and K cells are found throughout the GI tract. This is contrary to current established locations of these incretin cells. Surprisingly, there is no exclusive predominance

of either cell types in the proximal or distal regions of the small intestinal tract. Futher studies will need to be performed to confirm this finding.

(1) Holst JJ. Physiol Rev. 2007 Oct;87(4):1409-39.
(2) Ranganath LR. Clin Chem Lab Med. 2008;46(1):43-56. Review.
(3) Bryant MG, Bloom SR, Polak JM, Hobbs S, Domschke W, Domschke S, Mitznegg P, Ruppin H, Demling L. Measurement of gut hormonal peptides in biopsies from human stomach and proximal small intestine. Gut 24: 114–119, 1983.
(4) Eissele R, Goke R, Willemer S, Harthus HP, Vermeer H, Arnold R, Goke B. Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. Eur J Clin Invest 22: 283–291, 1992.

Nothing to Disclose: WAL, EOB, VY, DC

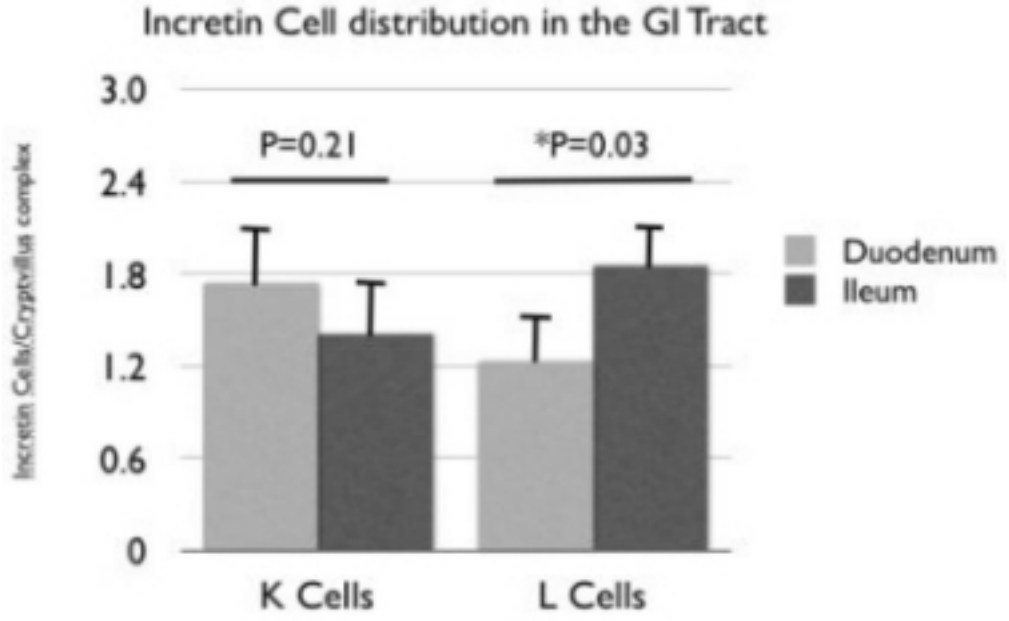
P2-503

Higher Density of GLP-1 Producing L Cells in the Distal Small Intestinal Tract of Diabetes Patients.

WA Lee DO[1], EO Beale MD[1] and D Cortez MD[1].

[1]USC, Keck Sch of Med Los Angeles, CA.

Background: Incretin deficiency has been shown in patients with diabetes.(1) Our previous mapping of incretin cells in non-diabetic patients revealed newly discovered, abundance of K(GIP) and L(GLP-1) cells throughout the small intestinal tract. Our aim in this study was to map the incretin cell distribution in diabetes patients and to discern if incretin deficiency is reflected in these cell density maps.
Methods: Through a query of the paraffin embeded small intestinal specimens at the Los Angeles County Medical Center, we selected normal reported biopsies from human gastrointestinal tracts. From this cohort, we selected for patients who were known to have diabetes. 6 patients with duodenal samples and 5 patients with ileum sample were identified. Manual method of counting using incretin cells(K or L)to Crypt Villus Comples(CVC) was employed.
Results:
Duodenum: 6 DM patient's duodenal samples. K/CVC was 1.74(+/-0.46). L/CVC was 1.23(+/-0.42). Unlike nondiabete patients, there was no significant difference between the K and L cell distribution.(P=0.07)
Ileum: 5 patient's terminal ileum samples. K/CVC was 1.41(+/-0.35). L/CVC was 1.85(+/-0.40). There was no statistical difference between K and L cells (p=0.10).
Comparing the K/CVC between the duodenum and ileum, there is no difference in the K cell density, (p=0.27).
The L/CVC difference between duodenum and ileum was statistically significant. (p=0.03) This was different compared with the nondiabetic pattern.



Discussion: This is the first human study showing the unique density of incretin cells in the distal small intestinal tract of diabetic patients. In diabetes patients, the K cell predominance over L cells is lost. In addition, the L cells are at a higher density in the ileum. More studies need to be done to confirm and evalaute these findings.

(1) Holst JJ. Physiol Rev. 2007 Oct;87(4):1409-39. Review.

Nothing to Disclose: WAL, EOB, DC

P2-504

Evidence in Support of Luminal Versus Circulating Octanoate for Ghrelin O-Acyl Transferase (GOAT)-Mediated Acylation of Ghrelin.

MO Thorner MBBS, DSc, FRCP[1], R Nass MD[1], BD Gaylinn PhD[1], J Liu MD[1], SS Pezzoli BA, MT[1] and ML Heiman PhD[2].

[1]Univ of Virginia Charlottesville, VA and [2]Eli Lilly & Co Indianapolis, IN.

Ghrelin is a 28-amino acid peptide with GH-releasing and appetite-stimulating properties, produced predominantly by stomach X/A cells. Ghrelin O-acyl transferase (GOAT) has been identified as the enzyme that attaches an 8-carbon fatty acid (octanoate) through a ester linkage to serine-3 of ghrelin (1,2); this acylation is necessary for its activity at the ghrelin receptor. We have previously demonstrated that fasting for >37.5 h in healthy young men (mean ± SD age 24.5±3.7; BMI 24±2.1 kg/m^2) leads to suppression of acyl-ghrelin and tonic secretion of desacyl-ghrelin, while total ghrelin secretion is not altered (3). In the same study, serum samples were collected hourly for 24 h in the fed state and during the last 24 h of a 61.5-h fast (from 37.5-61.5 h) for free fatty acid (FFA) analysis. Levels of medium chain (MCFA) and long chain (LCFA) fatty acids were measured in a highly sensitive LC/MS method developed for quantitative determination of C6-C18 FFA. Acidified plasma samples were extracted with ethyl acetate and separated using reverse phase HPLC column, coupled with mass spectrometer operated in negative APCI mode. Seven saturated and one unsaturated (C16:1) acid were detected using selected ion monitoring (SIM) technique and quantified in ratio to five stable-isotope labeled internal standards. 24-h mean FFA results are shown below.

FED ADMISSION

C6	C8	C10	C12	C14	C16	C16:1	C18
504 ± 22	404 ± 12	444 ± 23	776 ± 52	10 ± 0	98 ± 3	4 ± 0	31 ± 1

FASTED ADMISSION

C6	C8	C10	C12	C14	C16	C16:1	C18
602 ± 23	361 ± 14	372 ±12	1056 ± 43	16 ± 0	218 ± 6	17 ± 1	64 ± 2

Data are mean±SEM; n=8 (except C6 & C14 n=7)

24-h mean MCFAs were not lowered by fasting, however, LCFA concentrations were increased with fasting. Animal data suggest that luminal MCFAs provide the substrate for GOAT. Since acyl-ghrelin falls, but circulating MCFAs do not, this suggests that the decline in acyl-ghrelin during long-term starvation results from loss of substrate (octanoate) in the gastrointestinal lumen, rather than from blood. An alternative explanation is that long term starvation suppresses GOAT activity.

(1) Gutierrez et al. PNAS 2008; 105:6320
(2) Yang et al. Cell 2008; 132:387
(3) Liu J et al., JCEM 2008; 93:1980

Sources of Research Support: NIH Grants: RO1 DK076037 (MOT); MO1 RR00847 (UVA GCRC); K23 RR018770 (RN).

Nothing to Disclose: MOT, RN, BDG, JL, SSP, MLH

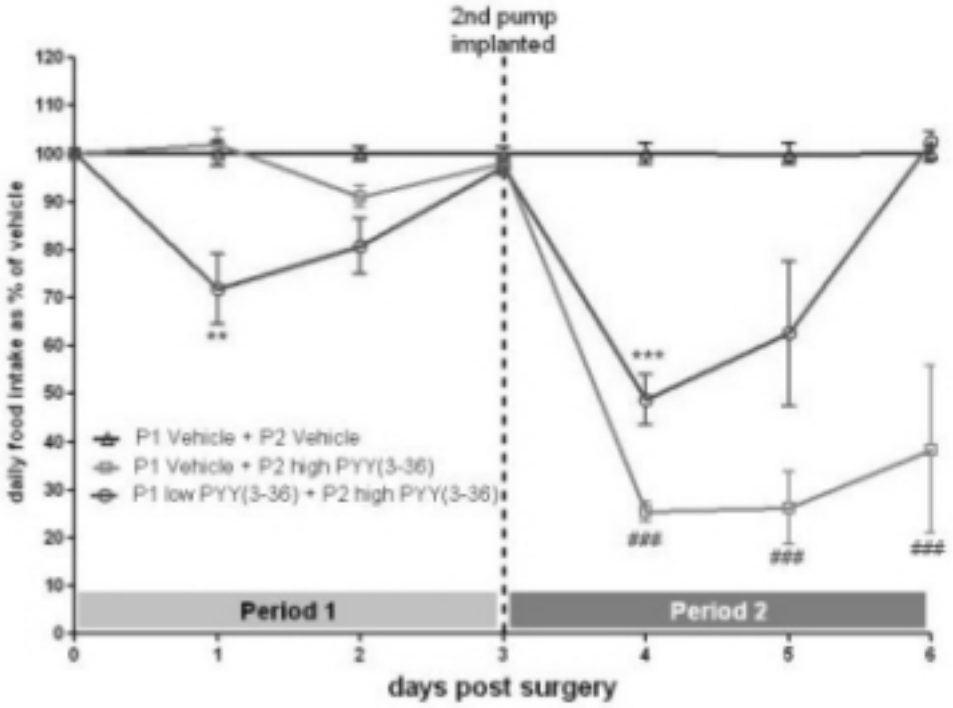
P2-505

Resistance to the Anorectic Effect of PYY3-36 during Continuous Infusion Is Independent from the Stimulation of Appetite Resulting from Weight Loss.

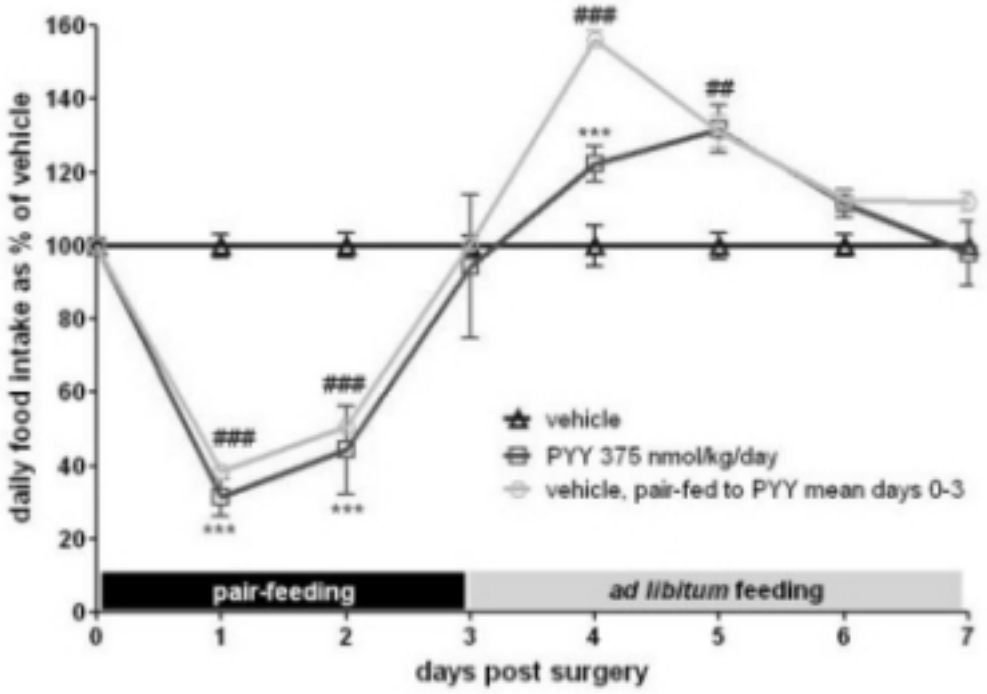
K Hostomska MSc[1], V Salem MBBS[1], BCT Field MBBS, PhD[1], KG Murphy PhD[1], MA Ghatei PhD[1] and SR Bloom MD, DSc[1].

[1]Imperial Coll London London, UK.

Introduction: Peptide YY (PYY) is an anorectic hormone released from L cells after the ingestion of food. Its active form, PYY3-36, activates Y2 receptors in the CNS to elicit anorectic effects. Intermittent administration of PYY3-36 significantly reduces food intake and body weight in rodents. However, continuous infusion by any route only transiently reduces food intake. Periods of fasting and weight loss are associated with a decrease in leptin levels which increases hunger to maintain body weight homeostasis. The anorectic effect of continuous PYY3-36 infusion is prolonged by co-administration with leptin, suggesting that the homeostatic defence of body weight driven by low leptin levels may be sufficient to overcome PYY-induced anorexia. Nevertheless, the contrast in outcome between continuous and intermittent administration suggests that tolerance and/or Y2 receptor down-regulation may also occur during continuous administration.
Hypothesis: Resistance to the anorectic effect of PYY3-36 that occurs during continuous infusion is independent from the stimulation of appetite resulting from weight loss.
Study 1: We used subcutaneous osmotic pumps to infuse mice (n=9) continuously with low dose (200 nmol/kg/day) PYY3-36 or vehicle for 3 days (Period 1). Mice then received a second pump, infusing either high dose (1500 nmol/kg/day) PYY3-36 or vehicle (Period 2). High dose PYY3-36 infusion partially overcame the loss of anorectic effect occurring after low dose pre-treatment (figure 1).



Study 2: To investigate whether attenuation of the anorectic effect of high dose PYY3-36 in study 1 resulted solely from defence of body weight set-point, we used subcutaneous osmotic pumps to infuse mice (n=9) continuously with PYY3-36 (375 nmol/kg/day) or vehicle. One group of vehicle-treated mice was pair-fed to the PYY group. When daily food intake returned to that of vehicle-treated controls, the pair-fed mice were allowed to eat *ad libitum*. In the following 24 hours, mice receiving PYY3-36 ate significantly less than the previously pair-fed mice, despite the two groups having lost weight to a similar degree (Figure 2). This suggests that PYY3-36 is continuing to exert an attenuated anorectic effect which is masked by the compensatory drive to conserve body weight.



Discussion: The homeostatic defence of body weight may obscure the anorectic effect of continuously infused PYY3-36. However, tolerance and/or Y2 receptor down-regulation cannot be excluded.851

Nothing to Disclose: KH, VS, BCTF, KGM, MAG, SRB

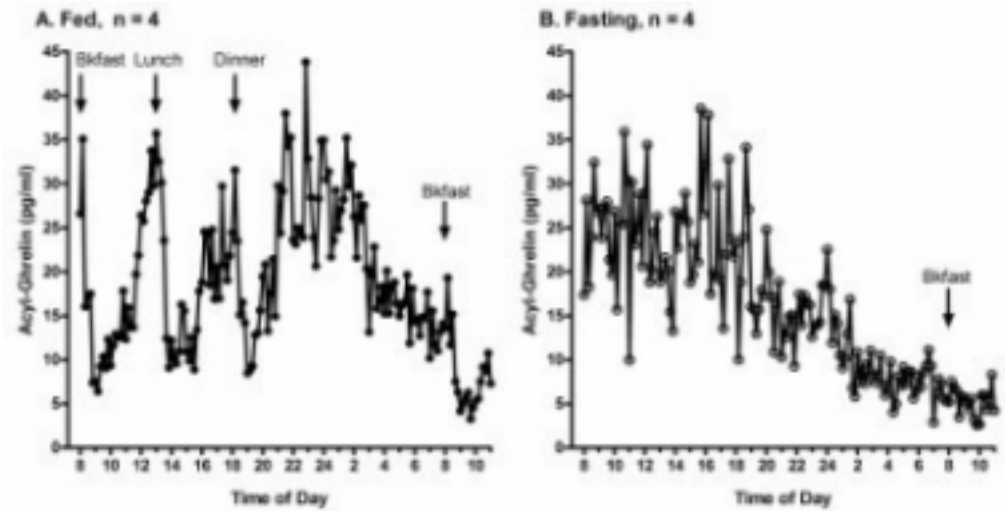
P2-506

Acyl-Ghrelin Secretion Declines over a 37.5-H Fast in Healthy Young Men.

R Nass MD[1], BD Gaylinn PhD[1], J Liu MD[1], SS Pezzoli BA, MT[1] and MO Thorner MBBS, DSc, FRCP[1].

[1]Univ of Virginia Charlottesville, VA.

Ghrelin is a 28-amino acid peptide with a unique acylation with octanoate by ghrelin O-acyl transferase (GOAT) at ser[3] of prepro-ghrelin which is necessary for its GH-releasing and origenic effects. It is produced predominantly by X/A cells in the stomach and small intestine. We have previously demonstrated that fasting for >37.5 h in healthy young men leads to suppression of acyl-ghrelin and tonic secretion of desacyl-ghrelin, while total ghrelin secretion is not altered (1). In order to determine how quickly acyl-ghrelin levels fall following the onset of fasting, we studied 4 normal men [mean (±SD) age 21±1.8; BMI 23.2±0.7 kg/m^2] on 2 separate admissions at least a month apart. On both admissions, subjects ate dinner from 1800-1830 h and then fasted overnight. Frequent blood sampling every 10 min for 27 h began at 0800 h the following morning. On the fed admission standardized meals were served at 0800, 1300 and 1800 h on Day 1 and at 0800h on Day 2. On the fasting admission, no meals were served until 0800 h on Day 2. Blood samples were assayed in duplicate in our in-house 2-site sandwich assay for acyl-ghrelin (1). A 27-h profile of acyl-ghrelin levels is shown in the figure.



At 0800 h acyl-ghrelin levels were similar on both admissions after the overnight fast. On the fed day the expected preprandial increase and postprandial decrease in acyl-ghrelin levels were observed, as well as a nighttime peak that occurred around midnight. During the fast, acyl-ghrelin remained at preprandial levels initially, but over the ensuing 24-h period acyl-ghrelin progressively declined, starting at about 2100 h. The nighttime increase seen on the fed admission was essentially abolished on the fasting admission. These data suggest that during fasting over 37.5 h, acyl-ghrelin secretion cannot be sustained, either due to inhibition of GOAT or to depletion of substrate (octanoate) which is likely of luminal origin.

(1) Liu et al JCEM 2008; 93:1980

Sources of Research Support: NIH Grants: RO1 DK076037 (MOT); MO1 RR00847 (UVA GCRC); K23 RR018770 (RN).

Nothing to Disclose: RN, BDG, JL, SSP, MOT

P2-507

Hepatic Changes in Metabolic Gene Expression in Old Ghrelin and Ghrelin Receptor Knockout Mice.

NH Rogers PhD[1], MP Van der Brug PhD[1], MR Cookson PhD[2], Y Sun MD, PhD[3] and RG Smith PhD[1].

[1]Scripps Florida Jupiter, FL ; [2]NIA, NIH Bethesda, MD and [3]Baylor Coll of Med Houston, TX.

Ghrelin knockout (GKO) and ghrelin receptor (growth hormone secretagogue receptor) knockout (GHSRKO) mice exhibit enhanced insulin sensitivity (1), but the mechanism is unclear. Insulin sensitivity declines with age and is inversely associated with accumulation of lipid in liver, a key glucoregulatory tissue. While the GHSR is not found in liver, central ghrelin can indirectly regulate peripheral (i.e hepatic) metabolism. In an effort to clarify links between ghrelin status and insulin resistance, we performed in depth hepatic gene expression studies in older C57Bl/6 WT, GKO and GHSRKO mice. We first isolated hepatic RNA from 10 month (mo) WT and GHSRKO littermates (n=3) and globally assessed gene expression using microarrays (Illumina) and RNA-Sequencing (Illumina Genome Analyzer II). Both methods showed higher *IGFBP2*, and lower lipid droplet-associated protein (*cidea, cidec, S3-12*), *acetyl-coA carboxylase (ACCβ)*, and *stearoyl-coA desaturase (scd1)* expression in GHSRKO compared to WT mice (Table 1). Moreover, RNA-seq methods revealed additional differentially expressed genes (underscoring array limitations). Genes more highly expressed in GHSRKO mice included *insulin receptor (insr)* and *ampkα2*, while *carnitine palmitoyl transferase (cpt1α)* abundance was lower.

We next used real-time-PCR and 2-way ANOVAs to assess hepatic gene expression differences in 10 and 15 mo GKO and GHSRKO mice (each vs respective WT littermates). Remarkably, whereas WT mice express markedly more (~40-fold) *cidea* at 15 mo compared to 10 mo of age, 15 mo GKO and GHSRKO mice show expression levels similar to the 10 mo WT mice (genotype x age interactions, $p<0.001$). Likewise, 15 mo WT mice express ~5-fold more *PPARγ* than 10 mo WT mice, but 15 mo knockout mice show only 2x more (GKO), or similar (GHSRKO) hepatic *PPARγ* as their 10 mo counterparts. Accordingly, older WT mice express more *cidec, S3-12* and *scd1*, and less *IGFBP2*, but knockout mice again show attenuated age-related changes. In summary, comprehensive hepatic gene expression analyses reveal altered metabolic profiles in older mice that are dependent on ghrelin signaling, albeit indirectly. Key findings, such as increased *insr* expression in GHSRKO mice, offer enticing clues to help explain enhanced insulin sensitivity in mice lacking ghrelin or the ghrelin receptor.

Fold Changes in GHSRKO liver

Gene	**Arrays**	**RNA-seq** (RPKM-based)
Cidea	-2.5	-2.7
Cidec	-2.4	-2.7
S3-12	-2.2	-2.2
ACCβ	-1.4	-1.9
Scd1	-1.3	-1.4
IGFBP2	+1.4	+1.7

(1) Sun Y et al., Cell Metab 2006; 3(5): 379-86

Sources of Research Support: NIA Grant R01AG19230 awarded to RGS; NIA Grant R01AG29740 awarded to RGS.

Nothing to Disclose: NHR, MPVdB, MRC, YS, RGS

P2-508

A Novel Active GLP-1 ELISA That Detects Fasting GLP-1 (7-36) Level without Extraction.

KB Scribner Doering PhD[1], DG Perregaux MA[2], S Scheibel BS[3], D Mather BS[2], JL Treadway PhD[2], C LaMarca MS[1], C Shaw MS[1] and P Gao MD[3].

[1]ALPCO Diagnostics Salem, NH ; [2]Pfizer Global Res & Development Groton, CT and [3]Epitope Diagnostics, Inc San Diego, CA.

Glucagon-like peptide-1 (GLP-1), an important regulatory incretin hormone, is an attractive therapeutic target due to its effects on glucose homeostasis. Commercial immunoassays specific for biologically active GLP-1 are available for a variety of detection systems, yet measuring accurate fasting levels of active GLP-1 in healthy volunteers without solid-phase extraction (SPE) has remained a challenge. To address this technical limitation, we have developed a 2nd generation ELISA specific for GLP-1 (7-36). The assay range is 2.1 - 54 pM with an analytical sensitivity (LOD) of 0.2 pM. Samples with high and low GLP-1 concentrations (n=2) diluted 1.2 - 6-fold yielded a mean recovery of 97%. Intra- and inter-assay precision were both <6.0 %.

In the current study, blood was collected from 8 healthy volunteers after an overnight fast and a week later within 2 hours following a midday meal. This study design allowed for the assessment of the fed/fasted stimulation index (SI) for each donor. EDTA plasma samples (using BD™ P700 tubes containing DPP-IV inhibitor) were held at -80°C until further analyses.

Samples were assayed using the new GLP-1 (7-36) ELISA either neat (nonextracted) or following SPE. All fasted and nonfasted samples were within the limits of detection, allowing for the calculation of individual SI (range 1.2 – 8.8). There was good correlation of GLP-1 (7-36) values for nonextracted vs. extracted samples (r=0.898), indicating that this 2nd generation ELISA provides an accurate assessment of circulating GLP-1 without SPE. Moreover, our assay values for nonextracted samples showed excellent correlation with extracted sample values from the Millipore GLP-1 (Active) ELISA (r=0.914). However, 6 out of 8 nonextracted fasted samples were undetectable in the Millipore GLP-1 (Active) ELISA.

In all, this 2nd generation GLP-1 (7-36) ELISA provides a long-awaited means for detecting low fasting levels of GLP-1 without SPE and should prove invaluable to the field of GLP-1 research.

Disclosures: KBSD: Employee, ALPCO Diagnostics. CL: Employee, ALPCO Diagnostics. CS: Employee, ALPCO Diagnostics. PG: Owner, ALPCO Diagnostics.
Nothing to Disclose: DGP, SS, DM, JLT

P2-509

Anti-Diabetic Properties of the Histamine H3 Receptor Protean Agonist Proxyfan.

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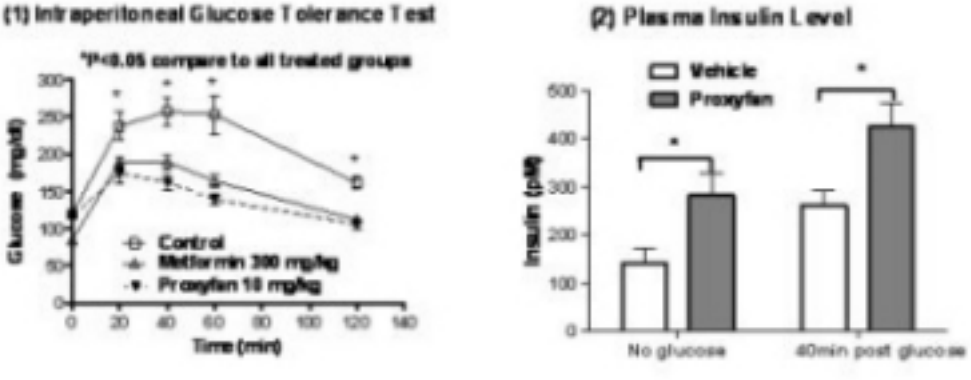
The histamine H3 receptor is a G-protein-coupled receptor that plays a regulatory role in many physiological and pathological functions. Proxyfan is a unique histamine H3 receptor protean agonist which has been documented to produce a spectrum of pharmacological effects including full agonist, inverse agonist and full antagonist. We have discovered that acute oral administration of proxyfan can significantly improve glucose excursion after an intraperitoneal glucose tolerance test (ipGTT) in lean or high fat/cholesterol diet-induced obese (DIO) ICR mice. The effect of proxyfan (10 mg/kg, po) was comparable to that of metformin (300 mg/kg, po, figure 1). The H3 receptor antagonist/inverse agonist thioperamide (20 mg/kg, po) can also improve ipGTT, while the H3 receptor agonist imetit did not reduce glucose excursion in the ipGTT. In addition, the proxyfan-induced reduction of glucose excursion was not observed in the H3 receptor knockout mice suggesting that proxyfan mediated this effect through the histamine H3 receptor. Both the selective H1 receptor antagonist chlorpheniramine and the selective H2 receptor antagonist zolantidine administered alone had modest effects on glucose excursion, and neither inhibited the glucose excursion reduced by proxyfan. Proxyfan significantly reduced glucose excursion by increasing plasma insulin levels *in vivo* (figure 2). Proxyfan can also increase insulin release in islets culture *in vitro,* suggesting H3 receptors may be present on islet cells. No significant difference in insulin sensitivity was observed when proxyfan was administered to ICR mice prior to an insulin tolerance test. Proxyfan significantly reduced non-fasting glucose levels in a non-genetic type 2 diabetes mouse model, while it had no effect on non-fasting glucose in the leptin-deficient ob/ob type 2 diabetes mice.
In conclusion, these findings demonstrate for the first time that manipulation of the histamine H3 receptor by proxyfan can significantly improve glucose excursion by stimulating insulin secretion. This effect is dependent on the presence of leptin and can be blocked by neither an H1 nor an H2 receptor antagonist.



Nothing to Disclose: JJH, MH, SZ, CD, BP, GV, CS, YZ, LK

P2-510

Diet-Induced Hepatic Insulin Resistance Is Mediated by Endocannabinoids Via CB_1 Receptor-Mediated Dephosphorylation of Akt-2 by PHLPP1.

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Insulin resistance is a common consequence of obesity and a major contributor to cardiovascular disease. Cannabinoid receptor-1 (CB_1R) blockade improves glucose tolerance and insulin sensitivity in obesity[1,2,3,4], suggesting a role for endocannabinoids in insulin resistance. Obesity is also associated with endoplasmic reticulum (ER) stress, which has been linked to insulin resistance[5]. Here we show that high-fat diet induces hepatic insulin resistance in wild-type mice but not in mice with global or hepatocyte-specific deletion of CB_1R. In wild-type mice and in $CB_1R^{-/-}$ mice with liver-specific transgenic expression of CB_1R, both high-fat diet and CB_1R activation elicit the ER stress response. In human and mouse isolated hepatocytes, CB_1R activation causes ER stress-dependent suppression of insulin-induced phosphorylation of the downstream signaling molecule akt-2, through stimulation of the ser/thre phosphatase PHLPP1[6,7]. These findings indicate that activation of hepatic CB_1R by endocannabinoids is both necessary and sufficient to account for diet-induced hepatic insulin resistance, and identify CB_1R-regulated PHLPP1 as a therapeutic target in insulin resistance.

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Nothing to Disclose: JL, LZ, KX, CB, GK

P2-511

A Novel GLP-1/GIP Dual Agonist Normalizes Glucose Tolerance and Fat Mass in Diet Induced Obese Mice.

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Glucagon, Glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) are hormones involved in the maintenance of glucose homeostasis and of sizable importance to the clinical management of diabetes. These three homologous peptides exhibit specific association with their native receptors through structural elements within different regions of their sequences to confer differential pharmacology. We explored the structure-activity relationship of these hormones through the use of single residue substitutions, hybrid peptides and conformational stabilization. We have identified a set of novel chimeric peptides which exhibit high potency and balanced co-agonism among these three receptors. The structural basis for this change appears to be a combination of local positional interactions and a preferred secondary structure. We have previously reported the combinatorial efficacy of receptor agonism at the glucagon and GLP-1 receptors to achieve potent satiety inducing and lipolytic effects in a single peptide of sustained duration of action (1). There is appreciable uncertainty pertaining to the relative wisdom of agonism or antagonism at the GIP receptor for the treatment of diabetes and obesity. We have here selected the approach to explore the combinatorial efficacy of GIP/GLP-1 co-agonism. Administration of select peptides from a series of newly designed high potency analogs with differential receptor agonism normalized adiposity and glucose tolerance in diet induced obese mice. Selective removal of GLP-1 agonism modulated but did not eliminate glycemic efficacy supporting the virtue of co-agonism. These in vivo observations establish a basis for testing in human subjects.

(1) Day, J.W. et al. (2009) Nature Chemical Biology 5 (10) pp 749-57

Sources of Research Support: Marcadia Biotech.

Disclosures: JG: Employee, Marcadia Biotech. LZ: Employee, Marcadia Biotech. RD: Scientific Board Member, Marcadia Biotech. MT: Scientific Board Member, Marcadia Biotech.
Nothing to Disclose: DP-T, MT, NO, PP, JH, JH, KH, TM, HK, KH, VG

P2-512

Ghrelin Receptor Null Mice Have Reduced Visceral Fat and Improved Insulin Sensitivity during Aging.

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Aging is associated with a higher incidence of Type 2 diabetes. Loss of lean mass and accumulation of fat, particularly visceral fat, during aging result in increased insulin resistance. Insulin resistance is a major pathogenic factor for Type 2 diabetes. Ghrelin is the only circulating orexigenic hormone known to increase growth hormone release, stimulate appetite and promote obesity. We have reported that ghrelin deletion increases glucose-induced insulin secretion and partially rescues the diabetic phenotype of leptin-deficient *ob/ob* mice.
Acute ghrelin infusion has been shown to induce lipolysis and insulin resistance. Ghrelin receptor null (*Ghsr-/-)* mice have reduced body weight and reduced abdominal fat. To determine whether deletion of the ghrelin receptor would improve age-associated insulin resistance, we performed insulin tolerance tests (ITT) on young, middle aged and old wild-type (WT) and *Ghsr-/-* mice to evaluate the effects of insulin on blood glucose. Young WT and *Ghsr*-null mice were both sensitive to insulin, and the difference between WT and *Ghsr*-null was not pronounced. As the mice aged, WT mice showed a marked increase in insulin resistance, whereas the *Ghsr*-null mice remained sensitive to insulin. Hyperinsulinemic-euglycemic clamps further demonstrated that the null mice have reduced glucose production, increased peripheral glucose infusion, and increased glucose uptake in skeletal muscle. In addition, we performed hyperglycemic and hypoglycemic clamps. *Ghsr-/-* mice secrete less insulin during hyperglycemic clamps, and require higher glucose infusion during hypoglycemic clamps. All functional studies support that *Ghsr-/-* mice have improved insulin sensitivity. In line with the functional data, old *Ghsr-/-* mice have significantly reduced epididymal fat (more than 50%) and reduced liver steatosis. Also, old *Ghsr-/-* mice have lower plasma cholesterol, triglyceride, free fatty acid and leptin. Consistently, the expression of adipocyte differentiation and lipid regulatory genes was reduced in the epididymal fat of old *Ghsr-/-* mice. Together, the data suggest that the reduced adipocyte differentiation in visceral fat may be, at least in part, the underlying mechanism that mediates the insulin-sensitive phenotype of *Ghsr-/-* mice. Hence, inactivation of GHS-R signaling reduces visceral fat and prevents age-associated insulin resistance, and GHS-R antagonists may have beneficial effects in preventing age-dependent onset of Type 2 diabetes.

Sources of Research Support: NIH/NIA 29740 (RGS), Vanderbilt Mouse Metabolic Phenotyping Center (U24DK05963)7, NIH/NIA grant 1R03AG029641-01 (YS) and USDA grant (YS).

Nothing to Disclose: YS, PKS, IO, LS, AGAS, OPM, LC, RGS

P2-513

Is Nesfatin-1 a Metabolic Hormone with Glucoregulatory/Insulinotropic Effects in Rodents?.

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Nesfatin-1 is a recently discovered hormone that has been shown to inhibit food intake in rodents and reduce blood glucose levels in hyperglycemic *db/db* mice. The main objective of this study was to characterize the effects of chronic infusion of nesfatin-1 on insulin release and metabolism in rodents. Nesfatin-1 infusion using subcutaneously implanted Alzet™ osmotic mini-pumps caused a significant increase in insulin secretion *in vivo* at 10-20 minutes during an oral and intraperitoneal glucose tolerance tests in Fischer 344 rats. Similarly, nesfatin-1 also caused an ~ 2 fold increase in glucose stimulated insulin secretion from MIN-6 cells and C57BL/6 mouse pancreatic islets *in vitro*. Nesfatin-1 co-incubation also increased preproinsulin mRNA expression in MIN-6 cells. These evidences provide strong evidence for insulinotropic actions of nesfatin-1 in rodents. High glucose administration stimulated nesfatin-1 release and prepronesfatin mRNA expression in MIN-6 cells, and prepronesfatin mRNA expression in the pancreas of Fischer 344 rats. These results indicate that nesfatin-1 is a glucose responsive hormone. Pronesfatin immunoreactivity and prepronesfatin mRNA expression was dramatically reduced in the pancreatic islets of streptozotocin induced type 1 diabetic mice. In contrast, pronesfatin immunoreactivity and prepronesfatin mRNA expression was significantly increased in diet induced obese, type 2 diabetic mice. Furthermore, subcutaneous infusion of nesfatin-1 using Alzet™ osmotic mini-pumps reduced cumulative food intake, increased physical activity and increased energy derived from fat during the dark phase. Together, these data indicate that nesfatin-1 is a metabolic hormone with insulinotropic actions, and suggests that nesfatin-1 biology is altered in experimental models of diabetes and obesity.

Sources of Research Support: Natural Sciences and Engineering Research Council (NSERC) of Canada, Canadian Institutes of Health Research (CIHR), Canada Foundation for Innovation (CFI).

Nothing to Disclose: RGG, BR, XG, MPG, RT, RC, SU

P2-514

Investigating the Metabolic Clearance and Degradation of the Anorexigenic Gut Hormone PYY3-36 as a Tool To Design Long-Acting Analogues of PYY3-36 for the Treatment of Obesity.

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Peptide YY (PYY) is a satiety hormone that communicates nutritional status to the central nervous system. PYY is released postprandially from endocrine L-cells. It is processed to generate the principle bioactive form PYY3-36, which acts on Y2 receptors in the brainstem and hypothalamus to reduce appetite. Chronic intravenous infusion of PYY3-36 induces weight loss in rodents and acute intravenous infusion to obese humans reduces food intake. Furthermore, obese humans have been reported to display a blunted postprandial rise in PYY3-36, suggesting PYY3-36 is a potential anti-obesity drug target. However, exogenous PYY3-36 is rapidly cleared and has a short circulating half-life. Additionally, at supraphysiological levels PYY3-36 has been found to produce nausea in humans. The development of potent, long-acting PYY3-36 analogues may circumvent these limitations. Metalloendopeptidases concentrated in the kidney brush border (KBB) have been implicated in PYY degradation and cachexic renal failure patients exhibit high levels of PYY. To investigate whether the kidney is a primary site of PYY3-36 clearance, we compared the pharmacokinetics of exogenous PYY3-36 in nephrectomised and sham-operated rats. The half-disappearance time of PYY3-36 after an intravenous injection increased from 25.9 (+/-0.03) to 45.4 (+/-0.7) min in nephrectomised rats vs. sham-operated controls. To further investigate the degradation profile of PYY3-36, purified metalloendopeptidase meprin β and KBB preparations were incubated with PYY3-36 *in vitro* and the cleavage products analysed by high performance liquid chromatography and mass spectrometry. Both meprin β and KBB membrane cleave PYY3-36 at multiple sites, including Glu10-Asp11 and Pro14-Glu15. This data is consistent with the substrate preference of meprin β, which initiates cleavage through electrostatic interaction between positively-charged residues in its active site and negatively-charged residues in the substrate. When KBB membrane was pretreated with the meprin inhibitor actinonin *in vitro*, its degradative effect on PYY3-36 was entirely prevented. Furthermore, mice administered actinonin with PYY3-36 displayed significantly higher PYY3-36 plasma levels compared to mice administered PYY3-36 only. In conclusion, these experiments suggest that meprin β may play a role in the degradation of PYY3-36 *in vivo,* providing data useful in designing long-acting PYY3-36 analogues in the development of a potential anti-obesity therapy.

Nothing to Disclose: MLA, JSM, JCS, TT, KGM, MGG, SRB

P2-515

Sex-Different Hepatic Glycogen Content and Glucose Output in Rats.

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Aim It is well established that male and female rat livers differ regarding gene expression and metabolism. We have tested the hypothesis that male and female rat livers differ regarding lipid and carbohydrate metabolism.
Methods Healthy male and female rats were compared regarding hepatic whole-genome mRNA expression, lipid and glycogen content, b-oxidation and insulin signalling. Hepatic glucose output (HGO) was determined by *in situ* perfusion of the liver, and the perfusates thereby generated analysed for glucose and other liver-derived metabolites using nuclear magnetic resonance (^{1}H-NMR) spectroscopy.
Results Out of 27 649 genes printed on the arrays, approximately 3 500 were detected in liver. With a 5% false discovery rate and a cut-off at 1.5-fold difference, 383 (11%) transcripts among these were higher in females, and 399 (11%) transcripts were significantly higher in males. The results pointed towards a female-predominant capacity for hepatic uptake of long-chain fatty acids, synthesis of triglycerides, and assembly of VLDL particles. In addition, a male-predominant capacity for mitochondrial and peroxisomal oxidation of fatty acids was revealed. A clear sex-difference was also observed in the expression of genes encoding proteins of importance for cholesterol and bile acid synthesis, with higher expression in female liver. Furthermore, male rats had higher levels of gene products for glucose uptake and synthesis of glycogen, fatty acids, retinoids and glutathione.
Based on differences in gene expression levels, hepatic lipid metabolism might differ between male and female rats, but no sex-differences were revealed in hepatic triglyceride content, FA oxidation rate, or blood ketone bodies. However, male rats had higher rates of glucose production, higher hepatic glycogen content, and higher ratios of insulin to glucagon levels. A trend towards higher levels of liver-derived lactate, glycerol and the gluconeogenic amino acids glutamine, valine and leucine was also observed in males. The effects of insulin on hepatic AKT-phosphorylation, gene regulation and HGO were similar between the sexes.
Conclusion Data presented in this report lend further support to the view of the liver as a sex-differentiated tissue with male rats having a greater carbohydrate and amino acid turnover. Glycogen storage and release of glucose are major functions of the liver, and the possibility that they might be regulated by sex-dependent mechanisms is intriguing.

Nothing to Disclose: CG, KY, EW, LC, JL, KB, C-GO, GN, PT-E

P2-516

Conserved Role for Insulin Receptor Signaling in Catecholaminergic Cells in Control of Energy Homeostasis.

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As the obesity epidemic, diabetes mellitus type 2, and associated comorbidities show no signs of abating, large efforts have been put into a better understanding of the homeostatic control mechanisms involved in regulation of body weight and energy homeostasis. While for the longest time the exact nature of these mediators remained elusive, the recent identification that peripheral hormones such as insulin, leptin, and numerous others, as well as nutrients directly can communicate to the hypothalamus to control energy homeostasis, has set the ground for detailed understanding of the neuronal circuitry underlying the control of body weight homeostasis.
Besides basic homeostatic hypothalamic circuits, food palatability and reward are major factors involved in the regulation of energy homeostasis. Previous studies have demonstrated that both insulin and leptin receptors are expressed and functional in dopaminergic neurons of the midbrain. Moreover, insulin, as well as leptin, is able to modulate reward-seeking behavior and drug relapse, which are known to be dependent on intact dopaminergic signaling in the mesolimbic dopamine system. To determine the role of insulin receptor (IR) signaling in this neurocircuit, we decided to alter IR signaling in tyrosine hydroxylase (Th)-positive catecholaminergic neurons. To this end, we first used the Cre-loxP system to selectively disrupt the IR in Th-expressing cells of mice. IR inactivation in Th-expressing cells results in a 15% increase in body weight and a 30% increase in epigonadal fat pad mass. Obesity in $IR\Delta^{Th}$-mice develops in the presence of hyperphagia but unaltered energy expenditure and locomotor activity. Furthermore, we demonstrate that in wildtype mice prolonged insulin action *in vivo* results in a decrease of excitatory inputs onto dopaminergic midbrain neurons. Next, we expressed either a dominant negative mutant of the IR or a constitutively active mutant of the IR in Th-neurons of *Drosophila*. Similar to the observations in mice, flies transgenic for the dominant negative version exhibited increased body weight, adiposity and food intake.
Taken together, these data provide novel *in vivo* evidence for an evolutionary conserved, essential role of insulin signaling in Th-expressing cells to alter their electrical activity to control food intake and energy homeostasis.

Nothing to Disclose: ACK, AM, TO, MS, TLH, JCB

P2-517

Ablation of Ghrelin Receptor in Leptin-Deficient Mice Has Paradoxical Effects on Glucose Homeostasis Compared to Ghrelin-Ablated Leptin-Deficient Mice.

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Background: Ghrelin is produced predominantly in stomach and known to be the endogenous ligand of the growth hormone secretagogue receptor (GHSR). Ghrelin is a GH stimulator and an orexigenic hormone. In contrast, leptin is an anorexic hormone, and leptin-deficient *ob/ob* mice are obese and diabetic. To study the functional antagonism between ghrelin and leptin, we generated ghrelin and leptin double knockout mice (*Ghrelin-/-.ob/ob*). We reported that deletion of ghrelin in *ob/ob* mice had no effect on obesity, but significantly improved the diabetic phenotype of *ob/ob* mice. We further demonstrated that the attenuation of the hyperglycemia in *Ghrelin-/-.ob/ob* mice was due to increased insulin secretion which was, at least in part, due to down-regulation of uncoupling protein 2 in pancreatic B-cells. Furthermore, we showed that *Ghrelin-/-* mice had improved glucose tolerance and increased insulin secretion.
Hypothesis: The deletion of GHSR in leptin-deficient mice improves glucose homeostasis of *ob/ob* mice.
Materials & Methods: GHSR and leptin double knockout mice (*Ghsr-/-.ob/ob*) were used. Glucose and insulin levels were monitored at different age. Glucose tolerance test (GTT) and insulin tolerance test (ITT) were performed in mice at 10-14 weeks of age.
Results: Similar to *Ghrelin-/-.ob/ob* mice, deletion of *Ghsr* did not rescue the obese phenotype of *ob/ob* mice. Surprisingly, *Ghsr-/-.ob/ob* mice showed reduced insulin and worsened hyperglycemia when compared to that of *ob/ob* mice. *Ghsr-/-.ob/ob* mice showed decreased glucose tolerance during GTT and increased insulin resistance during ITT. Furthermore, similar to *Ghsr-/-.ob/ob* mice, *Ghsr-/-* mice showed reduced insulin secretion during GTT and hyperglycemic clamp.
Summary: The glycemic phenotype of *Ghsr-/-. ob/ob* mice is in total contrast to that observed in *Ghrelin-/-. ob/ob* mice, implying ghelin's effect on glucose homeostasis in *ob/ob* mice may be independent of GHSR. The paradoxical effects of ghrelin and GHSR in leptin-deficient condition raise the possibility that there exists an as-yet-unknown additional ligand for GHSR, and/or an additional unknown ghrelin sub-type receptor, which are involved in glucose homeostatic regulation of ghrelin/GHSR signaling.

Sources of Research Support: USDA (YS), Vanderbilt Mouse Metabolic Phenotyping Center U24DK05963(OM) and the NIH/NIA grant 1R03AG029641-01 (YS).

Nothing to Disclose: YL, IO, XM, OM, RS, YS

P2-518

Glycemic Control during Post-PTCA Period Predicts the Progression of Atherosclerosis in the Follow-Up Angiography in Patients with Diabetes.

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Background : Several studies reported that HbA1c level is not a predictor of major adverse cardiovascular events (MACE). However, these studies assessed the initial HbA1c level, and not the trend of glycemic control during the follow up period. We investigated the effect of different degrees of glycemic control on a need for an additional percutaneous transluminal coronary angioplasty (PTCA) in the follow-up angiography.
Methods : From 887 diabetes patients who received a successful balloon angioplasty or drug eluting stent implantation, 232 patients who underwent a 9-month angiographic follow-up were enrolled in the study. Body mass index, blood pressure, ejection fraction, HbA1c, lipid profile, cystatinC level, medication list, and angiographic findings were reviewed serially from the initial intervention to the follow up angiography.
Results : Sixty-four patients received additional PTCA for a newly developed coronary lesion or restenosis of the previous intervention site while there was no need for an intervention in 168 patients. There were no differences in anthropometric and biochemical parameters between the two groups except for baseline HbA1c level (7.36% vs 7.76%, p = 0.041), follow up HbA1c (7.14% vs 7.53%, p = 0.02) and mean HbA1c (7.25% vs 7.64%, p = 0.012). Both group showed an improved HbA1c level through the follow up period, but the prognosis was dependent on the overall degree of glycemic control. Multiple logistic regression identified a poor glycemic control, representing higher level of mean HbA1c, to be an independent risk factor for predicting a need to redo PTCA (OR 1.59, 95% CI 1.06-2.36, P = 0.024).
Conclusion : Glycemic control at baseline as well as during post-PTCA period is an independent predictor of the need for additional PTCA.

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Nothing to Disclose: WHS, EHL, SHB, SAK, ESK, JSY, JSN, MC, JSP, CWA, BSC, EJL, SKL, KRK, HCL

P2-519

52-Week Treatment with Diet and Exercise + Testosterone Improves Non-Alcoholic Fatty Liver Disease and Cardiovascular Risks in Hypogonadal Men with the Metabolic Syndrome.

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Objectives: Men with the metabolic syndrome (MetS) and type 2 diabetes (T2D) often have low testosterone levels. Elevating low testosterone levels may improve features of the MetS and glycemic control. In this analysis we assessed effects of normalization of circulating testosterone on biomarkers of non-alcoholic fatty liver disease (NAFLD) , and cardiovascular risk .
Design and Methods: In a single-blind, 52-week clinical trial, 32 hypogonadal men with the MetS and newly diagnosed T2D were randomized to supervised diet and exercise (D&E) alone (n=16) or with additional transdermal testosterone gel (50mg QD; n=16). The MetS was defined by the Adult Treatment Panel-III and the International Diabetes Federation. Hypogonadism was defined as a total testosterone ≤12.0 mmol/L. Endpoint were baseline adjusted change in biomarkers of NAFLD (GPT, GOT, g-GT, CRP) and cardiovascular risk (homocysteine, PAI-1, fibrinogen, Apo(a) and TG).
Results: 52-weeks of treatment T administration resulted in a significantly larger improvement in all measured biomarkers of NAFLD in T treated patients as compared to supervised D&E alone. Levels of homocysteine, PAI-1, fibrinogen, Apo(a) and TG improved significantly in both treated groups, with PAI-1, fibrinogen and TG showing a significantly larger improvement in T treated patients as compared to supervised D&E alone.
Conclusions: Addition of testosterone to supervised D&E results in greater beneficial effects on biomarkers of NAFLD and cardiovascular risk. Our results invite to consider the significance of diagnosing and, if warranted, treating testosterone deficiency in men with diabetes type 2.

Disclosures: AEH: Speaker, Bayer Schering Pharma, Berlin, Germany. FS: Employee, Bayer Schering Pharma. LJGG: Speaker, Bayer Schering Pharma.
Nothing to Disclose: MCB

P2-520

Diabetes Risk Assessment in Mexicans and Mexican-Americans: Effects of Parental History of Diabetes Are Modified by Adiposity Level.

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Parental diabetes history (parental DM) is a well-known risk factor for type 2 diabetes mellitus (T2DM) and considered strong evidence for a genetic basis of T2DM. Whether this relationship is affected by other known risk factors - specifically obesity - remains unclear, possibly due to a relative paucity of lean diabetics. This issue was investigated using data from a high-risk population from Mexico (the National Health Survey 2000, n=27,349), with observations replicated using Mexican-American data from the NHANES 2001-2002, 2003-2004 (n=1,568).
As expected, positive parental DM was a significant T2DM risk factor, regardless of age, gender, or adiposity level. However, positive parental DM conferred greater risk among leaner individuals relative to their heavier peers (p=0.001). In other words, in the presence of parental DM history, the effect of BMI on T2DM risk was smaller.

Association of Type 2 Diabetes and Parental History of Diabetes, by Adiposity Status, ENSA, Mexico, 2000, and NHANES, United States, 2001-2004*

	ENSA 2000		NHANES 01-04	
	BMI < 25	BMI ≥ 25	BMI < 25	BMI ≥ 25
Parental history of diabetes	Odds Ratio (95% CI)	Odds Ratio (95% CI)	Odds Ratio (95% CI)	Odds Ratio (95% CI)
Mother only	3.30 (2.13, 6.00)	2.16 (1.85, 2.52)	3.42 (1.09, 10.80)	2.40 (1.32, 4.34)
Father only	3.37 (2.07, 5.49)	1.30 (1.02, 1.67)	10.40 (2.42, 44.40)	2.13 (0.84, 5.39)
Both parents	7.86 (3.15, 19.60)	3.87 (2.81, 5.33)	5.30 (1.30, 21.60)	5.40 (2.53,11.50)

* Participant age ≥ 30 years, age of diabetes diagnosis ≥ 30 years, adjusted for gender, age, and body mass index

These findings suggest that parental DM is a stronger risk factor for T2DM in the absence of obesity, thus lean diabetics could help identify T2DM susceptibility genes. This finding reinforces the concept that parental DM and body mass index are independent T2DM risk factors, and suggests that glycemic screening should be considered for individuals with positive parental DM regardless of their overweight status.

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Nothing to Disclose: HEVM, RWC, TP, AIB, GO, MHA, WCH

P2-521

The Effect of Losing Abdominal Fat on the Progression of Carotid Intima Media Thickness in Patients with Type 2 Diabetes.

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Background: Abdominal obesity has been proven to be a better marker for cardiovascular disease and metabolic syndrome than body weight or BMI. Although there are numerous cross-sectional studies have demonstrated close relationships between measures of obesity and atherosclerosis and also their predictive value in the progression of atherosclerosis, data on the effect of loosing weight loss or abdominal obesity on the progression of atherosclerosis is lacking. We aimed to assess the impact of reducing body weight, waist circumference, and various metabolic parameters on the progression of carotid intima media thickness (IMT) in Korean type 2 diabetes or prediabetes patients.
Methods: This study comprised of 297 type 2 diabetes or prediabetes patients. Anthropometric measurements, various metabolic parameters, and carotid IMT were measured at baseline and 1 year later.
Results: There were significant differences in waist circumference (86.4 ± 9.2 cm vs. 84.6 ± 8.8 cm, P = 0.001), HbA1c (7.5 ± 2.3 % vs. 6.9 ± 1.6 %, P < 0.001) and mean left and right IMT (0.63 ± 0.17 vs. 0.65 ± 0.17, P = 0.032; 0.60 ± 0.15 vs. 0.62 ± 0.16, P = 0.015, respectively) after 1 year. The change in waist circumference correlated with changes in HbA1c (r^2 = -0.115, P = 0.076) and changes in mean right IMT (r^2 = -0.145, P = 0.025), maximum right IMT (r^2 = -0.239, P = 0.001), mean left IMT (r^2 = -0.146, P = 0.024), and maximum left IMT (r^2 = -0.119, P = 0.066) after adjusting for age, sex, and medications that could influence IMT. In multiple regression analysis, the change in waist circumference was a significant predictor of the progression of mean right and left IMT, independent of changes in body weight, BMI, and HbA1c level. Meanwhile, changes in body weight and body mass index were not independent predictors of the progression of IMT.
Conclusions: In summary, controlling abdominal obesity seems to have a significant impact on the progression of subclinical atherosclerosis in patients with diabetes or prediabetes, and more emphasis should be given to the waist circumference.

Nothing to Disclose: JSN, WHS, EHL, SHB, SAK, ESK, JSY, MC, JSP, CWA, BSC, EJL, SKL, KRK, HCL

P2-522

Inflammation Is Worse in Non-Hispanic Black Adolescents with Metabolic Syndrome Than Non-Hispanic Whites or Mexican Americans: NHANES 1999-2006.

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Inflammation and Metabolic Syndrome (MetS) are linked and both are associated with cardiovascular disease and diabetes. Non-Hispanic black (NHB) adolescents are at greater risk for cardiovascular disease and diabetes than non-Hispanic whites (NHW) or Mexican Americans (MA) but NHB have lower rates of the Metabolic Syndrome (MetS). Therefore the value of MetS in NHB adolescents is uncertain. No data is available on whether the association between inflammation and MetS is ethnic-specific. High-sensitivity C-reactive protein (hsCRP) is an excellent marker of inflammation. Using National Health and Nutrition Examination Survey (NHANES) 1999-2006 data, our goal was to compare hsCRP levels in NHB, NHW and MA with and without MetS. The participants were 2,733 non-diabetic adolescents, age range 12-18y, 52% male. A modified pediatric NCEP-ATPIII definition of MetS was used. Prevalence of MetS was compared among ethnic groups via a chi-square test. Mean hsCRP levels were compared in a pairwise fashion among the ethnic groups via t-tests, both overall and by MetS classification status. The percent of NHB, NHW and MA with MetS was (4.3, 8.9, 9.6, $P<0.01$ for comparisons of NHW and MA with NHB). In all ethnic groups, hsCRP levels were higher in the presence of MetS than the absence. Among adolescents with MetS, hsCRP levels were twice as high in NHB adolescents as in NHW or MA. Furthermore, the difference in hsCRP levels in adolescents with and without MetS was twice as high in NHB as in NHW or MA. Overall, inflammation is more severe in NHB adolescents with MetS than NHW or MA adolescents with MetS. Consequently MetS could be an especially important indicator of inflammation of in NHB adolescents.

Table: hsCRP Levels According to Metabolic Syndrome Status and Ethnicity

	NHB (n=886)	NHW (n=778)	MA (n=1018)	p-value
hsCRP mg/dL (mean±SE)	1.22 (0.05)	0.95 (0.06)	1.21 (0.06)	a**, c**
hsCRP with MetS	3.09 (0.45)	1.73 (0.16)	2.00 (0.17)	a**, b*
hsCRP without MetS	1.06 (0.06)	0.89 (0.06)	1.14 (0.06)	a**, c**
Difference in hsCRP	2.02 (0.48)	0.84 (0.17)	0.86 (0.17)	a*, b*

a NHB vs. NHW; b NHB vs. MA; c NHW vs. MA; * p<0.05, ** p<0.01

Nothing to Disclose: MDD, MJG, AES

P2-523

Components of the Metabolic Syndrome Are Not Affected Differently by Regular Consumption of Sucrose or High Fructose Corn Syrup.

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Introduction: It has been suggested that excess consumption of fructose may be a causative factor in the increase in overweight and obesity and the development of associated metabolic complications. As the primary sources of fructose in the American diet, sucrose (table sugar) and high fructose corn syrup (HFCS) have been specifically singled out. Studies on the acute effects of these two sugars have shown them to have equivalent metabolic effects by every parameter yet measured in humans, but, to our knowledge, this is the first prospective study to examine the longer term metabolic effects of fructose whether consumed as a component of sucrose or high fructose corn syrup at normal population consumption levels.
Methods: Sixty-three overweight and obese individuals were placed on a eucaloric (weight stable) ADA exchange diet for 10 weeks (mean age 38.3 ± 11.9 years). Individuals were required to consume either sucrose or HFCS sweetened low-fat milk, with the added sweetener comprising either 10% or 20% of their daily calories, which approximates the 25th and 75th percentile population consumption levels of fructose, respectively. Metabolic rate was estimated using the Harris-Benedict equation, and the caloric target appropriately set to maintain a stable weight. Measurements of waist circumference and fasting blood samples were obtained prior to, and at the end of the 10 week intervention. This was a double blind study, so the identity of the sweeteners was hidden from the participants and investigators, with the milk being coded as either group X or group Z. At the time of the current analysis the blind had yet to be broken.
Results: There was no change in waist circumference (88.29 ± 90.58 vs 88.39 ± 14.96 cm), triglycerides (133.64 ± 73.29 vs 135.06 ± 77.44 mg/dl), systolic (107.58 ± 13.45 vs 110.47 ± 122.80 mmHg) or diastolic blood pressure (71.05 ± 10.34 vs 73.90 ± 9.85 mmHg), and no significant change in HDL or glucose in any group. Significantly, all parameters tested remained in the normal range during both pre and post testing.
Discussion: The absence of any statistically significant differences between the X and Z group together with the normal values of all parameters at both pre and post testing suggest that, when consumed in typical amounts and as part of a weight stable diet, there are no differences between sucrose and HFCS, nor does consumption of either sucrose or HFCS result in increased risk factors for the metabolic syndrome.

Sources of Research Support: Corn Refiners Association.

Nothing to Disclose: JL, DK, TJA, KM, JMR

P2-524

The Effect of a High Fat Meal on Postprandial Arterial Stiffness in Obesity and Type 2 Diabetes.

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Context: Postprandial dysmetabolism is emerging as an important cardiovascular risk factor. Augmentation index (AIx) is a systemic measure of arterial stiffness and independently predicts cardiovascular outcome.
Objective: To assess the effect of a standardized high fat meal on metabolic parameters and AIx in: i) lean controls, ii) obese non-diabetic and iii) subjects with type 2 diabetes (T2DM).
Design and Setting: Male subjects: controls (n=8), obese (n=10) and T2DM (n=10) were studied for 6 hours following a high fat meal and water control. Glucose, insulin, triglycerides and AIx (radial tonometry) were measured serially to determine the incremental area under the curve (iAUC).
Results: AIx decreased in all 3 groups following a high fat meal. A greater overall postprandial reduction in AIx was seen in controls and T2DM compared with obese subjects (iAUC AIx: 2219 ± 1204%•min, 2958 ± 1102%•min and 1255 ± 429%•min respectively; $p<0.05$). The time to return to baseline AIx was significantly delayed in subjects with T2DM (297±68 min) compared with controls (161±88 min; $p<0.05$). Postprandial metabolic responses are summarized in figure 2. There was a significant correlation between iAUC AIx and iAUC triglycerides ($r=0.50$, $p<0.05$).
Conclusions: Obesity is associated with an attenuated overall postprandial decrease in AIx. Subjects with T2DM have a preserved, but significantly prolonged, reduction in AIx following a high fat meal. The correlation between AIx and triglycerides suggests that postprandial dysmetabolism may impact upon vascular dynamics. The markedly different response observed in the obese subjects compared with those with T2DM was unexpected and warrants further evaluation.

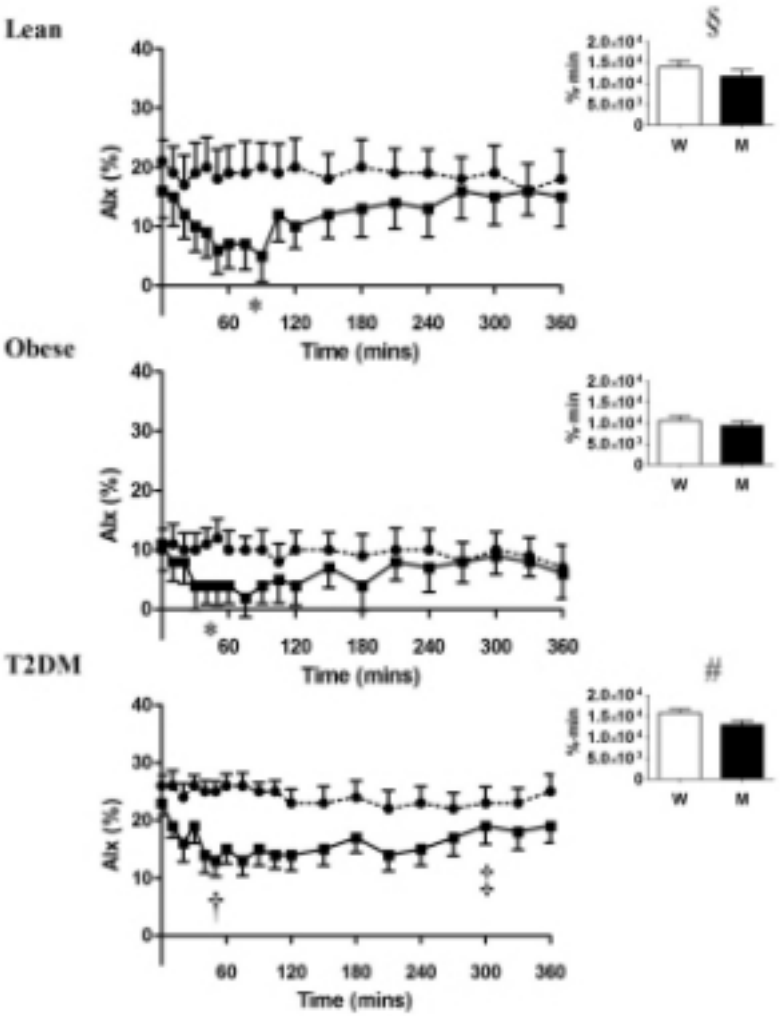

Figure 1. Postprandial augmentation index
Mean postprandial augmentation index following water (circle) and high fat meal (square) in lean controls, obese subjects and subjects with T2DM
Inset graphs represent the area under the curve for water (W) and meal (M) AIx
Error bars represent SEM
*p<0.05 vs. baseline AIx; †p<0.005 vs. baseline AIx; ‡p<0.05 time to return to baseline vs. controls; §p<0.05 iAUC AIx vs. obese subjects; #p<0.005 iAUC AIx vs. obese subjects

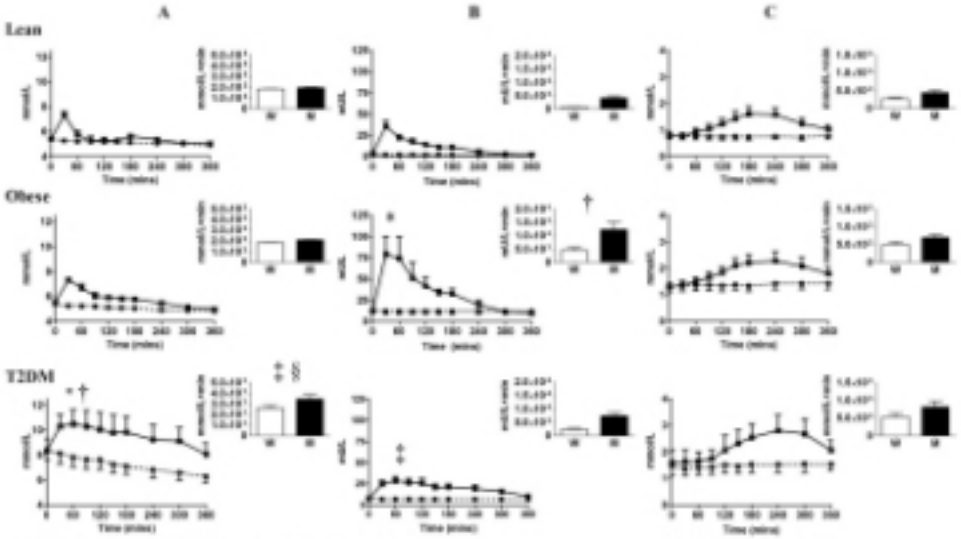

Figure 2. Postprandial metabolic responses
Postprandial mean metabolic responses following water (circle) and high-fat meal (square). Error bars represent SEM.
Inset graphs represent area under the curve (AUC) response following water (W) and meal (M).
A Glucose: *p<0.005 C_{max} glucose and p<0.05 T_{max} vs. controls; †p<0.005 C_{max} glucose vs. obese subjects; ‡p<0.005 iAUC glucose vs. controls; §p<0.05 iAUC glucose vs. obese subjects
B Insulin: *p<0.05 C_{max} insulin vs. T2DM; †p<0.05 iAUC insulin vs. controls; ‡p<0.05 T_{max} insulin vs. controls
C Triglycerides: Non-statistically significant trend for obese subjects and subjects with T2DM to have a higher C_{max} and later T_{max} compared to lean subjects (p=0.07 and p=0.06 respectively)

Sources of Research Support: National Health and Medical Research Council, Australia; Princess Alexandra Foundation, Brisbane, Australia.

Nothing to Disclose: LKP, JP, XZ, IJH, BEH, PS, SHL, JPW, JHM, JBP

P2-525

Diabetes Mellitus in Patients with Acute Ischemic Stroke.

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Introduction:Most studies report an increased risk of coronary heart disease in patients with altered glucose metabolism. Few studies have evaluated these changes in patients with acute ischemic stroke.
Objective: To evaluate the prevalence of diabetes mellitus and other cardiovascular risk factors in patients with acute ischemic stroke (AIS).
Methods: Prospective study of 125 patients consecutively admitted to the Stroke Unit during a one year period. HbA1c was determined at admission. In patients without a previous diagnosis of diabetes mellitus (DM), a 75g oral glucose tolerance test (OGTT) was carried out after the 4th day of hospitalization, to allow clinical stabilization. Changes in glucose metabolism were classified according to the American Diabetes Association criteria. The results were expressed in mean ± SD and statistical analysis used the t Student test.
Results: We evaluated 73 men and 52 women, mean age 63.86 ± 12.99 years. 26% had current or past history of smoking, 67% were hypertensive, 2% had peripheral vascular disease, 13% had previous history of stroke and 6% of acute myocardial infarction. In 25 (20%) patients, DM was already diagnosed but, in the remaining 100 (80%), glucose metabolism was unknown. Of the patients without a previous diagnosis of diabetes who underwent OGTT, 24% had DM , 41% Impaired Glucose Tolerance, 2% Impaired Fasting Glucose and 33% normal glucose tolerance. HbA1c was 5.15 ± 0.46% in normal, 5.48 ± 0.73% in those with Impaired Glucose Tolerance and 5.50 ± 0.51% in diabetic. Patients with previously diagnosed DM had higher HbA1c than the remaining (7.77% vs 5.54, $p < 0.001$).
Conclusion: In this series of patients with acute ischemic stroke who underwent OGTT, 67% had altered glucose metabolism and 24% undiagnosed DM. It seems important to implement strategies of early detection of altered glucose metabolism, in order to prevent events such as ischemic stroke, a major cause of morbidity and mortality in Portugal.

Nothing to Disclose: CM, FM, MLP, JRF, AF

P2-526

Uric Acid: Is It an Independent Risk Factor for Cardiovascular Disease in Diabetic and Non-Diabetic Recipients after Kidney Transplant?.

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Background: Vascular disease is a common cause of mortality after kidney transplant (KTX). Diabetic recipients, including post-transplant diabetes (PTDM), have greater vascular mortality after KTX. Uric acid is strongly associated with insulin resistance, hypertension, and renal insufficiency. Uric acid has also recently been suggested to be a non-traditional risk factor for vascular disease (1). Objective and Methods: We conducted a prospective, observational study of KTX recipients to determine whether there was a correlation between uric acid and a number of established and proposed vascular risk factors –age, BMI, AIC, lipids, urine albumin/creatinine, hsCRP, Vitamin D, PTH, HOMA-IR, GFR, and phosphate—as well as with a noninvasive marker of overall vascular disease, carotid intima media thickness (CIMT). Inclusion criteria included ≥19 years, and >6 mo after KTX. Exclusion criteria were GFR <30ml/min/1.73m², disease or conditions causing weight changes, or previous heart, lung or small bowel transplant. Results: We studied a cohort of 333 patients: type 1 (DM1), type 2 (DM2), and non-diabetes (Non-DM) groups. Uric acid was not different between diabetic and non-diabetic groups (p=0.39): 6.2 ±0.2 mg/dl (Mean±SEM) in DM1 (n=75); 6.0±0.3 mg/dl in DM2 (n=38) and 6.4±0.1mg/dl in Non-DM (n=205), where only 18 of 342 patients were taking allopurinol (5%). Allopurinol use was greatest in the non-DM group (3.8%; n=13) compared to the diabetic groups (<1%). As expected, uric acid correlated with serum creatinine (r=0.39, p<0.0001), PTH (r=0.25, p<0.0001), GFR (r=0.34, p<0.0001), BMI (r=0.16, p=0.002), and HDL (r=0.14, p=0.009). Uric acid did not correlate with age (p=0.44), AIC (p=0.92), LDL (p=0.84), or triglycerides (p=0.34). There was no correlation between CIMT and uric acid (p=0.94), even though CIMT did correlate with diabetes, BMI, age, and blood pressure (p<0.05). Conclusions: While uric acid does correlate with known vascular risk factors, decreased renal function, BMI, and HDL, it did not correlate with CIMT, suggesting it may not be an independent risk factor for vascular disease in this population.

1. Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. NEJM 2008;359:1811-1821.

Sources of Research Support: Grant RO1DK069919 (clinicaltrial.gov:NCT00374595).

Nothing to Disclose: UM, GCG, JMW, TLH, CEC, ERL, FY, RBS, JLL

P2-527

Effect of Cilostazol on Plasma Adipocytokine Levels and Arterial Stiffness in Subjects with Type 2 Diabetes and Metabolic Syndrome: A Placebo Controlled, Double-Blind, Randomized, Crossover Study.

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BACKGROUND: Cilostazol is a type 3 phosphodiesterase inhibitor used for peripheral vascular occlusive disease. In addition to the antiplatelet and vasodilator effects, cilostazol has been reported to have anti-inflammatory and anti-atherogenic effect in vitro study. The aim of this study was to investigate the effect of cilostazol on brachial ankle pulse wave velocity (baPWV), serum adipocytokine and inflammatory cytokine concentrations in type 2 diabetic patients with metabolic syndrome.
METHOD: This study was a placebo controlled, double-blind, randomized, crossover in design. A total of 40 type 2 diabetic subjects with metabolic syndrome were enrolled. In each treatment phase, patients received either cilostazol 50mg for 2 weeks and then 100mg for 6 weeks or matching placebo for the same periods. BaPWV and serum levels of adiponectin, interleukin (IL)-6, C-reactive protein (CRP), vascular cellular adhesion molecule (VCAM)-1, and intercellular adhesion molecule (CAM)-1, monocyte chemotactic protein (MCP)-1, tumor necrosis factor (TNF)-alpha were measured before and after 8 weeks treatment (Clinical trial reg. no. NCT00573950, clinicaltrials.gov.).
RESULT: After the study period, the baPWV did not change significantly in the cilostazol group than in the control group [mean difference +31.42 cm/s, 95% CI, +55.67 to -118.50 cm/s, p=0.469]. The serum VCAM-1 levels reduced significantly in the cilostazole group than placebo group [mean difference -105.18 ng/dL, 95% CI, -10.65 to -199.71 ng/dL, p=0.03]. Serum adiponectin and ICAM-1 showed tendency of improvement in the cilostazol group but there was no statistical significance. Serum levels of IL-6, ICAM-1, MCP-1 and TNF-alpha remained unchanged in both groups.
CONCLUSIONS: Cilostazol treatment significantly reduced serum VCAM-1 level, but this short term treatment was not associated with beneficial changes in arterial stiffness and other inflammatory cytokines except VCAM-1.

Nothing to Disclose: HYK, YJK, CRE, JHK, SJY, HJY, JAS, NHK, SGK, DSC

P2-528

Significance of Ambulatory Blood Pressure Monitoring and Its Relationship with Metabolic Factors in Children and Adolescents with Type 1 Diabetes.

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Purpose: Diabetes mellitus (DM) in children and adolescents is associated with atherogenic risk factors such as hypertension, dyslipidiema and obesity. Hypertension has a greater impact on cardiovascular disease in diabetic patients than non-diabetic individuals. Ambulatory blood pressure monitoring (ABPM) is better related to end organ damage and cardiovascular morbidity from hypertension than office blood pressure. In addition, carotid artery intima-media thickness (IMT) is a noninvasive marker of subclinical atherosclerosis. This study aimed to investigate the relationship between ABPM variables and IMT and to search for potential atherogenic risk factors in diabetic children and adolescents.
Method: ABPM and IMT were preformed in 57 diabetic children and adolescents (mean age 15.29±2.34 years). We reviewed hemoglobin A1c, 24 hour urine microalbumin, lipid profiles, DM duration, and BMI of subjects. BP index was defined as the mean BP divided by the 95th percentile BP for gender and height for ABPM pediatric norms and calculated for systolic BP (SBP) and diastolic BP (DBP) for 24 hour, daytime, and nighttime intervals.
Results: Of 57 patients, 15 (26.3%) were hypertensive confirmed by ABPM. IMT (hypertensive group, 0.48±0.07 vs. non-hyperensive group, 0.43±0.04 mm) and urine microalbumin (hypertensive group, 14.79±2.04 vs. non-hyperensive group, 4.57±6.92 mg/day) were increased in hypertensive group compared to non-hypertensive group. Positive correlations were observed between IMT and all BP indexes except daytime DBP index. The ratio of total and HDL cholesterol (TC/HDL) is positively correlated with IMT (r=0.400, *P*=0.003), whereas HDL cholesterol (HDL-C) is negatively correlated with IMT (r=-0.281, *P*=0.041). Age and all SBP indexes showed positive association with IMT in a multilinear regression model.
Conclusions: This study revealed a significantly increased IMT and urine microalbumin in a diabetic children and adolescents with hypertension. All SBP and TC/HDL were positively correlated with IMT, but HDL-C had negative correlation with IMT. Consequently, hypertension and dyslipidemia may strongly contribute to the progression of atherosclerosis in diabetic children and adolescents. Therefore, measurement of the ABPM and IMT could be good methods to detect macrovascular complications in diabetic children and adolescents. Longitudinal studies are needed to evaluate the relationship between ABPM variables and end-organ damage in diabetic subjects.

Nothing to Disclose: SHL, MJK, JHK, YAL, HHL, SWY, CHS

P2-529

Diagnostic Accuracy of Noninvasive Coronary Angiography Using 64-Slice Multidetector Computed Tomography in Patients with Type 2 Diabetes.

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Aims: The 64-slice multidetector computed tomography(MDCT) is an noninvasive imaging technique for assessing luminal stenosis of coronary artery and is expected to substitute for invasive coronary angiography. The aims of this study were to investigate the diagnostic accuracy and usefulness of 64-slice MDCT coronary angiography for detecting coronary artery disease(CAD) in patients with type 2 diabetes.
Methods: We studied 271 symptomatic patients undergoing invasive coronary angiography(ICS). Eighty-five patients were type 2 diabetes(M:F=45:40, 41.9±9.4 yrs). Results were analyzed for signigicant coronary stenosis(over 50% luminal narrowing) by segment, by artery, by patient.
Results: Of the 4,605 coronary segments studied, 4,603 segments(99.9%) were assessed quantitatively by both MDCT and ICS. 708 segments were detected as significant lesion in ICS. In patients with type 2 diabetes, there were 244 significant lesions of 1,274 segments in ICS. Sensitivity, specificity, and positive and negative predictive values for presence of significant stenosis were: by segment, 88.1%, 96.6%, 86.0%, and 97.2% respectively; by artery(n=254), 94.3%, 92.9%, 94.3% and 92.9%, respectively; by patients(n=84), 97.3%, 100%, 100%, and 83.3%, respectively. There were not different with results of non-diabetic patients.
In our results, obesity (≥ 25 kg/m2) and level of coronary calcium score did not affected diagnostic accuracy of MDCT significantly.
Conclusions: Our results indicate that 64-slice MDCT coronary angiography shows similar diagnostic accuracy compared with non-diabetic patients and may be useful for detecting CAD in patients with type 2 diabetes.

Nothing to Disclose: JEL, WJC, JSY, KCW, HWL

P2-530

Glucose Control and Lipid Metabolism in African American Patients with Type 2 Diabetes and Chronic Hepatitis C.

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Context: Metabolic abnormalities are common in chronic hepatitis C infection.
African Americans have a lower prevalence of metabolic syndrome despite higher prevalence of obesity and hypertension. Understanding the interaction between hepatitis C infection, serum lipids and glucose metabolism in African American population is an important question because of the prevalence of the hepatitis C in inner city African American population. The primary aim of this study was to compare the lipid profile and glucose control in the African American patients with type 2 diabetes (T2D) with and without chronic hepatitis C infection (HCV).
Methods: We conducted retrospective chart review on African American patients in an academic outpatient clinic setting. We compared data from 111 patients with T2D and HCV, 68 patients with HCV, and 104 patients with T2D. We also analyzed differences and correlations in serum levels of total, LDL and HDL cholesterol, triglyceride and hemoglobin A1C.
Results: The three groups were similar in term of age and gender distribution. The viral load and grade of infection were not different between the patients with HCV only and those with HCV and T2D. Chronic HCV infection was associated with lower total and LDL cholesterol levels, even in the patients who had T2D. In contrast, the elevated serum triglyceride levels associated with T2D were not reduced in patients with T2D and chronic HCV (158 ± 68.5 vs 128± 58, P = 0.01) , although diabetes control was better in the T2D group with HCV than in the T2D group without HCV (A1C: 7.1 $\pm$ 1.8% versus 8.8 $\pm$ 2.1%, P <0.001). The HDL cholesterol level was not different among the three groups. The mean body mass index (BMI) of the group with T2D without HCV was 35.5 kg/m2, which was significantly higher (P<0.001) than that of HCV (BMI=28.3) or HCV and T2D (BMI=30.1) groups. However, no correlation was found between BMI and HbA1c or lipid parameters.
Conclusions: Chronic HCV infection in T2D patients decreases serum levels of total and LDL cholesterol, but has no effect on triglyceride levels. HCV infection may differently alter the cellular pathways of cholesterol and triglyceride metabolism in patients with T2D. T2D patients with chronic hepatitis C infection may have a metabolic profile different from classic T2D.

Nothing to Disclose: JS, SZ, BS, AA-S

P2-531

Insulin Sensitivity, Metabolic Syndrome, and Cardiovascular Disease in an Elderly, Obese Population.

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Introduction

Elderly individuals who are mildly obese and have no history of cardiovascular disease (CVD) or diabetes pose challenges for physicians because although they may have only moderate cardiac risk, they frequently present with myocardial infarction or stroke. We hypothesize that insulin sensitivity will vary within this population and the degree of insulin sensitivity will vary inversely with atherosclerosis, as measured by carotid ultrasound, and with other CVD risk factors, such as parameters of the metabolic syndrome (MBS) and the Framingham risk score (FRS).

Methods

We studied 60 - 80 yo men and women with body mass indices 30-34 kg/M^2 and no history of CVD or diabetes. Assessments were made for components of the NCEP ATP III criteria for MBS and FRS. Oral Glucose Tolerance Test (OGTT) and Frequently Sampled Intravenous Glucose Tolerance Test (FSIVGTT) were performed. We calculated insulin sensitivity index (S_I) from the FSIVGTT using the Bergman Minimal Model. Carotid intima-media thickness and plaque score (sum of plaque heights) were measured by carotid ultrasound.

Results

We present data from the first 23 subjects of a planned study of 150 subjects. Carotid ultrasounds were performed on 14 subjects. Subjects were divided into tertiles based on S_I (see Table). The most insulin resistant subjects (Tertile 1) had lower total cholesterol, HDL-C, and had higher carotid plaque scores than subjects in the more insulin sensitive tertiles (Tertiles 2 and 3). We observed a strong inverse relationship between insulin sensitivity and plaque score (R^2 = 0.38, p = 0.02). To date, we have not found a relationship between the number of MBS parameters or FRS with plaque score.

	Tertile 1 (n = 8)	Tertile 2 (n = 7)	Tertile 3 (n = 8)
Si	2.1 ± .5*	3.85 ± .5*	7.5 ± 2.5
Age	69 ± 6	72 ± 5	65 ± 6
SBP	137 ± 13	143 ± 14	132 ± 12
BMI	32 ± 1.4	32.5 ± 1.5	32.9 ± 2.1
Fasting Glucose	96 ± 8	94 ± 7	92 ± 12
Total- C	146 ± 28*	176 ± 24	195 ± 37
LDL-C	89 ± 24	105 ± 16	117 ± 33
TG	111 ± 39	140 ± 47	122 ± 53
HDL-C	35 ± 6*	43 ± 9	53 ± 10
Plaque Score	14.3 ± 8.7 (n = 5)*	10.2 ± 7.5 (n = 5)	0.9 ± 1.8 (n = 4)
FRS	21 ± 7	23 ± 6	14.5 ± 7
MBS Parameters	2.8 ± 1	2.4 ± 1	2 ± .8

* $p < 0.05$

Conclusion

In healthy, mildly obese, elderly subjects, insulin resistance increases the risk of atherosclerosis. In this select population, which is at moderate risk for CVD, insulin resistance may offer additional information of risk beyond that provided by FRS or MBS.

Nothing to Disclose: GDC, RLP-R, KIC, SFF, JEN, JRL

P2-532

The Effect of Serum Lipids on VER and ERG in Asian American and Caucasian Diabetics.

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Both Visual Evoked Response (VER) and Electroretinography (ERG) have been used to assess visual function in diabetic retinopathy(1,2,3). Asians have increased prevalence of diabetes due to their predilection for the metabolic syndrome. It may be important to examine the ethnic specific role of serum lipids and glucose in diabetic visual function.
Purpose: To use VER and 30Hz Flicker ERG to analyze the relationship between visual function and serum glucose and lipids in Asian American and Caucasian type II diabetic patients with good vision.
Methods: Subjects: 54 patients, 54 eyes, 27 Asian American(AA) and 27 Caucasian(C) age-matched pairs. VER and 30Hz Flicker ERG(ISCEV protocol(4,5), LKC UTAS System). Inclusion criteria: 20/15-20/60 vision, lipid profile, FBS, HbA_{1c}, dilated funduscopic exam. Analysis: Retrospective chart review, Fisher's exact test and student's t-test.
Results: We first examine how serum lipids and glucose level affects VER and ERG in AA and C diabetics. The threshold values for lipid levels are(mg/dL except for HbA_{1c}): CH(cholesterol): ≥160 vs <160, TG(triglyceride): ≥120 vs <120, HDL: ≥50 vs <50, LDL: ≥100 vs <100, Non-HDL CH: ≥120 vs <120, FBS: ≥126 vs <126, HbA_{1c}: ≥7% vs <7%. The threshold values for amplitude and implicit time: VER: 9μV and 100ms; ERG: 80μV and 28ms. Among AA, VER implicit time is prolonged with increased TG and FBS while non-HDL CH and HbA_{1c} depress VER amplitude in C. Also, elevated FBS prolongs ERG implicit time in AA. Between AA and C diabetics, there are significant differences in VER amplitude, implicit time and ERG implicit time.

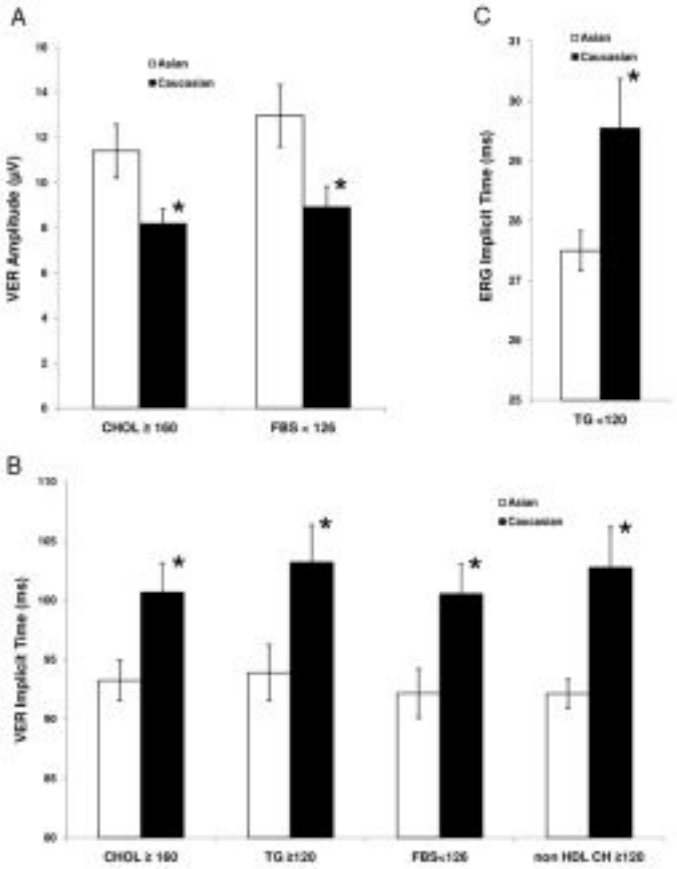

Figure1: VER and ERG comparison between Asian American (AA) and Caucasian (C) diabetics. AA or C diabetics were selected according to preset criteria (total chol ≥160, total chol<160, TG≥ 120, TG <120, HDL≥50, HDL<50, LDL≥100, LDL<100, non-HDL CH≥120, non-HDL CH<120, FBS≥126, FBS<126, HbA1c≥7.0%, or HbA1c<7.0%, respectively; mg/dL except for HbA1c) and compared using student's t-test. The significant effect of serum lipids and glucose level on VER amplitude (A), VER implicit time (B), and ERG implicit time (C) is shown. No significant difference is found in ERG amplitude between AA and C diabetics. *P<0.05

Conclusion: This pilot study is the first to suggest that serum lipids alter VER and 30Hz Flicker ERG significantly in diabetics. Moreover, we find that VER and ERG exhibit differential susceptibility to unfavorable serum lipid profile between AA and C diabetic patients. Interestingly, VER is more affected than ERG in both ethnic groups. C diabetics tend to have smaller VER amplitude and longer VER implicit time than their AA counterparts. Further large-scale studies are needed to evaluate the use of VER and ERG in diabetics with abnormal serum lipids.

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Nothing to Disclose: GW, LM, BH

P2-533

Classical and Novel Cardiovascular Risk Factors in Pregnant Filipino Women with and without Gestational Diabetes.

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Background*:* Nearly half of Filipino women with gestational diabetes mellitus (GDM) have been shown to develop not only type 2 diabetes but also the Metabolic Syndrome within 2 years from the index pregnancy. However, it is not known whether classical (hypertension, dyslipidemia, abnormal glucose tolerance) or novel (elevated hsCRP, hyperinsulinemia) cardiovascular risk factors already manifest during a GDM pregnancy.
Objective*:* To compare classical (lipid profile, blood pressure) and novel (inflammatory markers, insulin resistance) cardiovascular risk factors of pregnant women with and without gestational diabetes mellitus.
Design*:* Cross-sectional, comparative study
Methodology*:* Pregnant women consulting for prenatal care were recruited if they were >20 years old, between 20-28 weeks gestation, and had a singleton pregnancy. Prior diabetes, impaired glucose tolerance, or GDM; intake of steroids or beta-blockers during the current pregnancy; history of dyslipidemia or intake of lipid-lowering medications; significant past medical history; and ongoing or a recent history of infection or injury were exclusions. Participants were divided into lean and obese groups (BMI cut-off 27.7). Blood pressure, hsCRP, lipid profile and insulin levels during a 100-g oral glucose tolerance test were measured. Validated measures of insulin sensitivity in pregnancy were computed.
Results*:* Fifty-six women were recruited (11 with GDM, 8 obese and 37 lean). Total cholesterol levels were increased in GDM women (p=0.02) compared to those with normal glucose tolerance (NGT). Mean blood pressures did not significantly differ between groups, although more GDM women had systolic BP $\geq$140 mm Hg (p=0.00). Two-hour post-glucose load insulin levels were increased in GDM women compared to obese (p=0.02) and lean (p=0.00) NGT women, however, measures of insulin sensitivity were not significantly different. GDM women tended to have higher hsCRP levels (p=0.15), with a trend for hsCRP directly correlating with BMI and indirectly with fasting insulin.
Conclusion*:* During a GDM pregnancy, classical, but not novel, cardiovascular risk factors are manifested.

Sources of Research Support: Philippine General Hospital - Pfizer Lipid Research Unit.

Nothing to Disclose: MVH-G, ITIT, MVL, PCP, MAL-A

P2-534

The Effect of Definition on the Prevalence of Metabolic Syndrome and Its Components.

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Background: No studies have compared the prevalence of Metabolic Syndrome (MS) among Canadian adults using the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP III) and the International Diabetes Federation (IDF) definitions.
Objective: The purpose of this study was to examine the sex specific prevalence of MS in Canadian adults by using ATP III and IDF definitions and determine the effect of definition on the prevalence of the component factors
Methods: The Canadian Heart Health Survey was a cross-sectional probability sample survey of 23179 adults ages 18-74, conducted in all 10 Canadian provinces between 1986 and 1992 .The present study was based on 4724 men and 4712 women for whom full anthropometric measurements were obtained (provinces of Alberta, Manitoba, Ontario, Quebec and Saskatchewan) and for whom data on all components of MS were available. MS was defined according to ATP III and IDF definitions. A weighted analysis using SPSS PASW Complex Samples (version18) was conducted.
Results: According to ATP III, 17.9% and 15.3% of men and women have MS, while according to IDF, 23.8% and 17.3% of men and women have MS, respectively. Kappa agreement between the definitions was 72 % for men and 80% for women. In men who have MS according to IDF 68.3%, 86.8%, 78.3%, and 15.4% have low high-density cholesterol, high triglyceride, hypertension, and diabetes, respectively, versus 79.9%, 93.1%, 87.3%, 17.7% according to ATP III. In women, the comparable levels of prevalence are 78.8%, 77.6%, 76.2%, 15.3% according to IDF, and 83.3%, 79.2%, 82.6%, 20.5% according to ATP III.
Conclusion: In Canadian adults the prevalence of MS is higher when the IDF definition is applied but the metabolic derangement of individuals identified is less severe. This may have implications for the prognostic value of the two definitions.

Nothing to Disclose: KM, BR, PP

P2-535

Prevalence of Diabetes, Prediabetes and Metabolic Syndrome in a Selected Sector of a Rural Community in the Philippines: Phase II of the Community-Based Diabetes Self-Management Education (DSME) Program in San Juan, Batangas, Philippines.

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Background: This survey gathered data prior to the implementation of the community-based Diabetes Self-Management Education Program in San Juan, Batangas, Philippines. Prevalence data regarding diabetes, prediabetes and metabolic syndrome in the Philippine rural setting are limited.
Objectives: To determine the prevalence of diabetes, prediabetes and metabolic syndrome in this rural community, and to determine the risk factors associated with their occurrence
Study Design: Cross-sectional prevalence survey using three-stage stratified random sampling design
Participants: Recruited were adults at least 18 years old. Excluded were pregnant women and those taking glucocorticoids.
Methodology: Each participant answered a questionnaire. Blood pressure, height, weight, waist circumference and hip circumference were measured. Tests for fasting blood sugar, 75-gram oral glucose tolerance and lipid profile were done.
Results: A total of 365 adults were surveyed. Mean age was 49 years. Most were females, married, non-smokers and had elementary or secondary education. Nine percent were known diabetics, while 10% were newly diagnosed, giving a diabetes prevalence of 19%. Prediabetes was present in 26%. Metabolic syndrome was seen in 38%, with low HDL being its most prevalent component. Increasing age correlates with increasing prevalence of diabetes (OR 1.04), prediabetes (OR 1.02) and metabolic syndrome (OR 1.03).
Conclusion: Diabetes, prediabetes and metabolic syndrome are prevalent in this rural community, more prevalent than in the Philippine general population, with increasing age being a significant risk factor. A low HDL is the most prevalent component of the metabolic syndrome, compatible with data from the Philippine general population and other Asian countries.

Sources of Research Support: International Diabetes Federation - Bringing Research in Diabetes to Global Environments and Systems (BRIDGES) program; Philippine Council for Health Research and Development; Municipality of San Juan, Batangas, Philippines.

Nothing to Disclose: MAS, EPP, GJRA, FLL-A, NJ, EP

P2-536

Acetylsalicylic Acid Resistance in Patients with Type 2 Diabetes Mellitus, Prediabetes and Non-Diabetic Coronary Artery Disease.

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Background: Several studies have demonstrated a beneficial role of antiplatelet therapy with acetylsalicylic acid (ASA) in prevention of coronary heart disease. Antiaggregant effect of ASA is not uniform in all patients. The purpose of the present study was to evaluate the prevalence of ASA resistance in a consecutive group of patients with diabetes mellitus type 2 (T2DM), prediabetes and nondiabetic coronary artery disease (CAD).
Methods: The effect of ASA was assessed using the platelet function analyser (PFA-100) system. Patients with abnormal blood count results, hepatic and renal disease, taking a drugs affecting platelet function were excluded. Resistance to ASA was defined as a normal collagen/epinephrine induced closure time (CTCEPI) (82–165 s) after one week ASA therapy. According to manufacturer's recommendations, we accepted the prolongation of CTCEPI lower than up to at least 165 s as an ASA resistance. Age and sex matched 3 groups were including, nondiabetic CAD (group 1, n=70), prediabetes (group 2, n=34) and T2DM (group 3, n=155) were compared.
Results: Mean age (years), CTCEPI values and male/female ratios in groups were similar [(57.0±7.6, 55.0±10.7, and 54.5±9.0, p=0.157; 219.7±79.9, 252.1±60.9, and 234.7±71.6, p=0.121; 28/42, 14/20, and 63/92, p=0.599), respectively]. ASA resistance were obtained in 26 (37.1%), 6 (17.6%) and 41 (26.5%) patients in groups, respectively (p=0.154). ASA resistance were significantly higher in men, smokers, patients with hyperlipidemia and patients using insulin (37.0, 36.4, 35.5 and 43.3, respectively). Patients using beta blockers and angiotensin converting enzyme inhibitors had significantly lower ASA resistance (27.2, and 24.6). Altough patients using calcium channel blockers, sulfonylurea**,** thiazolidinedione and metformin had also lower ASA resistance, but the difference was not statistically significant. There was no correlation between CTCEPI and glycemic and lipid parameters, and high sensitive C-reactive protein.
Conclusion: Altough the different was not statistically significant, the finding that ASA resistance prevalance was higher in patients with nondiabetic CAD compared to patients with preDM ve T2DM made us think that prothrombotic risk factors for CAD (needed to be explained by genetic and molecular mechanisms) were more effective on ASA resistance compared to metabolic factors. Drugs influencing ASA metabolism can also contribute to ASA resistance through drug interaction mechanisms.

Nothing to Disclose: SI, MC, UO, HC, DB, OU, YT, ZGC, SG

P2-537

Relationship between Missing Teeth and Health Status Associated with Components of Metabolic Syndrome in Korean Rural Elderly.

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This study analysed the possible associations of dental disease with medical status during 6 years. The subjects were 1494 men and 2117 women elderly over age 60. They were examined the number of missing teeth and health status such as age, BMI, blood pressure, AST level, fasting plasma glucose, and cholesterol level.
In cross sectional study, both men and women subjects were sorted into three group. The group 1 had below 8 missing teeth, the group 2 had 8 ~ 18 missing teeth, and the group 3 had above 18 missing teeth. This study showed that the more missing teeth group have significant relationship between missing teeth and health status in both men and women. Age, blood pressure, AST level, fasting plasma glucose, and cholesterol level were significantly high in men. In women, Age, BMI, blood pressure, fasting plasma glucose and cholesterol level were significantly high.
The Multiple regression analysis results showed that in man, the most influence factors are age, blood pressure, fasting plasma glucose, cholesterol level, AST level respectively in the strength of associations between missing teeth and health status. In women, age, systolic BP, cholesterol level, fasting plasma glucose, BMI and AST level are in influential factors.
In summary, age, blood pressure, fasting plasma glucose, cholesterol were significantly associated with missing teeth in men. In women, age, systolic blood pressure, cholesterol, fasting plasma glucose, body mass index were significantly associated with missing teeth. So, components of metabolic syndrome may be a potential risk factor to dental health in Korean rural elderly.

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Nothing to Disclose: KCW, HKL, JSM, JEL, EDJ, JSY, HWL

P2-538

Hyperglycemia Is Linked with Prolonged Hospital Stay after Autologous Hematopoietic Stem Cell Transplantation.

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Hyperglycemia frequently occurs after hematopoietic stem cell transplantation. Although hyperglycemia has been reported to be associated with prolonged hospital stay after allogenous hematopoietic stem cell transplantation, little is known after autologous hematopoietic stem cell transplantation that occurs more often than allogenous hematopoietic stem cell transplantation. Parenteral nutrition and steroid are frequently used during hematopoietic stem cell transplantation and could raise blood glucose. In this study, we examined the impact of morning blood glucose concentration (BGC) on the length of posttransplant hospital stay in relationship to the use of parenteral nutrition and steroid after autologous hematopoietic stem cell transplantation.

We reviewed the medical records from the patients who received autologous hematopoietic stem cell transplantation from Jan 1, 2005 to Dec 31, 2007 at this institution. After exclusion of the patients with known diabetes before hematopoietic stem cell transplantation and random BGC ≥ 200 mg/dL before hematopoietic stem cell transplantation, this study included 671 adult (≥ 18 years old) patients (44% female, age 49±13 years old, BMI 28.33±5.75 kg/m^2, mean±STD). Among them, 72% received parenteral nutrition and 12% received steroid. Use of parenteral nutrition and steroid were associated with elevated morning BGC (P<0.0001 for both) and the length of posttransplant hospital stay (P<0.0001 for both). Morning BGC was positively associated with the length of posttransplant hospital stay (P<0.0001). This association remained significant after adjustment for age, gender, and BMI (P<0.0001), and also after adjustment for age, gender, BMI, use of parenteral nutrition and steroid (P=0.006). Based on the deciles of morning BGC, a significant increase in the length of posttransplant hospital stay was noted from the 8th to 9th deciles (16.6±2.8 vs. 19.2±2.5 days, P<0.0001, while 118±2 vs. 129±5 mg/dL for BGC) after adjustment for all covariates.

Our observations confirm the negative associations of parenteral nutrition and steroid with morning BGC and the length of posttransplant hospital stay. Hyperglycemia is significantly associated with prolonged length of posttransplant hospital stay after adjustment for covariates with a threshold effect. An interventional trial is required to confirm the causal relationship. Better glycemic control could potentially shorten the posttransplant hospital stay.

Nothing to Disclose: RK, MP, RJ, CK, KCC

P2-539

Different Correlations of hs-CRP Concentrations with Therapeutic Variables among Three Ethnic Groups of Singapore with Diabetes Mellitus 2.

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Introduction and Background: Proinflammatory markers such as high sensitivity C-reactive protein (hs-CRP) correlate with insulin resistance and cardiometabolic risk among type 2 diabetes individuals and show evidence of ethnic variations and correlations with various therapeutic variables. We therefore examined whether hs-CRP concentrations are correlated with antidiabetic agents, ACEi/ARB, statins and aspirin in our multiethnic population and whether any correlation differs by ethnicities.
Methods: We prospectively recruited 111 Chinese, 68 Malays and 67 Indians with type 2 diabetes mellitus and measured their hs-CRP in addition to standard laboratory tests. A multivariate regression model was built to analyze for any correlation of hs-CRP to the various therapeutic and demographic variables.
Results: A significant correlation was seen between hs-CRP and BMI, female gender and the use of metformin ($p<0.005$) in all three ethnicities collectively when adjusted for age, sex, BMI, smoking status, HbA1c, lipid profile, use of aspirin, ACEi/ARB, statins, metformin, rosiglitazone, sulfonylurea, glinides, acarbose and insulin. A subgroup analysis of individual ethnic groups revealed a correlation of hs-CRP only to female sex and BMI in the Chinese subgroup, with age, BMI, statins, ACEi/ARB and sulfonylureas in the Malay subgroup and with age, female sex, smoking status, ACEi/ARB and metformin in the Indian subgroup ($p<0.05$). Correlations with rosiglitazone and acarbose were omitted here due to a limited sample size on these medications.
Discussion and Conclusion: The above suggests from a therapeutic perspective that hs-CRP responds favorably to metformin and ACEi/ARB in the Indians, to ACEi/ARB and sulfonylurea in the Malays while having no significant associations to treatment modalities in the Chinese. Further larger scale, long term prospective trials will help to clarify these associations better.

Sources of Research Support: The Endocrine and Metabolic Society of Singapore.

Nothing to Disclose: RD, MJ, SPC, RWMC, MKSL

P2-540

Evaluation of the Relation between Metabolic Parameters and Subclinical Atherosclerosis in Patients with Gestational Diabetes.

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Objective: Gestational diabetes mellitus (GDM) is a common complication of pregnancy. The risk to develop type 2 DM later in life is 5-50%. Subclinical inflammation is an important component of atherosclerosis and pre-diabetic period. The aim of the study is to evaluate subclinical atherosclerosis and metabolic parameters in patients with GDM at diagnosis and first year postpartum.
Methods: 60 patients with GDM and 39 healthy pregnant women were included in the study. OGTT was applied to patients at diagnosis and first year postpartum. Their metabolic, subclinical atherosclerosis parameters were evaluated and their carotid intima media thickness (CIMT) was measured with B mode ultrasound.
Results: There were no differences with respect to age, parity, gestational age (week), weight gain in pregnancy (kg), history of previous GDM and large birth weight infant. The mean fasting blood glucose (FBG), fasting insulin, homeostasis model assessment insulin resistance (HOMA-IR), uric acid, fibrinogen, triglyceride (TG), CRP levels and CIMT in women with GDM were significantly higher than those in the control group ($p<0.05$ for all). There were no significant differences between the groups with respect to plasma levels of total cholesterol (TC), high density lipoprotein–cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL –C). At the first year postpartum; impaired fasting glucose (IFG) (2.5%) in 1 patient, impaired glucose tolerance (IGT) (12.5%) in 5 patients, IFG +IGT in 5 patients (12.5%) and DM in 4 patients (10%) were detected. The results of twenty five patients (62.5%) were normal. When metabolic and subclinical atherosclerosis parameters at diagnosis and first year of 40 patients who were able to be contacted at the first year postpartum were compared; while there were no changes with regard to BMI, FBG, HBA1c and CIMT, there was a significant recovery in TG, TC, LDL-C fibrinogen and CRP levels and insulin resistance.
Conclusion: Even though some of the metabolic anomalies which occur during pregnancy in pregnant women with GDM improved, the development rate of IFG, IGT and DM during the first year was detected to be high. Also due to not detecting any change in the first year post pregnancy in CIMT which is an indicator of subclinical atherosclerosis, the patients with gestational diabetes may require to be followed up with regards to subclinical atherosclerosis in future periods.

Nothing to Disclose: UO, SI, AA, GA, YT, DB, SG

P2-541

Insulin Sensitizing Agent, Pioglitazone, Decreases Blood Pressure along with Reduction of Insulin Resistance in Type 2 Diabetic Patients with Metabolic Syndrome.

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Background: The metabolic syndrome is a cluster of disturbances such as impaired glucose tolerance, hypertension, central obesity, dyslipidemia, and others for which insulin resistance and compensatory hyperinsulinemia have been proposed to be the underlying disorders.
Objective: This study was designed to elucidate the effects of PPAR gamma agonist, pioglitazone, on blood pressure and renin-angiotensin-alodosterone system with the insulin sensitizing effect in Japanese type 2 diabetic patients (T2DM) with metabolic syndrome (METS).
Design and Methods: 24 Japanese type 2 diabtic patients with METS (20-42 years of age (28.9 $\pm$ 5.3)) were enrolled in this trial, to who gave informed consent to participate in this study. These patients were treated with pioglitazone (15mg/day) for 3 months. Homeostasis model of assessment (HOMA-IR), free fatty acid (FFA), blood pressure, pulse rate, plasma renin activity (PRA), plasma aldosterone concentration (PAC) , atrial (ANP) and brain natriuretic peptide (BNP), were measured before and upon completion of the administrating drug. Diagnostic criteria of METS were according to IDF Asia.
Rsults:
1. HOMA-IR decreased significantly with treatment of pioglitazone from 5.68 $\pm$ 2.98 to 2.32 $\pm$ 1.36 (P<0.001).
2. Plasma free fatty acids (FFA) and triglycerides (TG) were decreased significantly after treatment of Pioglitazone, 0.79 to 0.358μEq/L, 146.7 to 96.5 mg/dl, respevtively. HDL-cholsterol (C) elevated significantly 37.5 to 52.3 mg/dl.
3. After administration of pioglitazone, systolic and diastolic blood pressures decreased significantly (from 138.8 $\pm$ 10.4/86.7 $\pm$ 6.5 to 125.3 $\pm$ 9.7/77.9 $\pm$ 7.7 mmHg, (p<0.01). Heart rate also decreased significantly with treatment (from 80.6 $\pm$ 10.6 to 70.5 $\pm$ 9.12/min (p>0.01) .
4. Plsma renin activity (PRA) decreased significantly with treatmnt from 1.86 $\pm$ 2.2 to 0.67 $\pm$ 2.1 to 0.90$\pm$ 1.3 ng/ml (p<0.01).
5. Body mass index, haematocrit, ANP and BNP did not changed significantly.
6. After treatment of pioglitazone, plsma AST and ALT are markedly decreased.
Conclusion: Monotherapy of PPAR gamma agonist, pioglitazone, may reduce elevated blood pressure, improve dyslipidemia, fatty liver in T2DM with metabolic syndrome along with reduction of insulin resistance.

Nothing to Disclose: SH, JY, HS, TW

P2-542

Testosterone Deficiency as the Cause of the Metabolic Syndrome.

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Testosterone has a favorable impact on the parameters most often disrupted in the metabolic syndrome, i.e. body mass, insulin secretion and sensitivity, lipid profile and blood pressure. Hypogonadism associated with fatigue is becoming more common in men and women as age and weight both increase over time. [1, 2] The age-related declines in testosterone and low IGF-I, are often associated with the deposition of visceral fat creating increased waist size, a major component of the metabolic syndrome.[3] This may be the first sign of overweight that most patients notice. Nonetheless, a low serum SHBG, low total and free testosterone is also associated with metabolic syndrome even in nonobese men.[4] Some clinical signs relating to androgen deficiency, such as increased fat mass over lean body mass plus the lack of motivation to exercise contribute to the sleep disruption and fatigue seen in the metabolic syndrome.[5] As testosterone modifies both fat metabolism and insulin activity through its effect on abnormal glucose tolerance, it creates beneficial changes regarding insulin sensitivity, HDL cholesterol levels and elevated blood pressure. [6,7] About one third of type 2 male diabetics, who are obese with poorly controlled blood glucose, also demonstrate hypogonadism. [8]Testosterone supplementation is safe and effective in all these conditions. Cultural obesity due the American diet of empty calories is not only a strong risk factor for type 2 diabetes, but is often found in conjunction with hypogonadism. [9] During improvement of glucose control with insulin, levels of free testosterone and its metabolites increase.[10] Testosterone replacement therapy has demonstrated the potential to slow or halt the progression from metabolic syndrome to overt diabetes and cardiovascular disease. [11] These finding in addition to the fact that hypogonadism is so often associated with the metabolic syndrome indicate that it could be the primary cause of this condition rather than just an associated finding.[12]

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Nothing to Disclose: AHK

P2-543

Prevalence of Lower Levels of C-Reactive Protein in Type 2 Diabetic Men Treated with Thiazolidinediones and Exenatide.

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It has been shown that low grade inflammation as reflected in increased C-reactive protein (CRP) concentrations may play a role in the pathogenesis of insulin resistance, type 2 diabetes and atherogenesis. We therefore, conducted a cross-sectional analysis of 297 males with type 2 diabetes, to investigate the effect of sulfonylureas (SU), metformin, thiazolidinediones (TZD**),** insulin, exenatide and sitagliptin, on CRP. CRP concentrations were log transformed and adjusted for age, BMI, HbA1c, co-morbidities (such as congestive heart failure, coronary artery disease, chronic kidney disease) using Charlson index. Table 1 and table 2 show multivariate analysis of individual medications and combination of medications, respectively, on CRP, after adjusting for other medications. We conclude that patients on TZD's alone, and in combination with metformin and exenatide have significantly lower CRP values. Further large studies need to be undertaken to look at the effects of various diabetes medications on inflammatory markers, including CRP.

	Non-Users	Users	P-Value
Metformin	5.2±0.8	3.5±0.6	0.13
Insulin	3.96±0.4	3.95±0.4	0.98
TZD	5.2±0.6	2.7±0.2	0.04*
Exenatide	4.8±0.4	2.6±0.2	0.07
SU	4.5±0.4	3.4±0.4	0.7
Sitagliptin	4.6±0.4	2.86±0.2	0.12

Table 1: Multivariate analysis of individual medications on CRP concentrations (mg/l), data is mean±SE, *=p<0.05

	Non-Users	Users	P-Value
TZD and Metformin	5.1±0.6	2.8±0.2	0.041*
TZD and Exenatide	5.2±0.6	2.6±0.2	0.029*
TZD and Sitagliptin	4.8±0.4	2.9±0.2	0.06
TZD and SU	4.4±0.4	3.1±0.3	0.11
TZD and Insulin	4.4±0.4	3.7±0.3	0.37

Table 2: Multivariate analysis of combination of medications on CRP concentrations (mg/l), data is mean±SE, *p<0.05

Disclosures: AC: Speaker, Lilly USA, LLC, Merck & Co., Novo Nordisk, Sanofi-Aventis; Research Funding, Sanofi-Aventis. PD: Speaker, Sanofi-Aventis, Lilly USA, LLC, GlaxoSmithKline, Amylin Pharmaceuticals; Research Funding, GlaxoSmithKline, Sanofi-Aventis, Amylin Pharmaceuticals.
Nothing to Disclose: AV, SB, MV

P2-544

The Short-Term Influence of Weight Loss on Metabolic Profiles and Cardiovascular Risk in Obese Korean Patients with Type 2 Diabetes.

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Backgrounds and Objectives: The obese patients with body mass index (BMI), over 25 kg/m^2 constitute more than about 47% of adults in Korean diabetes. They are gradually increasing and related with high risks of cardiovascular disease. So, this study aims to assess the effects of single-drug intervention without any lifestyle modification or specific combination therapy on body weight, glucose metabolism, and cardiovascular risk in obese Korean type 2 diabetic patients.
Methods: This randomized placebo-controlled double-blind, prospective clinical trial compared placebo with sibutramine mesilate hemihydrate as therapeutic drug. A total of 55 obese type 2 diabetic patients (female : 80%, mean age = 50.6 ± 9.8 years old, duration of diabetes mellitus = 4.8 ± 4.2 years, BMI = 29.9 ± 3.8 kg/m^2) suitable for the inclusion criteria were randomly allocated to receive either sibutramine mesilate hemihydrate 11.51 to 17.26 mg once daily (n = 29) or placebo (n = 26) for 12 weeks. Body composition, profiles of lipid and glucose, and other metabolic parameters were assessed at randomization and end of trials. The agumentation index (AIx), pulse wave velocity (PWV), subendocardial viability ratio (SEVR), and ejection duration (ED) were also evaluated by applanation tonometry (SphygmoCor-System).
Results: Significant weight change was observed in the treatment group compared with placebo. The reduction of BMI and the waist/hip circumference were also greater in the treatment group than placebo. Glucose profiles did not change in both groups, but A1C level and homeostasis model assessment-IR fell significantly in subjects who lost ≥ 5% of weight in the treatment group (n = 14). Neither total nor LDL cholesterol showed any significant changes in any treatment group, but triglyceride levels fell significantly by 42.9 mg/dL (27%) in the sibutramine group (P = 0.001). Pulse pressure decreased significantly by 4.6 mmHg (13%) in the treatment group (P = 0.017) at the end of trial. Also, the improvement of SEVR and ED were observed in the subjects who lost ≥ 5% of weight.
Conclusions: Several drug intervention trials in obese type 2 diabetes have shown that the improvement of metabolic profiles depends on the degree of weight reduction and may have ethnic and regional variations. This study showed that 5% reduction of body weight from baseline can be a useful marker of minimum weight reduction for the improvement of metabolic parameters and cardiovascular markers in Korean.

Nothing to Disclose: HRL, SHY, OHR, SJL, DMK, MGC, HJY, EGH

P2-545

Hyperglycemia Early after HLA-Matched Hematopoietic Stem Cell Transplantation: Does It Predict Acute Graft Versus Host Disease?.

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Introduction: Acute Graft Versus Host Disease (GVHD) is a major complication of allogeneic hematopoietic stem cell transplant (HSCT), affecting up to 66% of patients. Like stress hyperglycemia, acute GVHD is believed to be initiated by excessive inflammatory cytokines. We conducted a cohort study to determine whether new-onset hyperglycemia early after HSCT indicates increased risk of developing acute GVHD.
Methods: Subjects were consecutive adult recipients (200 males and 200 females) of primary, HLA-matched, allogeneic HSCT for acute leukemia at our institution during 2003-2009. HSCT conditioning was myeloablative or reduced intensity. To be eligible, subjects had to receive a peripheral blood graft and GVHD prophylaxis that included tacrolimus, sirolimus, methotrexate, or mycophenolate mofetil. They also had to be alive and free of GVHD and leukemic relapse on Day 10 post-transplantation, the starting point for study. Exclusion criteria were history of diabetes mellitus, hyperglycemia within two weeks before admission for HSCT, and recent use of corticosteroids. Data were collected retrospectively. Study outcome was acute GVHD grade 2-4 or competing event (leukemic relapse or death) on Day 11-100 after HSCT. The primary risk factor was hyperglycemia during Day 1-10 after HSCT, categorized as “severe” (10-day mean fasting blood glucose of 180 mg/dl or higher, or use of medication to control hyperglycemia), “moderate” (10-day mean above 150 but below 180 mg/dl), or “mild” (10-day mean of 110-150 mg/dl), evaluated using generalized linear modeling of the cumulative incidence-based proportional hazard of acute GVHD. Multivariate analysis adjusted for factors potentially related to hyperglycemia (body mass index before HSCT, definite or probable febrile infection during Day 1-10) and acute GVHD (unrelated versus sibling donor, age and sex of donor and recipient, lymphoid versus myeloid leukemia, stage of disease, intensity of conditioning, and regimen of GVHD prophylaxis).
Results: Data collection for the study is nearly complete, and full results of the statistical analysis will be submitted before March 1st, 2010.

Nothing to Disclose: EHG, BS, CB, PMP

P2-546

Diabetes and Disease Burden in the Aged (≥65 Years), Community-Dwelling Medicare Beneficiary Population.

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Background: The U.S. population is aging. Epidemiologic knowledge about diabetes (DM) and the burden of its complications in geriatric populations, however, is limited. **Methods:** De-identified, cross-sectional data were extracted from the 2004 Medicare Current Beneficiary Survey in which 15559 beneficiaries, residing either in the community or long-term care facilities, or their caregivers were queried via interviews. General questions provided data about demographic traits, functional status, and disease burden. Supplemental questions provided data about diabetes. Beneficiaries <65 years of age, eligible for Medicare by disability, residing in chronic care centers, with pre-/borderline diabetes, or lacking significant information for specific chronic disease conditions (CD), i.e., coronary heart disease (CHD), stroke (CVA), or significant visual difficulties (SVD), were excluded. Descriptive and analytic statistics were used to characterize the population. **Results:** 10606 beneficiaries were identified. Eye exams were common; >80% in the non-diabetic population and >87.9% in the diabetic population reported having an exam within the last 2 years. 26.5%, 16.9%, 11.1%, and 6.8% reported having CHD, DM, CVA, or SVD respectively. Frank blindness was reported in <10% of those with SVD. For each of the specified CD categories, the presence of DM increased the likelihood of CD: CHD (40.2 vs 23.8%), CVA (16.1 vs 10.2%), and SVD (9.8 vs 6.2%). Of the patients with DM, 48.9% reported having DM without the other CD. 37.9%. 11.6%, and 1.7% reported having 1, 2, or 3 of the CDs respectively. Of the patients without DM, 66.8% reported none of the other CDs. 26.8%, 5.8%, and 0.6% reported having 1, 2, or 3 of the CDs respectively. 41.1% of patients with DM, but without CDs, (vs 31.4% of the comparable non-diabetic population) reported difficulties with activities of daily living (ADLs) or instrumental activities of daily living (IADLs). The likelihood of ADL/IADL dysfunction was greatest for SVD, followed by CVA, followed by CHD. This pattern was present in beneficiaries with and without DM. The likelihood of ADL/IADL dysfunction increased with the number of CDs and/or the presence of DM. **Conclusion:** CHD is a common DM-related chronic disease. Conversely, SVD is markedly less prevalent, but is correlated with a greater perceived level of disability. The presence of multiple CDs contributes to cumulative decreases in overall functional capacity in the ambulatory setting.
This presentation reflects the opinions of the authors and does not necessarily reflect the position of the CMS or the US government.

Nothing to Disclose: EAK, GSA, RJB

P2-547

Near Infrared Spectroscopic Evaluation of Glucose as a Non-Invasive Alternative to the Finger Stick Technique.

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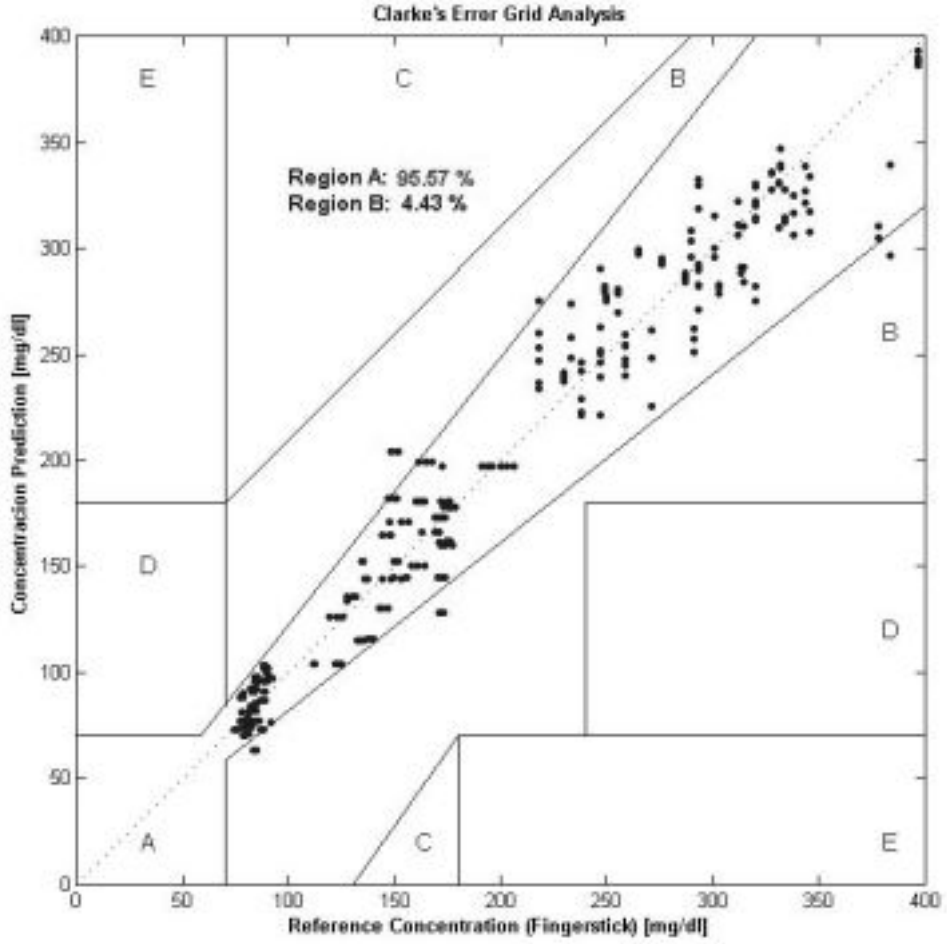
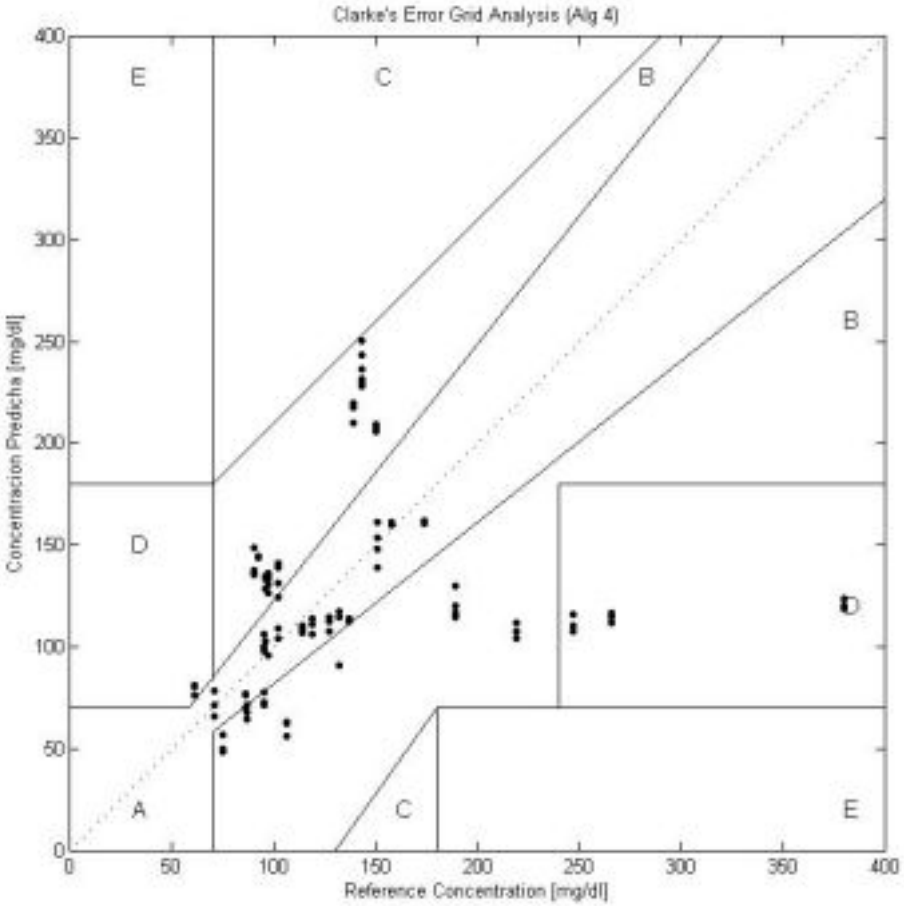
In 2007 the incidence of diabetes in patients under 20 was over 180,000. Lifestyle changes involving: diet, exercise, medication and self glucose monitoring follows (SGM). SGM, recommended for all diabetics, involves multiple fingersticks daily. Compliance is always challenging. Attempts at non-invasive techniques have been attempted for decades with little success. Here we describe a technology currently in clinical trial at our facility using Near-Infrared light (NIR) directed at the space between the index and middle finger.
Method: Children aged 11-18 are recruited. After informed consent/assent, baseline glucose is measured using a standard home glucometer and venus blood (grey top tubes). The space between the index and middle finger of the right hand is placed into a hand cradle, near infrared light is passed through the tissue measuring reflectance from glucose. A regression is developed using the spectra captured and measured glucose values. This is repeated at different glucose values using oral glucose loads if needed. Once developed a new set of subjects and scans challenge the algorithm for accuracy. Results are compared to measured values.
Results: 5 subjects were used to collect 270 unique glucose values and spectra to develop an early algorhythm. 95.57% of the data is within the clinically significant zone.



5 different subjects were used to collect 90 unique spectra to test the algorhitm (glucose range: 61-406). 38.46% and 47.11% fall in zone A and B respectively.



Conclusion: Preliminary data shows that near infrared directed between the index and middle finger is ideal for glucose monitoring. The early dearth of data may explain extradynamic values. Adjustments have since been made to the hand cradle. We anticipate this will further increase the accuaracy and precision of the data.

Sources of Research Support: All-Protect LLC.

Nothing to Disclose: AP, AB, ST

P2-548

Association of Depression with Baseline Characteristics and Response to Diabetes Management in a Low-Income Hispanic Population.

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Background: Depression is reported to be common in low-income Hispanic patients with T2DM and to be associated with poor response to diabetes management but response to antidepressant therapy has been mixed.
Aim: To ascertain the prevalence of depression as diagnosed on the PHQ9 score in patients attending a clinic serving predominantly low-income Hispanic patients and to characterize the association of this diagnosis with baseline and outcome variables.
Method:All patients attending the Roybal Diabetes Management Clinic in East LA have a 2-item depression screen on admission and if either response is positive a full PHQ9 test is offered. We compared patients who attended the clinic in 2009 with and without depression using t-test and chi-square comparisons.
Results:Of 459 patients 89(19%)had a positive 2-item screen. PHQ9 was not done/not available in 17(incl.3 patients already on treatment for depression),20 subjects had a score of <7 consistent with mild depression and 52(11%)≥7 consistent with moderate to severe depression. These 52pts were compared to 370pts with a negative screen. See table.Pts with depression were significantly less likely to be discharged for reaching the clinic A1c goal of<8%(38.5 vs 56.3%; p=0.007).There was no difference in other BL or outcome variables, either demographic(incl gender)or clinical(incl. weight, BMI and BP) with the notable exception of a significantly higher A1c on discharge in the patients with depression and documentation of more neuropathy and foot problems. There was a trend to a higher dose of insulin on discharge in the depression group but no difference in metformin,sulfonylureas or TZD, statin or ACE/ARBs use.

Comparison of Patients Diabetes with and Without Depression

	Age(yrs)	Duration of DM(yrs)	A1c on admission(%)	Change in A1c(%dec)	Neuropathy(%)	High Risk Feet(%)	Insulin Dose on Discharge(IU/d)
Depression	50.9(9.9)	11.2(8.1)	10.0(1.7)	-0.9(1.5)	40(50)	30(50)	43.5(45.4)
No Depression	51.5(10.2)	10.7(8.5)	9.8(2.2)	-1.5(2)	20(40)	20(40)	30.4(45.4)
p	0.67	0.65	0.5	0.04	<0.001	0.019	0.056

Values are mean(sd)

Conclusions: We confirm a high prevalence of depression in low-income Hispanic patients attending a community diabetes clinic and an association with poorer response to glycemic control.Neuropathy and foot care should be investigated as therapeutic targets in this group.

Nothing to Disclose: DN, W-AL, CS, AG, AP, EOB

P2-549

Sex Hormone-Binding Globulin Attenuates the Age-Related Increase in Adiponectin Levels in the Baltimore Longitudinal Study of Aging.

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It has been shown that adiponectin increases with age, but whether such an increase is primarily due to aging or age-related confounders is unknown. Testosterone (T) decreases with age in men, and men with hypogonadism have significantly elevated adiponectin, which normalized with testosterone replacement. It is unclear whether this effect is due to T changes or to parallel sex hormone-binding globulin (SHBG). We tested whether T and/or SHBG contribute to the increase in adiponectin with age using data from the Baltimore Longitudinal Study of Aging.
Nine hundred and seven subjects had data on adiponectin, total T, SHBG, and anthropometrics (Table 1). Participants on sex hormone therapy or sex hormone modulating agents were excluded. Biochemical markers were log-transformed for analyses.

Table 1

	Women	Men
Characteristic	Median(25th-75th precentile)	
N	419	488
Age,yrs	66(58-76)	71(61-79)
Weight,kg	69.4(60.0-79.9)	83.1(75.1-94.0)
BMI,kg/m²	26.1(23.0-30.4)	26.7(24.6-30.2)
Adiponectin,µg/mL	12.9(8.2-20.4)	8.9(5.0-15.6)
Total T	19.0(13.0-27.0)	432.5(328.0-558.0)
SHBG	79.0(55.0-111.0)	57.0(42.0-81.0)

All characteristics differ at $P<0.01$.

Table 2. Linear regressions

		Adiponectin			
		Women		Men	
Model	Variable	β	R²	β	R²
1	Age	0.12*	0.01*	0.34†	0.11†
2	Age	0.11*	0.08*	0.30†	0.16†
	BMI	-0.26†		-0.22†	
3	Age	0.13*	0.09*	0.30†	0.16
	BMI	-0.26†		-0.22†	
	Total T	0.12*		-0.02	
4	Age	0.08	0.17†	0.22†	0.18†
	BMI	-0.14*		-0.17†	
	Total T	0.08		-0.07	
	SHBG	0.32†		0.20†	

β=standardized coefficient. *$P<0.05$, †$P<0.001$

Adiponectin increased with age in both men and women, and the strength of the association was slightly attenuated after adjusting for body mass index (BMI). This association was not affected by adding total T to the model. The addition of SHBG to the model attenuated the effect of age on adiponectin by 27% in men and 38% in women, and the independent association of age with adiponectin was no longer significant in women. Furthermore, the introduction of SHBG significantly improved the fit of the models (Table 2). These results support the hypothesis that SHBG plays a role in the modulation of adiponectin. Further studies are needed to understand the mechanism by which SHBG affects circulating adiponectin.

Sources of Research Support: Intramural Research Program of the NIH, National Institute on Aging.

Nothing to Disclose: KSG, RR, AAW, ODC, LF, JME, CWC

P2-550

Differential Diagnosis of Type 1 Diabetes (T1D) and Type 2 Diabetes (T2D) in African American (AA) and Hispanic American (HA) Pediatric Patients Aged 2 – 18.

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INTRODUCTION Incidence of pediatric T1D and T2D is rising, with T2D increase attributed mostly to rising overweight in children and adolescents. Genetic predisposition puts AA and HA children and youth particularly at risk for T2D. Differentiation of T1D from T2D at presentation has been made more challenging due to: 1) the decreasing age of T2D patients at onset; 2) differences among ethnic groups and between genders.
OBJECTIVE: Develop a multivariate logistic model that will assist in the initial differential diagnosis of T1D and T2D.
METHODS Retrospective patient data from 227 AA and 91 HA charts, spanning birth years from the 1980s to 2000s, were used to assess differences in T1D and T2D patients. Variables used to develop the model included gender, age at diagnosis (agedx), and BMI. Hyperpigmentation and family history were not included due to sparse retrospective data.
RESULTS Model: Age, gender, and BMI z-score from 150 randomly selected AA patients were utilized to create a model that was tested in 74 AA and 89 HA patients. In AA, sensitivity (correct prediction of T2D) was 91%, and specificity (correct prediction of T1D) was 93%. In HA, sensitivity was 79% and specificity was 87%. The model, developed with AA data, was relatively poor in predicting T2D in HA patients but could predict T1D with a better accuracy. Differences between AA and HA results may be due to a significant ($p < 0.0001$) difference in gender distribution of T2D HA and AA patients. Agedx decreased with time for both T1D and T2D. Agedx decreased faster in AA T1D patients than in AA T2D patients ($p = 0.0437$). BMI z-score exhibited a non-significant increase with year of birth in T2D patients; conversely, T1D patients exhibited no increase in BMI z-score. The rate of increase in AA T2D girls was significantly ($p < 0.0015$) different from the mild rate of decline in AA T1D girls.
CONCLUSIONS: These data suggest that differential diagnosis of T1D and T2D in minority pediatric patients can be achieved with good sensitivity and specificity using parameters easily and rapidly assessed at presentation. These data show a decrease in age of onset with time for both T1D and T2D and an increase in BMI z-score over time for T2D, but not T1D patients. These data also show gender differences in T2D risk in AA, but not HA, pediatric patients. A large, prospective study is needed and being planned to replicate these data, develop a model for HA, and refine the differential diagnosis models.

Nothing to Disclose: NK, SB, JNB, GG, JJ, RY, TT, NG, ER, JAN

P2-551

Risk Awareness of Conversion of Gestational Diabetes Mellitus into Diabetes among Women with Previous History of GDM and Treating General Practioners.

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[1]Indraprastha Apollo Hosp New Delhi, India.

OBJECTIVE—
To examine risk awareness for future risk of diabetes amongst women with history of gestational diabetes mellitus (GDM) and treating general practitioners (GPs) at primary healthcare level, as the first contact point in our country is usually a GP.
RESEARCH DESIGN AND METHODS—
We did a retrospective analysis of 144 women with previous history of GDM who presented for the first time with diagnosis of Type 2 diabetes in our endocrine outpatient department. We assessed whether post-partum oral glucose testing (PPOGT) was done by attending GPs and advice given to the patients' regarding future risk of diabetes. The patients' perceptions and self -knowledge about future risk of diabetes and modification of lifestyle behavioral practices were enquired. Multivariate models included participant characteristics as well as potential modifiers of risk perception (knowledge of diabetes risk factors and beliefs in the benefits and barriers of lifestyle modification).
RESULTS—
Only 16 out of 144 (11%) women were advised PPOGT during subsequent follow up; the same group were educated regarding future testing and risk of developing diabetes. Only 2 of these patients followed up as advised. 8 (6%) of women recognized that GDM was a risk factor for future diabetes, but only 14 (10%) believed that they were in high risk group for developing diabetes. Women who perceived themselves to be at moderate/high risk more often planned to modify their future lifestyle behaviors.
CONCLUSIONS—
Despite availability of robust literature evidence of a strong association between GDM and subsequent conversion to diabetes, GPs at primary level healthcare do not advise regarding future risk of conversion of GDM into diabetes. Women with histories of GDM usually do not perceive themselves to be at elevated risk; conversely, women who were educated regarding the same were willing to undertake lifestyle modification.

Nothing to Disclose: MG, MAS, SKW

P2-552

ABCC8, KCNJ11, GLUD1, GCK Gene Analysis and Clinical Phenotypes in Korean Patients with Congenital Hyperinsulinism.

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Background: Congenital hyperinsulinism (CHI) is a clinical and genetic heterogeneous disease. There have been few studies in Korea.
Aim: In order to identify genetic causes and clinical characteristics in Korean CHI patients, we performed the first large scale mutational analysis of Korean CHI patients.
Methods: We investigated 18 CHI patients by performing a direct sequence analysis of the entire coding sequence of *ABCC8, KCNJ11, GLUD1* and *GCK.*
ABCC8 and *KCNJ11* were sequenced in all patients without hyperammonemia. *GLUD1* mutation was saught in CHI with hyperammonemia. If no mutations were identified, *GCK* gene was sequenced.
Results: We found mutations in 16 patients (88.9%), which were within the *ABCC8* (75%), *KCNJ11* (18.8%), and *GLUD1* (6.2%). Ten novel mutations were identified, eight within *ABCC8* (E100X, W430X, D812N, Q922X, Q1133H, H1134W, E1208RfsX1220, A1366D), and two within *KCNJ11* (W91X, R136C). Three out of five diazoxide-responsive patients showed *ABCC8* mutations. All six patients undergoing a subtotal pancreatectomy were diagnosed histologically a diffuse form. Four of them had *ABCC8* heterozygous mutations.
Conclusions: A genetic diagnosis was possible in 88.9% of Korean patients. *ABCC8* mutations were the most commonest cause. The novel mutations in *ABCC8* and *KCNJ11* suggested genetic heterogeneity in Korean patients.

Nothing to Disclose: S-EP, C-HS, S-WY

P2-553

An Algorithm for the Clinical Assessment of Post-Islet Transplant Insulin Therapy.

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Introduction/background - Timely recognition and correction of islet exhaustion resulting from inadequate exogenous insulin therapy may play a vital role in the long term success of islet transplantation. This abstract reports on a quantitative method for predicting insulin therapy requirements in post-islet transplantation subjects with type 1 diabetes.
Methods - A neural-network-based computer algorithm has been developed to generate a non-linear regression function that reflects the correlation between several pre- and post-transplant subject parameters and reported subject insulin intake in the post-transplant period. After program training, parameter weights are calculated and used to predict post-transplant insulin requirements and graft survival estimates (GSE).
Results - The program's predicted insulin requirements (PIR) post-transplant were accurate within < 0.1 U/kg/day compared to reported actual insulin intake based on traditional clinical management practices. These minor differences between reported actual insulin intake and PIR values for all subjects (n = 11) were used to estimate the degree of hyper- and hypoinsulinization at each subject visit (41 observations). Subjects that were reportedly insulin-free but would be required to take supplemental insulin were classified into two groups based on whether the average calculated insulin deficit (CID) was greater or lesser than -0.03 U/kg/day within the first year post-transplantation. Although glycosylated hemoglobin (A1c) and average daily glucose were not different between the two groups during the first year post-transplant, they become significantly different ($p < 0.05$) during the second year, with subjects requiring ≤ 0.03 U/kg/day having A1c levels < 6% and average glucose of 111.7 mg/dl as compared to A1c of > 6.5% and average blood glucose of 127.9 mg/dl for subjects requiring > 0.03 U/kg/day.
Conclusions - The algorithm is accurate in identifying instances of inadequate post-islet transplant insulin therapy that can be associated with later increases in A1c serum levels and average daily blood glucose. The program can be used prospectively to ensure adequate insulinization post-islet transplantation in order to prevent deterioration in islet graft function, and thereby improve long-term islet transplant outcomes.

Sources of Research Support: National Institutes of Health (U42-RR16607); Juvenile Diabetes Research Foundation(31-2008-616).

Nothing to Disclose: CRO, JS, IAR, MYA-S, CVS, ME-S, DCD, YM, IHA-A, FRK

P2-554

Continuous Glucose Monitoring in Pregnant Women with Polycystic Ovary Syndrome: A Case/Control Study.

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Background: Women with PCOS are at increased risk for diabetes, and also likely gestational diabetes(GDM). We hypothesized that women with PCOS would display ambient hyperglycemia (as measured by a continuous glucose monitoring system) early in pregnancy that would progressively exacerbate with pregnancy.
Design and Methods: Case/Control study during singleton pregnancy of 17 women with PCOS and 17 healthy women all without diabetes at baseline. A 75-g oral glucose tolerance test (OGTT) followed by 24-h continuous glucose monitoring(CGM) was obtained at four times during pregnancy (Visit 1 [V1] at 6-10 wks; Visit 2 [V2] at 12-16 wks; Visit 3 [V3] at 24-28 wks; Visit 4 [V4] at 34-38 wks).
Results: At baseline, demographics were comparable except that women with PCOS had higher mean BMI (31.9 vs 26.3 for controls, p=0.03) and testosterone (95 and 60ng/dL, respectively, p=0.01). Eight women with PCOS (47%), and 2 (12%) controls developed GDM according to the WHO criteria. PCOS women had higher area under the curve (AUC) for glucose at OGTT at V1 and V2. Significantly higher AUC for insulin at OGTT was found for PCOS at V4 compared to V1, and for controls at V3 and V4 compared to V1, with no difference between the groups. No difference was found between the groups for 24h CGM AUC, mean, median, SD, CV, lunch and dinner postprandial values; day and night glucose values; and beta cell function. In 24h postprandial values, the only difference found was in lower glucose 30 minutes after meal at V4 compared to V1, in PCOS (p=0.04), which in the control group did not reach significance (p=0.08). Free androgen index (FAI) significantly decreased over the course of the study in both groups mediated by decreases in testosterone and increases in SHBG, but this decrease in FAI was stronger in PCOS group at V1 (p=0.002), V2 (p=0.08) and V3 (p=0.04) vs controls. There was no significant difference in birth weight between the groups, 3347g in PCOS and 3597g in Controls (difference in means -250, 95% CI -541 to 40; p=0.09), nor in gestational age. Birth weight correlated with the 24-hour glucose mean and median in controls.
Conclusions: During pregnancy 24-hour glucose monitoring, unlike OGTT, revealed only subtle changes in glucose metabolism between PCOS and healthy women. The observed changes imply that advancing pregnancy may ameliorate ambient hyperglycemia in women with PCOS, perhaps mediated by a relative marked improvement in hyperandrogenemia in women with PCOS.

Nothing to Disclose: RD, KIH, CB, ARK, RSL

P2-555

Evaluation of 1,5-Anhydroglucitol as a Marker for Glucose Variability in Patients with Type 2 Diabetes Mellitus.

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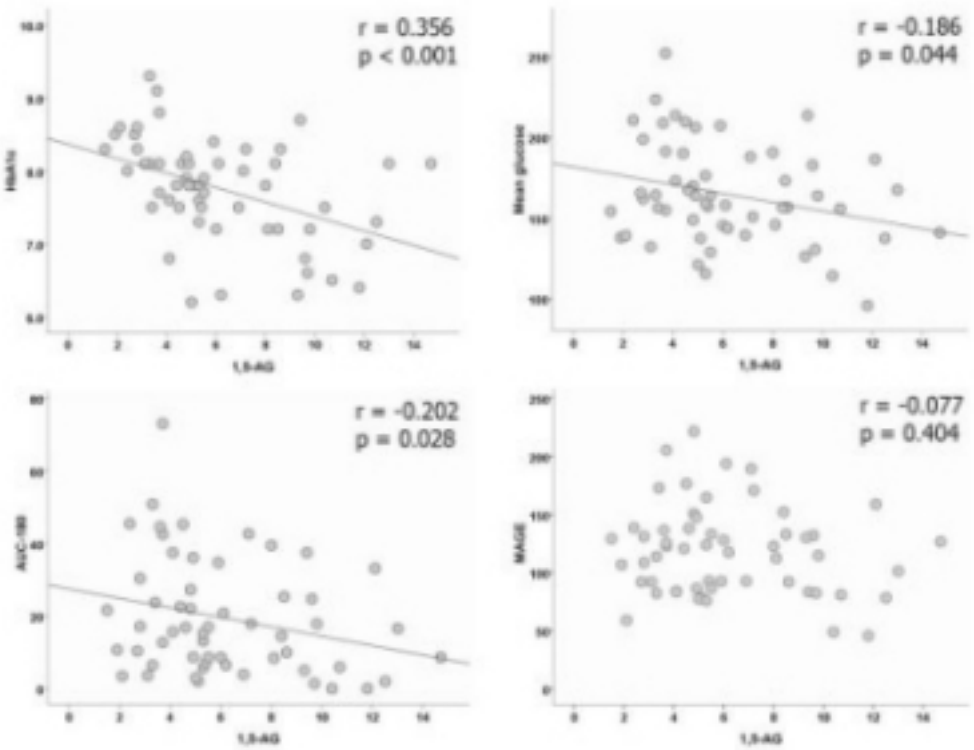
Aims: 1,5-Anhydroglucitol (1,5-AG) is a circulating polyol and has been suggested as a marker of short-term glycemic control and postprandial hyperglycemia. However, the role of 1,5-AG in glucose variability has not been established. The aim of this study was to demonstrate the usefulness of 1,5-AG as a marker of glucose variability in type 2 diabetes patients.
Methods: In 56 patients with type 2 diabetes, 1,5-AG was measured. Continuous glucose monitoring system (CGMS) was applied to the patients for 72 hours, and mean amplitude of glycemic excursion (MAGE) was calculated for glucose variability, and mean glucose, area under the curve for glucose above 180 mg/dL (AUC180), and mean postmeal maximum glucose (MPMG) were calculated. We analyzed the relationships among 1,5-AG, HbA1c, and the variables from CGMS measures.
Results: Characteristics of the enrolled subjects were as follows: age, 60±10 years; male sex, 39%; BMI, 24.5±2.9 kg/m^2; duration of diabetes 18±10 years; HbA1c, 7.8±0.7%. 1,5-AG levels were 6.4±3.3 mg/L (range 1.5~14.7), MAGE was 118±39 mg/dL, and mean glucose levels were 164±31 mg/dL, respectively. 1,5-AG levels were correlated with HbA1c, mean glucose levels and with AUC180.



The correlations were more significant in subgroup analysis with those having HbA1c ≤ 8.0% (n=33). However, 1,5-AG and MAGE was not correlated in these subjects.
Conclusion: Our data suggested a limited usefulness of the level of 1,5-AG in estimating glucose variability in moderately controlled patients with type 2 diabetes, while it can represent mean glucose and postprandial hyperglycemia.

Nothing to Disclose: MJK, BYH, YL, SHK, HJC, HSJ, YJP, SYK, KSP

P2-556

Combined Use of Body Mass Index and HbA1c Improves Specificity of Diagnosing Type 2 Diabetes Mellitus.

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Background: The criteria of glycated hemoglobin (HbA1c) ≥6.5% was recently recommended by the American Diabetes Association (ADA) for diagnosing type 2 diabetes mellitus (T2DM). In the absence of unequivocal hyperglycemia, HbA1c on 2 separate occasions should be done for confirmation of T2DM.
Aim: To evaluate the validity of using an easily obtainable parameter, the body mass index (BMI), to improve the specificity of a single HbA1c result for diagnosing T2DM.
Methods: Cross-sectional study involving 90 predominantly Chinese Asians (54 males, 36 females; mean age 60±17 (SD) years) with no known T2DM. Each subject was evaluated with a 75g oral glucose tolerance test (OGTT) and a corresponding HbA1c. Receiver operating characteristic (ROC) analysis was used to obtain an optimal HbA1c cut-off point for the diagnosis of T2DM, defined by 2-hour post-load (2HPG) glucose ≥11.1mM. Multiple ROC curve analysis was used to obtain the BMI cut-off that conferred the best specificity when paired with this HbA1c cut-off.
Results: The area under the ROC curve (AUROC) was 0.770 for HbA1c in predicting T2DM (95% CI 0.674-0.866, $p<0.001$). The optimal HbA1c cut-off point for the diagnosis of T2DM was 6.2% (AUROC 0.705; 95% CI 0.595-0.814, $p=0.001$), with a specificity of 65.3% and sensitivity of 75.6%. Specificity improved when HbA1c≥6.2% was paired with increasing cut-off of BMI. The paired values of HbA1c ≥6.2% and BMI ≥34kg/m^2 gave a specificity of 100% in predicting T2DM.
Conclusion: In our cohort, HbA1c in combination with BMI was a specific and stronger predictor of DM than HbA1c alone. A repeat blood test to confirm T2DM in patients with HbA1c ≥6.2% and BMI ≥34kg/m^2 may not be necessary. Larger multi-centered, multi-ethnic studies are needed to confirm this result.

Nothing to Disclose: ET, RC, L-WC, VA, J-PF, T-LT, JK, S-BS

P2-557

HbA1c May Not Be a Specific Determinant of Diabetic Status in the Elderly.

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Background:
Glycated hemoglobin (HbA1c) has been recommended as a means of diagnosing type 2 diabetes mellitus (T2DM) by the American Diabetes Association (ADA). We aim to determine the impact of age on the appropriate cut off value of HbA1c in relation to the diagnosis of T2DM in our local population.
Methods:
Cross sectional study of 90 predominantly Chinese patients without prior history of T2DM (mean age 60.9 years, range 20 to 93) who had a 75g oral glucose tolerance test (OGTT) and HbA1c done as a screening test. Patients were stratified into 4 quartiles: age <50 (n=20), 50.1-59 (n=24), 59.1-72 (n=23) and >72 years (n=23). Age, gender, race and past medical history were ascertained. Triglycerides, HDL cholesterol and fasting plasma glucose (FPG), as well as height and weight for calculation of body mass index (BMI) were measured. Receiver operating characteristic (ROC) curve analysis was used to derive the HbA1c cut off for diagnosis of T2DM.
Results:
FPG and lipid profile were not significantly different between the 4 groups (one way Anova). Of the 90 patients, 44.4% (n=40) were diagnosed with T2DM based on a 2-hour post-load glucose (2HPG) of >11.1mM. In our study, HbA1c of 6.2% was most optimal for diagnosing T2DM (sensitivity 75.6%, specificity 65.4%, area under ROC curve (AUROC) 0.705, 95% CI 0.595-0.814, p=0.01). The HbA1c cut off for each age subgroup was determined as well. The HbA1c cut off remained at 6.2% for subjects aged <72 years (yrs). However, for those aged >72, the HbA1c value was found to be 6.0%. At the specified HbA1c of 6.2%, the AUROC had a decreasing trend in older patients (age<50 yrs: AUROC 0.85, 95%CI 0.665-1.035, p=0.008; age 50.1-59 yrs and 59.1-72 yrs: AUROC 0.705, 95%CI 0.595-0.814, p=0.001; age >72 yrs: AUROC 0.697, 95%CI 0.475-0.918, p=0.110) suggesting that the specificity and sensitivity of HbA1c decreases as age increases. Sensitivity and specificity of HbA1c of 6.2% in age <50 yrs was 90% and 80% respectively, compared to 54.5% and 66.7% in those >72 yrs. Through linear regression, BMI was found to be significantly predictive for DM (p=0.001) besides HbA1c.
Conclusion:
HbA1c had a low specificity for diagnosing T2DM in older Asian subjects, suggesting that caution needs to be exercised in using HbA1c in isolation. Other metabolic parameters such as BMI are predictive of T2DM, and should be taken into consideration, especially in older subjects.

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(2) Saydah SH et al., Diabetes Care; 25: 1940-1945
(3) International Expert Committee, ADA., Diabetes Care; 32;(7)
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Nothing to Disclose: T-LT, RC, L-WC, JK, J-PF, ET, VSCA

P2-558

The Role of A1C in the Investigation of Diabetes in Patients with Impaired Fasting Glucose.

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Recently, the validity of A1C as a screening tool for diabetes (DM) has been examined and the cut point of ≥6.5% would be diagnostic of DM while between 5.7 and 6.4 % “pre-diabetes”, if confirmed. A1C has been considered as a substitute for fasting plasmatic glucose (FPG) and for the oral glucose tolerance test (OGTT). However, other reports have questioned the use of A1C because of low sensitivity and confounding aspects of assay. **Objective:** The aim of this study was to correlate the A1C values with the OGTT in individuals without DM but with IFG (FPG 5.6- 6.9 mmol/l). **Research Design and Methods:** We investigated 119 subjects with IFG. Exclusion criteria included: previous history of DM or FPG ≥ 7.0 mmol/L; chronic renal and liver diseases; TSH level >5 UI/ml; use of glucocorticoid; infection by HIV or hepatitis C virus; hemoglobinopathies or anemia; and pregnancy. After 12 hours of fasting they were submitted to OGTT with measure of plasma glucose 2 hours after overload (2-hPG). A1C, lipid profile, creatinine, uric acid and complete blood cell counts were also determined. The relation among A1C, FPG and 2-hPG were evaluated by a Pearson's coefficient. Furthermore, the patients were divided, according to the levels of A1C, in 3 groups (≦5.7%; 5.8-6.4% ≥6.5%). The other variables were compared among these groups using x^2 and ANOVA followed by Newmans-Keuls. Kappa coefficient was used to test agreement between A1C values and 2-hPG for the diagnosis of DM. A *P* value < 0.05 was taken as significant. **Results:** Mean age was 54.22 ± 14.63 years and 70.5% were women. The body mass index (BMI) was 30.16 ±5.46. A1C had a good correlation with 2-hPG in patients with IFG (P=0.0001). There was a weaker correlation between A1C and FPG (P=0.02). While levels of A1C ≥6.5% were associated to abnormalities in the 2-hPG in 86.7%, just 28.6% of these patients had 2-hPG >11.1 mmol/l. 31.6% of the patients with diagnosis of DM by the OGTT had A1C levels ≥6.5%. 64.3% of the patients with A1C ≤ 5.7% had a normal OGTT. There were no correlations among A1C level, age, BMI, waist circumference, red blood cell count, lipids, uric acid. The Kappa coefficient of reliability between A1C and 2-hPG to diagnose DM was 0.71. **Conclusion:** A1C is a useful tool in evaluating IFG patients and it correlates better to 2-hPG than to FPG. Yet, the agreement between A1C and OGTT to diagnose DM in this group was moderate (71%). It may be too soon to consider A1C as a substitute exam for OGTT.

Nothing to Disclose: MVL, MMS, MWL, APGD, JBB, INBD, AVR, GVC, VEB, EPD

P2-559

Hemoglobin A1c Collection in Hospitalized Patients as Part of the Diabetes Intervention Opportunity Study (DIOS).

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Assessing hemoglobin A1c (HBA1c) in hospitalized patients with type II diabetes has been proposed to be a useful test in guiding diabetes management after discharge. However, there is limited data documenting how this test is utilized in clinical practice. We report an initial experience as part of a multicenter DIOS study, in obtaining HBA1c in hospitalized patients and its potential use for diabetes management after discharge

Methods: We conducted a retrospective electronic medical record review of patients with type II diabetes at our medical center admitted from October 1, 2007 through October 1, 2008. Data from the first known hospitalization was used. Patient demographics, initial HBA1c, discharge anti-diabetic agents, length of stay and subsequent HBA1c after discharge were recorded.

Results: A total of 3,676 patients (54% female) were identified with a mean age of 63±14 years. The average length of stay was 4.6±25.7 days. There were 2114 pts (58%) who had HBA1c assessed during hospitalization with a mean value of 7.2±1.8%. Of these pts, 945 (45%) had a HBA1c ≥7%; 271 (13%) had a HBA1c ≥9%; 172 (8%) had a HBA1c ≥10%; and 6 (3%) had a HBA1c ≥12%. Upon discharge, 70% of pts with HBA1c ≥9, 71% of pts with HBA1c ≥10%, and 73% of pts with HBA1c ≥12% were prescribed regimens that included some type of insulin therapy. There were 265 pts in whom a follow-up outpatient HBA1c was available with a 3.7±2.7 months (range 1-11) following discharge. The mean outpatient HBA1c was 7.2±1.7%. There was no significant difference between the inpatient HBA1c and the first outpatient HBA1c after discharge. Among the pts with HBA1c ≥7% in the hospital, only 25% achieved HBA1c <7 in the outpatient setting.

Conclusion: Hospital HBA1c was obtained in the majority of pts (58%) and showed a significant fraction (45%) were uncontrolled (≥7%). One-third of pts with HBA1c ≥9% were discharged without insulin therapy. Only 25% of uncontrolled pts achieved HBA1c <7% after discharge. Despite its potential utility, 42% of our pts did not have a HBA1c done in the inpatient setting. This study demonstrates not only the opportunity to improve adherence in obtaining inpatient HBA1c but also an avenue to better utilize the hospitalization time to redesign the diabetic outpatient regimen. The data clearly shows the importance of our future prospective study using the inpatient HBA1c as a tool to improve long-term outpatient diabetes control.

Nothing to Disclose: JM, DB, JM, BP, LF

P2-560

Effectiveness of Continuous Glucose Monitoring in Patients with Diabetes Mellitus: A Systematic Review and Meta-Analysis.

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Real-time, continuous glucose monitoring devices (rt-CGM) is coming into widespread clinical use but results of studies comparing rt-CGM to conventional self-monitoring of blood glucose (SMBG) have not been consistent across study populations. Therefore, we conducted a systematic review and meta-analysis of the effects of rt-CGM on glycemic control in studies meeting all of the following criteria: 1) type 1 diabetes (T1DM) or type 2 diabetes (T2DM); 2) rt-CGM with glucose results visible to subjects; 3) inclusion of a comparison group; and 4) reported change in glycemic outcomes. We searched MEDLINE and EMBASE from January 2005 to December 2009. Titles, abstracts, and articles were reviewed independently by two authors. RESULTS: Of 492 titles, 181 articles were reviewed, identifying 12 studies that met inclusion criteria for our systematic review: 8 studies included only T1DM, 1 only T2DM, and 3 combined T1DM and T2DM. Two studies, while including a comparison group, were non- randomized prospective design. Study duration ranged from 10 days to 18 months. The definition and assessment of hypoglycemia and hyperglycemia varied across studies in non-standardized ways. However, of the 9 studies reporting hypoglycemic outcomes, 3 favored the rt-CGM group, while 1 favored SMBG and 5 reported no difference in the likelihood of hypoglycemia. Of the 6 studies reporting hyperglycemic outcomes, 3 favored the rt-CGM group, whereas 3 reported no difference between groups. Five randomized controlled trials (RCTs) with continuous use of rt-CGM, and ≥ 3 months of follow-up were included for meta-analysis of HbA1c changes (pooled N=815 from 7 sub-group estimates, all T1DM). Pooled weighted-mean difference (WMD) in change of HbA1c was calculated using the DerSimonian-Laird method for a random-effects model due to a high degree of between-study heterogeneity (Cochran Q statistics =30.6 on 6 df; I-squared = 80.4%; p<0.0001). Compared to SMBG, the use of rt-CGM significantly improved glycemic control with WMD in change of HbA1c = -0.25% [95% confidence interval (CI): -0.45% to -0.05%, p=0.013]. Both the Begg's funnel plot and the Egger plot did not identify significant publication bias. CONCLUSION: RCTs suggest that the use of rt-CGM may provide benefit in glycemic control without increasing hypoglycemia or hyperglycemia, although the additional reduction of HbA1c from rt-CGM demonstrated by current studies is small.

Sources of Research Support: NIDDK Grant T32DK62707; NIDDK Grant P60DK079637.

Nothing to Disclose: HCY, ASE, TTB, JCB, CDS

P2-561

Currently Available Continuous Glucose Monitoring Systems Lower Hemoglobin A1C for Adult Patients with Type 1 Diabetes: A Systematic Review and Meta-Analysis of Randomized Trials.

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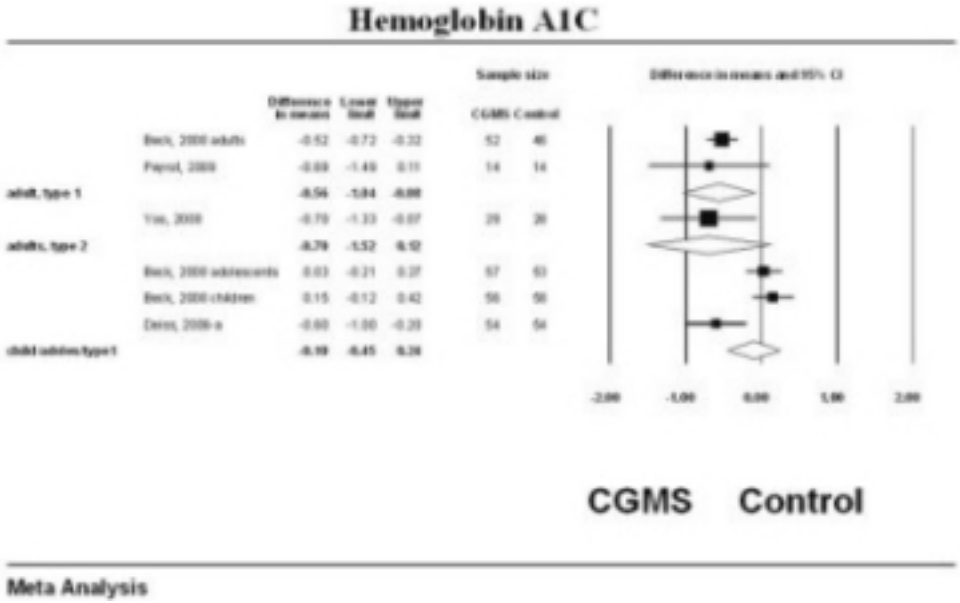
[1]Mayo Clin Jacksonville, FL and [2]Mayo Clin Rochester, MN.

Objective: Continuous glucose monitoring (CGMS) is a promising new technology that may revolutionize diabetes care. We conducted a systematic review and meta-analysis to assess the efficacy of CGMS in improving glycemic control and safety in reducing hypoglycemia compared to self monitored blood glucose (SMBG) devices.
Methods: We searched MEDLINE, EMBASE, Cochrane Central, Web of Science, and Scopus from 1996 through September of 2009. Included studies were randomized controlled trials of adults and children with diabetes. Pairs of reviewers worked independently to assess methodological quality and extract data. Meta-analytic estimates of treatment effects were generated using random-effects model.
Results: Of 870 articles generated initially during the search, 18 met inclusion criteria. 12 trials were ultimately excluded because they used CGMS devices that are no longer currently available. The included trials were heterogeneous with the largest trial including 322 patients followed for 26 weeks. CGMS was associated with a significant reduction in mean HbA1c of 0.6% (95% CI 0.1 to 1.0) in adults with type 1 diabetes. There was a nonsignificant reduction in children/adolescents or in patients with type 2 diabetes (figure). The quality of supporting evidence is considered low due to imprecision (wide confidence intervals) and inconsistency across studies (I^2=80% for type 2 DM, non estimable for other analyses). Data for the incidence of severe or nocturnal hypoglycemia and hyperglycemia was very sparse, poorly reported and imprecise. In studies that reported patient satisfaction, most felt confident about the device and gave positive reviews. Subgroup analysis and meta-regression demonstrated no effect of the length of study follow up, duration of CGMS use, allocation concealment and adherence monitoring on the weighted mean difference of hemoglobin A1c (p>0.05 for all analyses).
Conclusion: CGMS may reduce HbA1c for adults with type 1diabetes. Larger, more rigorous studies are needed to assess the efficacy of CGMS in reducing patient-important complications without significantly increasing burden of care for patients with diabetes.



Nothing to Disclose: MAK, KMW, HMM, MBE, MJG, MSB, CGC, YCK, PJE, VMM, GYG

P2-562

Clinical Utility of Office-Based Continuous Glucose Monitoring in the Care of Patients with Uncontrolled Diabetes.

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Objective: To determine the clinical utility of office-based continuous glucose monitoring (OBCGM) in the care of patients with uncontrolled diabetes.
Methods: Patients between 16 to 81 years with type 1 (T1DM) or type 2 diabetes (T2DM) were included in the study. A total of 122 patients completed a 72 to 120 hour OBCGM between November 2006 and September 2009. Of the 122 patients, 62 were male and 60 female patients, 84 were T1DM and 38 were T2DM. Based on the indications for the study, patients were assigned to one of two groups: hyperglycemia with or without hypoglycemia (Group A, n=97) or hypoglycemia alone (Group B, n=25). Hemoglobin A1C (A1C) were drawn prior to and after OBCGM study and intervention. A healthcare provider reviewed data from OBCGM and made appropriate recommendations.
Results: Among the patients within the hyperglycemia group, there was a small but significant drop of A1C (0.2%, p=0.03). A larger improvement in average A1C was seen in the following subgroups: 1. All patients with A1C > 8% (n=61, p=0.0007), 2. Patients with T2D (n=32, p=0.02), 3. Patients with T2D and A1C >8 % (n=23, p= 0.006), 4. Patients on multiple daily insulin injections (n=52, p=0.008), 5. Patients non-compliant with fingersticks prior to OBCGM (n=55, p=0.04). Individual improvement in A1C of more than 0.5% was noted in 30 of 97 patients in group A. Of 25 patients in group B, 11 reported resolution of symptomatic hypoglycemia. The average A1C in this group did not significantly change (p=0.67).
Conclusion: Office-based continuous glucose monitoring can have a beneficial role in the management of uncontrolled diabetes.

Disclosures: EAN: Consultant, Lilly USA, LLC; Medical Advisory Board Member, Medtronic Minimed.
Nothing to Disclose: NP, SSB, KP, SET, MCL

P2-563

Continuous Glucose Monitoring System (CGMS) as a Diagnostic Tool To Assess Post Partum Glycemic Status.

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Introduction
Glucose tolerance test (GTT) has always been considered as the gold standard diagnostic test for diabetes (DM). However, the reproducibility and validity of GTT has often been in question. We report the concordance of post partum GTTs with continuous glucose monitoring system (CGMS) in a cohort of patients with gestational diabetes who had high probability of persistent diabetes.

Methods
This analysis involves a cohort of 8 women who had gestational diabetes. Their postpartum assessment with GTT revealed normal or impaired glucose tolerance (IGT). However, given their high risk for having diabetes, based on antenatal insulin requirement (>70 units, n=4), obesity (n=3), family history (n=3), macrosomia (n=1) or symptoms (n=1) their glycaemic status was further evaluated on clinical grounds. Following the initial GTT at 6-8 weeks post partum, a repeat GTT within the following 6-8 weeks was arranged for those who had dysglycaemia on the first GTT (five patients). In addition, all 8 patients had a 72 hour CGMS (to collect real-time glycaemic excursions in response to routine diet and activities). The GTT results were classified as diabetic if patients had values more than 11 mmol/L on the CGMS.

Results

Results of GTT and CGMS

Patient	Risk for Diabetes	First GTT	Second GTT	CGMS
1	Insulin requirement, family history	IGT	DM	Positive
2	Insulin requirement, symptoms	IGT	DM	Positive
3	Insulin requirement	Normal		Positive
4	Macrosomia	IGT	IGT	Negative
5	Family history	IGT	Normal	Negative
6	Obesity, family history, insulin	IGT + IFG	Normal	Positive
7	Obesity	Normal		Negative
8	Insulin requirement, obesity	Normal		Negative

Out of the 8 patients, 5 had IGT at the first GTT, one of whom had IFG as well. On the second GTT, two patients had DM and one had IGT. The results of the second GTT concurred with the first GTT in one case only (20%).
The CGMS was positive in 4 out of 8 patients. The CGMS was concordant with the first GTT in 2 cases only. However, it identified four patients with glucose levels in the diabetic range, whilst they had IGT (n=3) or normal first GTT. Paradoxically, one patient with IGT had normal CGMS.

Conclusion
This data confirm the significant discordance between two repeated GTTs in postpartum women who had gestational diabetes. CGMS could be an alternative option to establish hyperglycemia or euglycemia in such high risk population, as a diagnosis of diabetes has huge implication on future health care needs.

Nothing to Disclose: FWFH, LV, ST, RI, AAF

P2-564

Do In-Hospital Blood Glucose Levels Influence the Short-Term Risk of Infections? A Prospective Observational Cohort Study of Hospitalized Subjects with Medical Illness.

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Background Long-term hyperglycemia and its adverse microvascular, immunologic effect leading to increased infection is well-known. There are conflicting study results regarding short-term glycemic control and its effect among critically ill patients, and data is very limited among non-critically ill patients. Our study investigated the relationship between in-hospital hyperglycemia and its short-term infection risk. **Methods** A prospective observational cohort study of consecutive adult patients was done in patients admitted to general medical wards in a community hospital from October 2007 to January 2008. Patients were divided into hyperglycemia (glucose >200mg/dl at least once), non-hyperglycemia (glucose <200mg/dl all the time) groups. Patients were followed up for 90 days after discharge. Data analysis was conducted using student t-test, chi-square test, and adjusted by multivariate logistic regression for confounders. Odds ratio with 95% confidence intervals are reported, and a p-value of <0.05 was considered as significant. **Results** Among 1149 enrolled patients, 726 (63%) completed the study. Of the 726 patients, 259 (35.6%) patients met the criteria for hyperglycemia group. Mean age was 56 years (age range 19-100), 401 (55.2%) were women, and 489 (67.3%) were Hispanics. Within 90 days, 12 (1.6%) died, 170 (23.4%) were readmitted, 67 (92%) were readmitted with infection. Multivariate analysis revealed no significant relationship between hyperglycemia and infection (P = 0.42, odds ratio 0.73, 95% CI = 0.33-1.58). **Conclusion** Our study suggests that hyperglycemia in the non-critically ill patients is not associated with clinically higher risk for infections and may be physiologic response to stress without further immunologic implications in the short term post-discharge period.

Nothing to Disclose: JL, JAV, JCF, YG, BK

P2-565

Chronic Diabetes Control, Infection and Adverse Outcomes in Hospitalized Inner City Minority Patients – Is There a Correlation?.

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Background: Although there are studies linking adverse outcomes amongst hospitalized patients with diabetes mellitus (DM), there is no substantial evidence correlating higher infection with chronic diabetic control. Our aim was to investigate if significant infection requiring hospitalization among diabetics is higher in those that are chronically uncontrolled, as correlated by HgA1c levels in an inner city hospital setting.
Methods: A prospective study was conducted on 191 diabetics admitted to a University based inner-city hospital in NY between July 2008 and December 2009. We defined a composite end point consisting of death, infections, and transfer to either acute, chronic or rehab facilities. Subjects were divided into a fairly controlled DM group (CDM, n=78) if HBA1c ≤9% and an uncontrolled DM group (UDM, n=113) if HBA1c >9%. HBA1c (%, +/- 6 months from admission), BMI and demographic data were then compared to this composite endpoint to analyze if any association exists. Odds ratio (OR) and 95% confidence interval were calculated using regression analysis, and a p value ≤0.05 was considered significant.
Results: Table 1 shows baseline characteristics of the patients. Infection requiring hospitalization was found in 19.2% (15/78) of the CDM group and 19.4% (22/113) of the UDM group. The 3 deaths that occurred came from the UDM group, which also had more serious complications (ICU/sepsis/shock) from infection 45% vs. 33%. In those who had a composite outcome, there was no significant increased odds of having uncontrolled diabetes (OR 1.087, p 0.807, CI 0.557-2.120) after adjusting for confounding variables including age, BMI, ethnicity and gender. However, the odds of obesity were significantly greater in those with a composite outcome.
Conclusions: Our data shows that diabetic hospitalized minority patients who suffered a composite adverse outcome including infections had no significant increase odds of having chronic uncontrolled diabetes, as measured by HBA1c. This suggests that the conventional ideas about infection, outcomes and chronic uncontrolled diabetes need to be further investigated.

Baseline characteristics

	CDM Group, HGA1c ≤ 9.0 (N=78)	UDM Group, HGA1c > 9.0 (N=113)
Mean age (yrs)	64	61
Gender (M:F)	29:49	43:70
Hispanic: Other	63:15	86:27
Mean LOS (days)	3	4
Mean HBA1c	7.62	13.3
Transfer to: acute facility/chronic facility/Rehab (# of patients)	6/0/2	1/4/6
Discharges	70	99
Deaths	0	3

Nothing to Disclose: JH, JAV, NL, MT, JDA, BK

P2-566

The Role of the Endocrinologist in Improving Diabetes Care.

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Objective: To determine the effect of diabetes care by endocrinologists on glycemic control, management of cardiovascular risk factors, and adherence to American Diabetes Association (ADA) guidelines.
Methods: The study is a retrospective chart review of 178 patients with diabetes, either type 1 or type 2, who presented for initial visit at the Hallett Center for Diabetes and Endocrinology between June 1, 2004 and May 31, 2005. Patients' weight, medications, HbA1c value, blood pressure and cholesterol levels, as well as their creatinine and urine microalbumin, and vaccination status were recorded at initial visit and at 12-18 months follow-up.
Results: One hundred seventy eight patients, 104 (58%) females and 74 (42%) males, were identified and included in the study. Mean HbA1c at the initial visit was 8.56 ± 1.95% and was improved to 8.02 ± 1.72% during the follow-up period (p=0.007). Patients' systolic blood pressure improved from 132.1 ± 20.5 mmHg to 128 ± 17.9 mmHg (p=0.048), diastolic blood pressure improved from 75.1 ± 12.0 mmHg to 71.8 ±11.8 mm Hg (p=0.009), while their LDL levels were improved from 106.4 ± 38.5 mg/dl to 92.5 ± 32.6 mg/dl (p=0.0007). At follow-up, 33.1% of patients had HbA1c <7% vs 24.2% at initial visit (p=0.034); 60.1% had LDL<100 mg/dl at follow-up compared to 43.3% at initial visit (p<0.0001), and 57.9% had blood pressure <130/80 mmHg at follow-up vs. 48.3% at initial visit (p=0.037). A significant number of patients were started on Aspirin (66.3% vs 33.1%, p<0.0001), ACE-inhibitors (55.1% vs 41.6%, p<0.001) or ARBs (21.9% vs 14.6%, p=0.006) as well as statins (66.9% vs 43.3%, p<0.0001). Weight and renal function were unchanged during the follow-up period.
Conclusion: Referral of diabetic patients to an endocrinologist significantly improves glycemic control, management of cardiovascular risk factors, and adherence to published standards for diabetes care.

Nothing to Disclose: JVM, MS, MJL

P2-567

Detection and Management of Hyperglycemia during Enteral Nutrition Therapy in Non-Critically Ill Hospitalized Patients.

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Hyperglycemia is reported to be one of the most frequently encountered complications of enteral nutrition (EN) therapy in hospitalized patients with and without diabetes. Recognizing that hyperglycemia can adversely impact patient outcomes, it is important that systems be in place to identify and treat patients who develop this complication of therapy. To address this, we sought to determine the frequency of orders for bedside blood glucose monitoring (BSBG) and insulin therapy among inpatients receiving EN. We also sought to investigate potential associations between glycemic measures and length of stay (LOS). All non-critically ill inpatients seen for evaluation by Nutrition Services within 24 hours of initiation of EN during a 6 week time period were included. The following information was collected: age; prior history of diabetes; indication of an order for BSBG and/or insulin therapy; BSBG results during the initial 24 hours of EN and at a follow-up visit conducted 3 to 4 days later. A total of 26 patients were identified, 20 of whom had no prior history of diabetes. Age was similar between those with and without diabetes (64±17 vs 64±17years). All patients with diabetes had an order for BSBG monitoring, and 83% had orders for insulin therapy. Of those without diabetes, 75% had an order for BSBG monitoring and 70% had an order for insulin therapy. Mean BSBG results were (DM vs no DM) 170±46 vs 133±16 mg/dl ($p = 0.054$) at the initial visit and 205±82 vs 133±14 mg/dl ($p = 0.08$) at the follow-up visit. BG values >200 mg/dl were observed in 75% of patients with diabetes and 5% of those with no prior DM history. The mean maximum recorded BG was 283±148 vs 154±26 mg/dl ($p = 0.09$), respectively. There was no association between BSBG measures and hospital LOS. There were no hypoglycemic events recorded in either group. In summary, we observed that the majority of patients receiving enteral nutrition in the hospital have orders for BSBG monitoring and insulin therapy. While glycemic control was maintained within desired guidelines in patients without a prior history of diabetes, hyperglycemia was a common event in those with diabetes. This suggests the presence of clinical inertia in adjusting insulin therapy in the hospital.

Nothing to Disclose: EK, KAD, KMB, ARP, MMC, CAA, ACD, MTK

P2-568

Neuropad Test Is a Useful Diagnostic Tool for Autonomic and Peripheral Neuropathy in Diabetes.

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Diabetic autonomic dysfunction has principally evaluated centrally by assessing heart rate variability and peripherally by assessing sweating using complex and expensive equipment. The aim of the present study was to compare the Neuropad test, a visual simple indicator for sodomotor function, with heart rate variability by coefficient of variation of R-R intervals (CVR-R) and clinical examinations. We examined 87 diabetic patients (55 men/ 32 women) with a mean age of 61.1±8.8 yrs, a mean diabetes duration of 13.0±7.5 yrs and a mean HbA_1c of 8.8±1.7%. All 87 patients underwent assessment of neurological deficits using modified Toronto clinical neuropathy score (mTCNS), 4 g monofilament and CVR-R. The Neuropad test was applied on the plantar aspect of the great toe and removed after 10 min to evaluate the color change as normal (blue color turned completely pink), patchy (patches of blue and pink), abnormal(remained blue). Twenty-eight patients showed normal, 45 patchy and 14 abnormal in Neuropad test. Patients with an abnormal response had significantly longer diabetes duration (19.9±7.9 yrs) than those with normal (10.9±6.9 yrs) or patchy (11.7±7.0 yrs) response, but HbA_1c levels were similar among three groups. The CVR-R in patients with an abnormal response (1.87±0.67) was significantly lower than those of normal (3.49±1.45) and patchy (2.76±1.17) response, respectively. Patients with an abnormal response showed significantly higher mTCNS and lower monofilament results than those with normal and patchy response. We conclude that Neuropad test is a useful diagnostic tool for diabetic neuropathy.

Nothing to Disclose: KY, HO

P2-569

Identifying Initial Laboratory Measurements in Children with Diabetic Ketoacidosis That Predict Correction of Acidosis.

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Background/Objective: Diabetic ketoacidosis (DKA) is a severe form of morbidity and a common form of mortality in Type 1 diabetes mellitus (T1DM) and Type II diabetes mellitus (T2DM) patients requiring immediate recognition, careful management, and correction of the ketoacidosis. In the pediatric population, initial pH, serum bicarbonate and blood glucose at presentation of DKA can predict time to correct acidosis using a standard treatment protocol. The aim of this study is to assess the relationship between initial level of acidosis (serum bicarbonate, pH value, and blood glucose) at presentation of DKA with the time to correction of acidosis to aid in determining which pediatric patients can be safely managed as outpatients.
Methods/Materials: All patients $\leq$ 18 years old with DKA who presented to a tertiary care pediatric center ED were retrospectively identified over a period of five years. Demographic, biochemical, and therapeutic data were collected for each patient. We used regression trees and linear regression models with time to correct acidosis as the outcome. The distribution of variables and the relationship with other variables was explored using plots like box plots, scatter plots and summary statistics like the mean, median, standard deviation and range.
Results: Three hundred and fifty visits were reviewed and multiple linear regressions were performed. The mean time to correction of acidosis using a standard protocol was 11.9 hours. Correction time and pH are greatly negatively related ($r=-0.994$, $p<0.0001$). Correction time and initial bicarbonate level are negatively related ($r= -0.913$, $p=0.011$).
Conclusions: This is an investigation of the relationship of time to correct acidosis to specific factors affecting this, and the ability to develop an algorithm to assist in evaluation of the patient on presentation. Initial laboratory values of venous pH and bicarbonate upon presentation of pediatric T1DM and T1DM patients with DKA can aid in determining potential time to correction of acidosis using a standard treatment protocol, and which patients can be safely managed as outpatients.

Nothing to Disclose: PK, PP-S

P2-570

Using Focus Groups and Informant Interviews To Identify Novel Approaches To Preventing Type 2 Diabetes in Women with a History of Gestational Diabetes.

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Background: Women with gestational diabetes mellitus (GDM) have a 7-12 fold risk for subsequent type 2 diabetes (T2DM). Despite this window of opportunity for the primary prevention of T2DM, few intervention studies have targeted postpartum women with a history of GDM. The majority of lifestyle intervention studies conducted in the postpartum period attempted to use a group-based face-to-face format and identified poor retention as a major barrier.
Objective: To learn about preferences for the delivery of lifestyle modification programs in women with a previous history of GDM, as well as to identify barriers to, and motivators for, lifestyle changes in the postpartum period.
Methods: We interviewed women with a history of GDM within the previous ten years using focus groups and informant interviews. Using grounded theory, we used open-coding to code and categorize data by themes.
Results: Our study included 29 women (mean age 36 (range 26-45), mean pre-pregnancy BMI 29 (range 15-50); 52% Caucasian, 28% African-American, 10% Asian, 7% Native-American). We initially hoped to conduct focus groups with all participants, but since 25/35 eligible women were unable to attend a focus group, we invited these women to participate in telephone interviews. This resulted in ten women participating in focus groups and 19 women completing telephone interviews. All women reported access to the internet; 97% used the internet daily, and the majority expressed interest in an intervention program delivered primarily on-line, with some face-to-face contact. Commonly cited barriers to the adoption of healthy lifestyle behaviors after pregnancy included time and financial constraints, duties related to childcare, lack of motivation, fatigue, and obstacles at work. Motivators for lifestyle change included fear of future GDM and T2DM, with one woman citing “pricking fingers,” as a powerful motivator to avoid T2DM. Lifestyle changes were facilitated through encouragement from peers, access to gyms with childcare, and home exercise equipment.
Conclusion: In our study, time constraints proved to be a major barrier to attending a single focus group, and were also cited repeatedly as a barrier to adopting healthy lifestyle changes in the post-partum period. Our findings suggest that using the internet to deliver an intervention program should be tested as a novel approach to prevent T2DM in postpartum women with a history of GDM, incorporating support from peers and access to gyms.

Sources of Research Support: CDC MM-1094-09/09.

Nothing to Disclose: JMN, EWS, CAZ, ZSA-R, NDR, SEL

P2-571

Pathobiology of Prediabetes in a Biracial Cohort (POP-ABC): Recruitment Sources and Yields.

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Introduction: The *POP-ABC* study aims to dissect the natural history of early dysglycemia in healthy African American and Caucasian offspring of parents with type 2 diabetes. Subjects (aged 18-65 years) were enrolled between September 2006 and January 2010, and undergo repeated metabolic assessments for ~5yr. The primary outcome is occurrence of prediabetes. Other endpoints include biobehavioral data, energy expenditure, insulin action and secretion. We here report the recruitment sources and relative yields for the first 300 (170 Black, 130 White) study enrollees.
Methods: All ecruitment sources were classified into 3 major categories: Advertisements, Community Outreach, and Clinical Facilities. Advertisements included mass media, Internet, distributed flyers, Utility Bill Insert, and direct mailing. Community outreach involved direct contacts during religious gatherings, Health Fairs, and other encounters. The Clinical Facilities group included the Health Center, area hospitals, and referrals by health workers.
Results: The chief recruitment sources and their relative yields are shown in table 1. Advertisements generated the highest yield: 55% overall, 69.4% of Whites and 51.5% of Blacks. Advertisements accounted for 73.5% of White male vs. 43.8% of Black male enrollment (P=0.03). Within the advertisement category, the yield from Newspapers and Utility Bill Inserts were tied at 15% each, despite the 4-fold higher cost of the former.

Table 1 Major Recruitment Sources and Relative Yields by Gender and Ethnicity

Source	All Subjects	Men	Women	Afr. Americans	Caucasians
Advertisement (%)	59.23	56.10	60.49	51.53	69.35
Community Outreach (%)	34.15	37.80	32.68	41.72	24.19
Clinical Facilities (%)	6.62	6.10	6.83	6.75	6.45
Total	100%	100%	100%	100%	100%

Community outreach accounted for 50% of Black male enrollment, compared to 20.6% of White men. Within the community outreach class, church gatherings were the major source of African American males (30%). Only 8 subjects (2.7%) joined through the Internet.
Conclusion: Advertisements and community outreach are highly effective, whereas clinical sources are relatively ineffective, for recruiting healthy African-Americans and Caucasians. Advertisements are less demanding than organizing community events; however, because their yields vary by race/ethnicity, both strategies should be considered when recruiting a diverse cohort.

Nothing to Disclose: CE, TH, DAA, NE, SD-J

P2-572

Implementing Postprandial Glucose Monitoring into Lifestyle Modification Benefits Patients with Type 2 DM.

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Objective: Postprandial hyperglycemia contributes to poor glucose control and is associated with increased cardiovascular risk in Type 2 DM. The objective of the study was to determine the effect of postprandial self blood-glucose monitoring (pp-sbgm) on glucose control, lipids, and body weight, and cardiovascular events.
Method: Subjects with Type 2 diabetes, A1c between 6.5 to 7.0 were randomized into the study group (at least two pp-sbgm a day and dietary modification based on glucose readings) and control group (no mandatory pp-sbgm) for a 6-month observational study. Anti-DM drug or insulin regimen was unchanged in either group if A1c remained less than 7.0 during the study. Endpoints included A1c, lipids, and weight.
Results: 170 subjects, mean age 63 yr, and body weight 194 lb were recruited. A1c, weight, and TGs were similar in the groups at baseline. By the end of 6 months, A1c (6.7±0.1 to 6.4±0.1, $p<0.05$), body weight (195±17 to 188±14, $p<0.002$), and TGs (141±21 to 96±17, $p<0.05$) decreased in the study group, but did not change in the control group. No cardiovascular events were observed in either group during the 6-month study period.
Conclusions: In Type 2 diabetes who had already reached the A1c goal, pp-sbgm at least twice a day was associated with further improvement in glycemia, lipids, and weight. We assume that life style modification promoted by postprandial hyperglycemia awareness may underlie these findings. These results substantiate the importance of implementing pp-sbgm into life style modification, and emphasize that pp-sbgm is critical in the control of Type 2 DM.

Nothing to Disclose: DZ, ML

P2-573

Differentiating Monogenic Diabetes from Type 2 Diabetes in Children: Guidelines and Importance.

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Monogenic Diabetes (MGD), or Maturity Onset Diabetes of the Young (MODY), is inherited in an autosomal dominant pattern. Originally described in young adults, it is increasingly presenting in children and can mimic type 1 (T1DM) or type 2 (T2DM) diabetes. Although the lack of obesity and evidence of insulin resistance are traditionally used to distinguish MGD from T2DM, the increased prevalence of obesity has complicated the differentiation of other types of diabetes from T2DM presenting during childhood or adolescence. We report 3 patients (2 males) with MGD (2 cases of glucokinase (GCK) gene mutation and 1 with HNF1α mutation) who were initially diagnosed as T2DM. Age at diagnosis was 4.7-12 years. All patients had no measurable antibodies against insulin, glutamic acid decarboxylase (GAD) or islet cell antigen (ICA-512). None presented acutely or developed ketoacidosis and HbA1C's at diagnosis were 6.1-12% with C-peptides 1.9-2.4 ng /ml (normal 0.8-3.1). All were initially treated with metformin alone or in combination with insulin, and required relatively low insulin doses to maintain satisfactory glycemic control, except for one patient who had very poor compliance and experienced profound weight loss without improved glycemia.
All patients had a three generation history of diabetes, containing non-obese relatives with diagnoses of T2DM or gestational DM. In all cases, our patients were the first family member identified as having MGD. Following correct diagnosis, the patients with GCK gene mutation had therapy stopped and the one with the HNF1α mutation was well controlled with sulfonylurea therapy.
Our experience suggests that MGD in children and adolescents can be misdiagnosed as T1DM or T2DM. Absence of immune markers helps the distinction from T1DM, but separation from T2DM, especially in overweight or obese individuals is more difficult. Careful examination of family history, lower C-peptide levels, and therapeutic response have been helpful for us in deciding who to test for MGD. Establishment of the correct diagnosis is important for choice of therapy, appropriate genetic counseling, and family education.

Nothing to Disclose: CD, MM, KLJ

P2-574

Philadelphia Experiment: Need for Guidelines for Self-Monitoring of Blood Glucose (SMBG) Frequency in Patients with Type 2 Diabetes.

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Aim: To assess the recommendation trend of our community physicians on the issue of SMBG in patients with type 2 diabetes.
Background: Despite increase in type 2 diabetes at an alarming rate, there are no established guidelines on SMBG frequency. The advice to these patients is mainly based on their physician's knowledge and perceived severity of the disease stage. A systemic review on SMBG in type 2 diabetes patients not using insulin showed a statistically beneficial effect on HbA1c but no difference in quality of life, well-being and patient satisfaction. Most studies are inconclusive on the benefits of this practice.
Methods: Survey of recommendation patterns was carried out using a questionnaire based on different HbA1c values, as a marker of advice for SMBG frequency and management therapy of type 2 diabetes patients. The questionnaire was distributed to physicians at different local hospitals.
Results: 358 questionnaires were sent and 245 responses were received. Table 1 summarizes the percentage response of primary care physicians regarding advice on SMBG to their type 2 diabetes patients. Majority of the physicians were split in their SMBG recommendations to these patients on oral medications alone, though they were a bit more aggressive with addition of insulin. No significant difference was found on advice patterns based on whether the physician was working in an academic center or in private practice.
Conclusion: Our results reemphasizes a dire need for established guidelines, on the issue of SMBG in type 2 diabetes patients, since majority of the physicians look for such guidance. To answer the question if these patients might benefit from SMBG, a randomized controlled trial with the help of a major endocrine society with strong voice and impact is needed. The trial would also need to show long-term benefits of SMBG on the prevention of complications and its impact on patient's lifestyle and psychological well-being.

Survey results

Medication	HBA1c	Frequency of Blood glucose monitoring/day			
		Once daily %	Twice daily %	3 times/day %	>3 times/day %
DM 2 patients on oral hypoglycemic agents only	≤7.5	61	30	7	2
	≤8.5	45	45	9	1
	≤9	13	60	20	7
DM 2 patients on oral hypoglycemic agents and once a day long acting insulin	≤7.5	33	52	12	8
	≤8.5	11	66	18	5
	≤9	7	76	10	7
DM 2 patients on insulin only	≤7.5	9	59	27	5
	≤8.5	5	65	29	1
	≤9	2	53	40	5

Nothing to Disclose: NUC, MKM, CMM, GV, ZS, TG, IA

P2-575

Advanced Glycosylation End Products and Trans Fatty Acids Measured in Typical and Greater Consumption Food in Mexico.

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INTRODUCTION. Advanced glycosylation end products (AGEs) are formed continuously in the body and they are increased in diseases like diabetes mellitus, renal failure and aging. Food is the main exogenous source, its contents depends on the nourishing content, temperature and cooking methods. Trans Fatty Acids (TFAs) are formed during partial hydrogenation of vegetable oils; the consumption of both promotes inflammation, increases the risk of coronary heart disease and inducing potentially insulin sensitive. The Mexican foods display a high possibility of containing.
OBJECTIVE. To measure contents of AGEs and TFAs in some of foods of greater consumption in Mexico.
MATERIAL AND METHODS. 176 representative foods of different regions from Mexico were studied. AGEs were measured like Carboxymethyl-lysine (CML) by ELISA method; TFAs (elaidic acid) were extracted with solvents and they were measured by Gas Chromatography; protein, fat and humidity were measured by the AOAC Methods.
RESULTS. The contents of AGEs resulted from the combination of factors like humidity, temperature, cooking method and nutrimental content, as examples: Chips 101 µg AGEs/100g; Milk cake 71; Nopal stewed 65; Shrimp Coctel 56. Products of bakery and bovine origin are those of greater content of TFAs: Bread with caramel sugar 7% TFAs of total fat; Goat meat 6%; Chocolate cake 6%; Flour tortillas 3%
CONCLUSION. The AGEs and TFAs content in different foods can provide important information to develop appropriate diets and help vulnerable people to prevent damage caused by these compunds.

Sources of Research Support: CONACyT Nº Agreement CA4O100108.

Nothing to Disclose: CVQ-G, MEG-S, GB-S, KW-Z, CR-F, MB-J

P2-576

Clinical and Epidemiologic Features of Patients with Diabetes Mellitus and Diabetes Insipidus in Tashkent City (Republic of Uzbekistan).

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Aim of the research: to study clinical and epidemiologic features of patients with diabetes mellitus (DM) and diabetes insipidus (DI) in Tashkent
Patients and methods: investigated 22 patients (19 w and 3 m) with DI and DM and with impaired glucose tolerance (IGT) in Tashkent. Examinations included clinical investigation, biochemical evaluation, RIA hormones, CT and MRI imaging , visual fields.
Results and discussion: Of these patients, 1 women had 1 type DM (4.5%), 17 patients (77.3%) have 2 type DM and 4 patients (18.1%) had IGT. Age of patients varied from 26 to 73 years-old (mean age 57 ± 1.4). Duration of DI was 2-49 years, whereas duration od DM 3-43 years. 20 patients (90.9%) had central DI and 2 patients (9%) had renal DI.
The most common symptoms in patients were thirst (94.8%), headache (75.0%), dry mouth (80%), dizziness (75%) and weakness (81.8%).
Manifestation factors were neuroviral infection in 12 patients (54.5%), stress in 10 cases (45.4%), brain trauma in 5 patients (22.7%), pregnancy in 2 women (22,7%), and surgery, bleeding and radiotherapy in 22.7%, 22.7% and 22.7% respectively. Thus, disease have subtle beginning, without any clear causes and associated with psycho and brain trauma.
Disease history revealed association with IHD in 13 patients (59%), chronic hepatitis and gallbladder disease in 19 patients (86.3%), chronic pyelonephritis in 14 cases (63.6%), intracranial hypertension in 6 patients (27.2%), brain vessels atherosclerosis in 8 patients (36.3%), and empty sella turcica, pituitary microadenoma, stroke and myocardial infarction in 7 (31.8%), 4 (18.1%), 1 (4.5%) and 1 (4.5%) of cases respectively. Diabetic encephalopathy(DE) developed in 13 patients, diabetic neuropathy in 5 patients, retinopathy in 8 patients, hypertension in 12 patients. According to severity of the disease 15 patients (68.1%) were subcompensated and 7 patients (31.8%) were decompensated.
Conclusions: 1) analysis of possible provocating factors of DI in association with DM showed that manifestation of disease was associated with psychotrauma and stress in 68.1%, neuroviral infection in 54.5% and pregnancy, surgery, bleeding and radiotherapy in 22.7%. 2) the most common complications developed in DI and DM were DE in 13 cases, neuropathy in 5 patients, and retinopathy and hypertention in 8 and 12 of patients. According to severity of the disease 15 patients (68.1%) were subcompensated and 7 patients (31.8%) were decompensated.

Sources of Research Support: IP RosFarmTur, Uzbekistan, Tashkent.

Nothing to Disclose: ZYK, MMK, YMU, DAA, AOK, GDN, KKN, GOA, SMS

P2-577

Regulation of the TRH Receptor Is Dictated by Regions of the Receptor outside the Phosphorylated C-Terminal Domain.

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The pituitary thyrotropin-releasing hormone receptor (TRHR) is a G protein-coupled receptor (GPCR) that triggers the synthesis and secretion of thyrotropin and prolactin in response to TRH. Modulation of TRHR sensitivity is a critical control mechanism in the hypothalamic/pituitary/thyroid axis. Desensitization of the TRHR is initiated when GPCR kinases (GRKs) phosphorylate activated receptors in the cytosolic C-terminal tail. Cytosolic arrestin then associates with phosphorylated TRHRs, effectively uncoupling them from G protein signaling and promoting receptor endocytosis. Resensitization requires dissociation of arrestin and dephosphorylation and recycling of receptors. The goal of these experiments was to identify the role of different regions of the TRHR in regulation of receptor sensitivity. To this end, we generated chimeras swapping the C-terminal domains of the TRHR and the prototypical 2-adrenergic receptor (2AR). We explored the basis for the marked differences between TRHR and 2AR in rates of phosphorylation and dephosphorylation and affinity for arrestin, capitalizing on highly reliable ELISAs and phosphosite-specific antibodies to quantify phosphorylation at distinct sites. The TRHR-2AR chimera signaled normally to G proteins and, like TRHRs, underwent extremely rapid phosphorylation by GRKs in the C-terminal tail. On the contrary, this chimera internalized without arrestin, like the 2AR. Results for dephosphorylation mirrored those for phosphorylation, i.e. replacement of the TRHR C-terminus with the 2AR tail did not alter the pattern of dephosphorylation typically exhibited by the TRHR. Although no GPCR phosphatase has ever been identified, these results show that the responsible enzyme interacts not only with phosphosites in the TRHR tail but also with regions of the receptor outside the C-terminus. We conclude that the C-terminal domain of the TRHR defines arrestin affinity, whereas the transmembrane and cytoplasmic segments influence how the receptor is acted upon by kinases (initiating desensitization) and phosphatases (initiating resensitization). Ultimately, these interactions may prove important in shaping the TRHR response to the oscillatory concentrations of TRH encountered in vivo.

Sources of Research Support: NIH Grant DK19974.

Nothing to Disclose: AUG, PMH

P2-578

Thyrotropin Stimulates Na^+/I^- Symporter Gene Transcription by Phosphorylation of p65 at Serine 276.

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The Na^+/I^- Symporter (NIS)-mediated iodide uptake is the main rate-limiting step in thyroid hormonogenesis. Thyrotropin (TSH), a prime regulator of thyroid gland growth and hormone synthesis, constitutes the main physiological regulator of NIS expression. The Nuclear Factor-κB (NF-κB) is an ubiquitously expressed transcription factor activated in response to different signals roled in cell survival, proliferation and gene expression. Among the five members composing the NF-κB family, p65 constitutes the main effector and the most studied subunit. Activation of NF-κB in thyroid cells by TSH or TSH receptor stimulating antibodies has been reported, although the function of NF-κB in normal thyroid physiology has not been explored. Therefore, we sought to investigate the role of NF-κB in the TSH-induced NIS gene expression in thyrocytes.
We observed a nuclear recruitment of the p65 NF-κB subunit in response to TSH in FRTL-5 cells, suggesting NF-κB activation. Inhibition of protein kinase A by H89 blocked the TSH-induced p65 nuclear recruitment. Phosphorylation of p65 at Ser-276 was induced following treatment with TSH. By contrast, no induction was observed in the presence of H89. TSH induced activation of NF-κB reporter gene. Such effect did not occur under conditions that blocked p65 phosphorylation. To uncover the participation of NF-κB in the TSH-induced NIS gene expression, we performed TSH treatment in the presence of Bay 11-7082, a specific NF-κB inhibitor. Bay 11-7082 significantly reduced the TSH-induced NIS expression. The blockage of NF-κB signaling also reduced the TSH-stimulated NIS promoter activity, thus indicating a transcriptional event. Bioinformatical analysis revealed the presence of a NF-κB consensus site in the NIS-upstream enhancer region. Disruption of the κB site diminished TSH effect. Silencing p65 expression corroborated its role in the TSH-induced gene expression. Furthermore, chromatin immunoprecipitation assay confirmed the binding of the p65 NF-κB subunit to the NIS promoter induced by TSH. We established a link between p65 phosphorylation and trans-activation of the TSH-responsive gene NIS by inactivating the PKA phosphorylation site Ser-276 which substantially reduced the TSH-induced NIS expression.
Concluding, these findings provide evidence that NF-κB constitute a novel mediator of crucial importance in the TSH-induced NIS gene expression, a relevant finding of potential physiological and pathophysiological implication.

Nothing to Disclose: JPN, MN, AMM-R

P2-579

3-Iodothyronamine as a Novel Endogenous Chemical Messenger: Tissue Distribution and Catabolism.

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3-iodothyronamine (T1AM) is a novel relative of thyroid hormone, able to interact with specific G protein-coupled receptors, known as trace amine-associated receptors. Exogenous T1AM has been shown to produce functionally effects, e.g. reducing body temperature and cardiac contractility. However, a quantitative and comprehensive analysis of T1AM distribution and metabolism has not been performed in any tissue. In the present study we describe a novel liquid chromatography tandem mass spectrometry method that allowed to evaluate endogenous T1AM distribution in different rat tissues and its catabolism in cardiac preparations. In addition, we prepared isotope labelled T1AM (125I-T1AM) which was administered to mice (100 µCi, i.v.) to elucidate T1AM bio-distribution and pharmacokinetics. T1AM was detected in rat serum, at the concentration of 0.3±0.03 pmol/ml and in all tested organs (heart, liver, kidney, skeletal muscle, stomach, lung, and brain), at concentrations which were significantly higher than the serum concentration, ranging from 5.6±1.5 pmol/g in lung, to 36.1±10.4 in kidney and 92.9±28.5 pmol/g in liver. In H9C2 cardiomyocytes and in isolated perfused rat hearts significant uptake of exogenous T1AM was observed, and at the steady state total cellular or tissue T1AM concentration exceeded extracellular concentration by over 20-fold. In both preparations T1AM underwent oxidative deamination to 3-iodothyroacetic acid (TA1) and our data suggest that TA1 production occurred intracellularly and was followed by TA1 release. T1AM deamination was inhibited by iproniazid but not by pargiline or semicarbazide, suggesting the involvement of both monamine oxidase and semicarbazide-sensitive amine oxidase. Our preliminary in vivo biodistribution studies indicated that [125I]-T1AM was predominantly localized in the gastrointestinal tract, kidney and liver. The specificity of [125I]-T1AM uptake was confirmed in blocking studies. Coinjection of unlabeled T1AM (25 microg/kg) reduced the activity of [125I]-T1AM by more than 90%.
In conclusion, our results suggest that T1AM is an endogenous compound, which is widely distributed and is a substrate of amine oxidases. The highest T1AM levels are detected in the liver, kidney and gastrointestinal tract.

Nothing to Disclose: GC, AS, MM, SG, SF, PE, AR, TSS, RZ

P2-580

Transcriptional Profiling in Fibroblasts of Patients with Mutations in *MCT8* and Comparative Analysis with the Human Brain Transcriptome.

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Thyroid hormone (TH) is crucial for normal brain development. TH transporters importantly control TH homeostasis in brain as evidenced by the complex endocrine and neurological phenotype in patients with mutations in monocarboxylate transporter 8 (MCT8). The mechanisms underlying the neurological abnormalities in MCT8 patients are unknown, because (i) knowledge of the normal spatiotemporal expression pattern of MCT8 in human brain is largely lacking; (ii) genes downstream of MCT8 have not been identified; (iii) *Mct8 knockout* mice lack neurological abnormalities. We aimed to overcome these limitations by comparative transcriptome analysis of gene expression in patient fibroblasts with the human brain transcriptome.
Most over-represented categories in the differentially expressed (DE) genes in patient fibroblasts were related to actin cytoskeleton processes and cell adhesion. Studying *MCT8* and its transcriptional context in different comprehensive spatiotemporal brain transcriptome data sets, composed of in total 381 human brain samples, revealed distinct region-specific *MCT8* expression. Furthermore, *MCT8* expression demonstrated a clear age-dependent decrease, suggesting its importance in post-natal brain development. Performing comparative transcriptome analysis, we linked the DE genes in patient fibroblasts to the human brain transcriptome. DE genes in patient fibroblasts were strongly over-represented among genes which highly correlated with *MCT8* expression in brain. Furthermore, genes which highly correlated with both MCT8 and the T3-receptor (TRα or TRβ) were strongly enriched among DE genes in patient fibroblasts. This specifies genes in the classical TH signalling pathway which are likely disrupted in brains of MCT8 patients. Finally, our findings strongly suggest that the TRα2 variant is more closely connected to MCT8 than the classic T3-receptors.
The present study provides a molecular basis for understanding which pathways are likely affected in brains of patients with mutations in *MCT8*. Our data suggesting a functional relationship between MCT8 and TRα2, may imply a novel role for TRα2 in the (patho)physiology of TH signaling in brain. This study is the first to show how genome-wide expression data from patient-derived non-neuronal tissue related to the human brain transcriptome may be successfully employed to improve our understanding of neurological and endocrine disease.

Nothing to Disclose: WEV, SMAS, ZO, RS, FV, WFJvI, PJvdS, TJV

P2-581

Retinoic Acid Regulation of the Thyroid Hormone Transporter, Monocarboxylate Transporter 8 (Mct8), in Neural Differentiation.

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Thyroid hormone is required for neural differentiation. Monocarboxylate transporter-8 (Mct8) gene, expressed predominantly in brain, mediates thyroid hormone uptake and is required for normal neural development. Mct8 gene mutations in humans are associated with neurological abnormalities and severe mental retardation. Retinoic Acid (RA) induces differentiation of F9 mouse teratocarcinoma cells towards neurons and induces Mct8 expression. The 5' flanking region of mouse Mct8 was evaluated for basal promoter activity and for the presence of RA response elements (RAREs). Progressive deletions of the 5' flanking region, from -976 to -226 upstream of the transcription start site, were inserted into firefly luciferase (Luc) reporter vectors and expressed in F9 cells. Significant promoter activity (7.9 to 13.1-fold compared with the background) was observed in the constructs containing the fragment between -659 and -54, while the deletion of the sequence between -659 and -227 completely abolished promoter activity. Deletion of the region from -146 to -54, containing an Sp1 site, significantly decreased the promoter activity. The sequence from -659 to -54, including the proximal Sp1 site, is necessary for full promoter activity but is not enhanced by RA. To identify an RA responsive enhancer in the Mct8 locus, we inspected the mouse genomic sequence for consensus RARE elements, direct repeat (DR)-1, DR-2, and DR-5 configurations. Based on the half-site sequences and configuration, we identified 3 putative elements, which were inserted into a Luc reporter vector upstream of a heterologous SV40 promoter and tested for an RA-response. One of the 3 putative elements identified by sequence, a DR5 element located approximately 6kb upstream of the transcription start site, was induced by RA (~4.2 fold). To confirm that the DR5 element enhances the mouse Mct8 promoter, we fused the element upstream of the 5' flanking sequence (-976 to -54 or -836 to -54) and transfected the construct into F9 cells. RA significantly enhanced the DR5 RARE (6.2 to 7.3 fold). We have identified an RARE in the mouse Mct8 gene that confers RA-induction to a heterologous promoter and the Mct8 promoter. It is likely that this element mediates RA-enhancement of mouse Mct8 expression. Modulation of thyroid hormone uptake through the transcriptional up-regulation of Mct8 by RA may play an important role in neural differentiation.

Nothing to Disclose: KM, TK, GB

P2-582
Transport of T4 and T3 in Intestinal (Caco2) Cells.

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Introduction Millions of people in the world are treated with thyroxine for primary hypothyroidism. Where and how the uptake of thyroxine in the intestine takes place is not exactly known. We studied the characteristics of T4 and T3 uptake by Caco2 (colon carcinoma) cells, a regularly used model for uptake studies of the small intestine.
Methods Caco2 cells were cultured until confluence and after 2 weeks of differentiation. They were incubated for 5-180 min at 37 °C with 125I-labeled T4 and T3 in Dulbecco medium (pH 5.3 or 7.4) containing 0.1 % BSA and sodium or choline and in the absence or presence of non-radioactive iodothyronines and aromatic amino acids. Affinity-labeling of proteins was carried out using 125I-labeled BrAcT4 and T3. Quantitative PCR was used to determine the mRNA expression of the thyroid hormone transporters MCT8, MCT10, OATP2B1 and OATP4A1.
Results Uptake of T4 and T3 increased over time, with T3 uptake being two-fold higher than T4 uptake. No difference in thyroid hormone uptake was seen between confluent and differentiated Caco2 cells. There was no Na+- dependence of the thyroid hormone uptake. T4 transport was highly influenced by the acidity of the medium, with a 2-3 fold higher uptake of T4 at pH 5.3 compared to pH 7.4. Kinetic studies showed saturation of T4 and T3 transport in the µM range. Inhibition of T4 and T3 uptake was seen in the presence of aromatic amino acids, especially tryptophan. Incubation with BrAcT4 and T3 showed no clear labeling of a thyroid hormone transporter. Quantitative PCR revealed high MCT10 and OATP2B1 mRNA expression compared to the household gene cyclophilin A.
Conclusions Characteristics of thyroid hormone transport by Caco2 cells suggest the involvement of transporters. The pH dependence and sodium independence correspond to characteristics of OATP2B1 located in the apical membrane of intestinal cells. Inhibition by aromatic amino acids, especially tryptophan, could be explained by contribution of MCT10 located in the basolateral membrane. mRNA expression of both these transporters was high with qPCR. OATP2B1 and MCT10 could act in conjunction to translocate thyroid hormones across intestinal cells.

Nothing to Disclose: NB, WEV, TJV

P2-583

Non-Transcriptional Stimulation of Mitochondrial Metabolism by Thyroid Hormone Can Be Attributed to Regulation of Fatty Acid Oxidation Via the Mitochondrial Trifunctional Protein.

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Thyroid hormone (TH) has an important role in regulating whole body metabolism and energy expenditure. Recently, many groups have described the ability of TH to stimulate mitochondrial fatty acid oxidation (FAO), but the mechanism of this effect is unknown. GST-pull down assays in our laboratory revealed an interaction between one of the shortened thyroid hormone receptor (TR) isoforms and the Mitochondrial Trifunctional Protein (MTP), which is known to regulate the oxidation of long chain fatty acids. We hypothesized that this interaction was responsible for TH-stimulated mitochondrial metabolism. To this this hypothesis, we cultured mouse embryonic fibroblasts (MEF) from wild type and MTP knockout mice. We discovered that TH treatment of the MEFs rapidly stimulated FAO and ATP production wild type cells, but not in the null MEFs (MTP -/-). In addition, we determined that TH stimulated ATP production was observed only in adult wild type fibroblasts, and not in fibroblasts cultured from TR double isoform knockout mice (TRα-/-β-/-). Interestingly, western blot analysis revealed that TH treatment rapidly increased the expression levels of the MTPα protein, even in the presence of cycloheximide. Taken together, these data indicate that TH stimulates mitochondrial metabolism by binding to a shortened isoform of the TR located in mitochondria, stabilizes its MTP protein levels, which in turn, increases FAO. This non-transcriptional mechanism of action provide new insights into the physiopathology frequently attributed to this hormone.

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Nothing to Disclose: ESC, NS, JAI, LQD, XZ, SYC, JDL

P2-584

Using Power Analysis To Determine Sample Size To Assess Rat Thyroid Hormones.

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Thyroid hormones regulate body metabolism, consumption of oxygen and the production of heat and are also involved in the circadian rhythms that govern sleep. The two most important thyroid hormones are thyroxine (T4) and triiodothyronine (T3); these hormones are produced in response to thyroid stimulating hormone (TSH) that is released by the pituitary gland. Various chemicals induce drug metabolizing enzymes in the liver, including UDP-glucuronosyltransferase (UDPGT) that also influences the clearance of T4 and T3. As a result, an unintended consequence of enzyme induction is the observation of thyroid follicular cell hypertrophy. We have used the data from 4 studies that produced enzyme induction and secondary effects on the thyroid gland to determine what level of thyroid hormone change is necessary to observe this microscopic change in the thyroid gland. In the rat, 35 to 40% decreases in T3 or T4 and/or a 60% increase in TSH are sufficient to induce follicular changes in the thyroid gland. Based on this information, we have used power analysis to evaluate the number of animals needed to detect a significant effect on these hormones at an alpha = 0.05. As an example, to observe the above mentioned T3/T4 changes in female rats, a study requires 18 to 14 females/group using either multiplex or radioimmunoassay (RIA). Inclusion of TSH with T3/T4 increases the N to 39 females/group using the multiplex or 18 females /group using the RIA method.

Nothing to Disclose: RK, KFW, ARA, CLK, DJS

P2-585

Temporal and Spatio-Regulation of Tissue-Specific Thyroid Hormone Response Genes during Intestinal Metamorphosis in Xenopus Laevis.

Guihong Sun PhD[1], Biswajit Das PhD[1], Liezhen Fu PhD[1] and Yun-Bo Shi PhD[1].

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During amphibian metamorphosis, the small intestine is extensively remodeled from the larval to adult form under the control of thyroid hormone (TH). The remodeling of the small intestine during amphibian metamorphosis comprises first the apoptosis of the larval epithelia and then a burst of cell proliferation followed by differentiation. The resulting adult intestine is analogous to the mammalian intestine. We are using the intestinal metamorphosis in Xenopus laevis as a model to study vertebrate intestinal development. Previous studies have suggested that the cell fate change during TH-dependent frog intestinal metamorphosis is under the control of TH through tissue-specific gene regulation. To understand how TH-regulated tissue-specific gene expression affects cell fate during frog intestinal metamorphosis, our group has identified through cDNA array a number of genes falling into the categories of larval epithelium-specific, larval connective tissue-specific, adult epithelium-specific and adult connective tissue-specific, respectively. Here we have carried out detailed analysis on some of the genes to determine their spatio- and temporal-expression profiles. The results showed some genes are upregulated in connective tissues with little expression in the epithelia and some genes are mainly upregulated in the epithelia; some genes are downregulated in the connective tissue with little expression in the epithelia and some genes are downregulated in the epithelia with little expression in the connective tissues. Notably among those, hyaluronan synthase (HAS2), which catalyzes the synthesis of an extracellular matrix component, is down-regulated while matrilin-2 (MATN2), which is a component of extracellular matrix, and a Ras-like GTPase (RERG), which regulates cytoskeletal reorganization and gene expression, along with other extracellular matrix remodeling related genes are up-regulated in the connective tissues during the intestinal remodeling from larval to adult form when the connective tissues proliferate and remodel dramatically accompanying larval epithelial apoptosis and adult epithelial development. The results suggested that spatio-temporal regulation of these genes by TH is important for tissue-specific changes during the intestinal remodeling.

Nothing to Disclose: GS, BD, LF, Y-BS

P2-586

Expression Profiles and Transgenic Study Suggest a Coordinating Function of MMPs and TIMPs during Thyroid Hormone-Dependent Frog Metamorphosis.

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The extracellular matrix (ECM) plays a critical role in cell fate and behavior in multi-cellular organisms by functioning as a structural support for cells and a medium for cell-cell interactions. The matrix metalloproteinases (MMPs) are a family of Zn2+-dependent proteases capable of degrading various proteinaceous components of the ECM. Numerous studies have provided strong correlation between MMP expression and many different developmental and pathological processes. However, relatively few studies have been carried out to investigate the function of MMPs in vivo and particularly during postembryonic organogenesis in vertebrates largely due to the difficulty to manipulate mammalian embryos. Using the thyroid hormone-dependent metamorphosis of the South African clawed toad Xenopus laevis as a model, we have previously shown that the expression of the MMP stromelysin-3 (ST3) is tightly associated with intestinal remodeling during metamorphosis and that ST3 plays a role in promoting tadpole larval epithelial cell death and adult epithelial cell development through direct or indirect ECM remodeling. To investigate if the functional significance of ST3 expression during frog intestinal metamorphosis is unique to the ST3 and how its activity is regulated post-translationally, we extended our research to other MMPs and tissue inhibitors of matrix metalloproteinases (TIMPs), putative endogenous inhibitors of MMP activity. We found that the expression of several other MMPs is also temporally and spatially correlated with the TH-dependent frog intestinal metamorphosis and TIMP2 is temporally and spatially co-regulated by TH with MMP2 and MMP14 within the intestine. Transgenic studies revealed that overexpression of MMP14 caused distinguished morphological phenotypes in addition to larval epithelial apoptosis in the intestine of premetamorphic tadpoles and overexpression of TIMP2 disrupted natural or TH-induced frog metamorphosis, though it didn't cause obvious phenotype in the premetamorphic tadpoles. Double transgenic overexpression of MMP14 and TIMP2 canceled each other's phenotypes from transgenic overexpression of the individual genes. These results suggest that multiple MMPs participate synergically in cell fate regulation and TIMP2 may play a coordinating role in regulating MMP activity during frog metamorphosis.

Sources of Research Support: Intramural Research Program of NICHD, NIH.

Nothing to Disclose: LF, GS, Y-BS

P2-587

Sex and Tissue Differences in Glutathione S-Transferase α Gene Regulation by Thyroid Hormones.

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As previously reported, in liver, glutathione S-transferase α(GSTa) expression is down regulated by thyroid hormones (TH). GSTa is also expressed in kidney but not much is known about its regulation. Here, we studied the regulation of GSTa gene expression in kidney and liver by T3 in hypothyroid and hyperthyroid mice, as well as mice carrying 337T mutation in the TRβ gene, which prevents T3 binding. Hypothyroidism was induced in 8 week-old mice by feeding them a 0.15 % 5-propyl-2-thiouracil (PTU) diet for 5 weeks. After that, wild type (wt) and homozygous (ho) received daily sc. injections of a low (0.2µg/100 g BW), medium (0.5µg/100 g body BW), or high (1.0µg/100 g BW) dose of L-T3 for 7 days throughout the PTU treatment. Daily sc injections of 50µg/100g BW of L-T3 were given for 15 days to induce hyperthyroidism. We evaluated the mRNA expression and protein levels of GSTa using real-time PCR and Western Blot. Results were normalized for 36B4 mRNA and cyclophilin protein levels and expressed relative to the values for wt expression. As expected, TH deficiency induced higher liver GSTa mRNA (baseline-BL: 1± 0.04; hypo: 15.1±1.1; P<0.001) and protein (BL: 1±0.04; hypo: 1.7±0.15; P<0.01) levels than control animals. No sex differences were seen. Interestingly in kidney, we observed important sex differences. In males, there was an increase of mRNA (BL: 1± 0.01; hypo: 5.5±0.6, P<0.01) and protein (BL: 0.27±0.03; hypo: 1±0.15, P<0.05) levels after PTU diet when compared to BL. However, no clear evidence of GSTa regulation by TH was seen in female wt mice. GST mRNA (wt: 1±0.24; ho: 3.84±1.7; p<0,05) and protein levels (wt: 1±0.02; ho: 1.3±0.1; P<0.05) were higher in the liver of ho compared to wt. In contrast, in the kidney, GST mRNA was expressed in lower levels in ho mice (wt: 1±0.13; ho: 0.5±0.2, P<0.05), suggesting a tissue-specific regulation. After escalating doses of T3, GST mRNA levels in ho were only suppressed after the highest T3 dose (hypo: 0.6 ±0.1; high dose: 0.12±0.02; P<0.05), which indicated reduced sensitivity to TH inhibition, suggesting a possible role of TRα1 at highest dose. Our results showed that GSTa exhibits different patterns of gene regulation by T3 in liver and kidney. We've showed for the first time that kidney GSTa is regulated by T3 in a gender dependent way.

Sources of Research Support: CNPq - Brazil; FAPERJ - Brazil; CAPES - Brazil.

Nothing to Disclose: LCF, ACL, RMP, AC, LLS, TO-C

P2-588

Extracellular Signal-Regulated Kinase (ERK) Is Inhibited by Thyroid Hormone in Pressure Overload-Induced Hypertrophied Mouse Hearts.

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Pathological cardiac hypertrophy is a result of pressure overload-induced cardiac hypertrophy (CH). Certain signaling cascades activated in this process, like the extracellular signal-regulated kinase (ERK) pathway, promote negative remodeling and decrease contractile function. A contrasting process that is mediated by thyroid hormone results in a physiologic type of hypertrophy with increased contractility. It has been observed that thyroid hormone action is diminished in the case of pressure overload CH. We hypothesized that ERK activity might then be modulated by thyroid hormone levels, and consequently, that administration of thyroid hormone could alter the activity of this kinase in the process of pressure overload-induced CH. Activation of ERK happens via phosphorylation; therefore, phosphorylation of ERK was assessed through Western blots in hearts from control, hyperthyroid (2 weeks), and hypothyroid (4 weeks) mice. Additionally, we investigated the effects of ERK phosphorylation on hypertrophied hearts of mice submitted to transverse aortic constriction (TAC) for 10 weeks and treated with T3 (T3 administration was started at 8 wk after TAC and continued for 2 wk at a dose of 3.5 ng/g body weight, ip daily). Results show a 45±3% ($p<0.05$, $n=4$) decrease of phosphorylated ERK (p-ERK) in hyperthyroid mice, whereas hypothyroid mice exhibited a 25±3% ($p<0.05$, $n=4$) increase, and in comparison to control mice, TAC mice exhibited 55±8% ($p<0.05$, $n=4$) higher levels of p-ERK. However, TAC mice treated with T3 demonstrated a dramatic inhibition of ERK phosphorylation, which was 45±3% ($p<0.05$, $n=4$) lower than the normal control group. Additionally, activation of Raf-1, which is upstream of the ERK pathway, was explored by analyzing phosphorylation of the activating site, serine 338. Results showed that TAC mice presented a 44±4% ($p<0.05$, $n=4$) increase in p-Raf-1. In contrast, treating them with T3 resulted in a 37±3% ($p<0.05$, $n=4$) decrease in p-Raf-1 when compared to the normal control group, therefore inhibiting this maladaptive effect of pressure overload.
It can then be concluded that thyroid hormone has an inhibitory effect on the Raf-1/ERK pathway in TAC mice. The implications of this are that inhibiting the Raf-1/ERK pathway via treatment with T3 may result in improvement of contractile function and amelioration of pressure overload-induced CH.

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Nothing to Disclose: JASR, JS, BTS, WHD

P2-589

Cellular Localization and Function of Carboxy-Terminal Truncation Mutants of Pendrin.

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Background: Iodide efflux at the apical membrane of thyrocytes, an essential step in the biosynthesis of thyroid hormone, is stimulated by thyroid stimulating hormone (TSH). The mechanisms involved in apical iodide efflux remain, however, poorly characterized. Pendrin (SLC26A4) is a hydrophobic protein located at the apical membrane of thyroid follicular cells and it is thought to be involved in mediating iodide efflux. Biallelic mutations in the SLC26A4/PDS gene lead to Pendred syndrome, an autosomal recessive disorder characterized by sensorineural deafness, goiter and a partial defect in iodide organification. The intracellular carboxy-terminus of pendrin contains two putative protein kinase A (PKA) sites, which could be of importance in regulating the localization and function of the protein. Pendrin also contains a carboxy-terminal STAS (sulfate transporter and antisigma factor antagonist) domain, which may play a role in nucleotide binding and/or interactions with other proteins.
Methods: We characterized a series of carboxy-terminal truncation mutations of pendrin and determined the effect of these truncations on cellular localization and the ability to transport iodide.
Results: Time-lapse imaging of living cells demonstrates that the majority of GFP-tagged wild-type pendrin is located in intracellular compartments in the absence of forskolin. Within fifteen minutes after addition of forskolin, a significant amount of wild-type pendrin translocates to the plasma membrane. In contrast, GFP-tagged truncation mutants lacking the putative PKA phosphorylation sites and/or the STAS domain fail to reach the plasma membrane in response to forskolin. Functional analyses are consistent with the localization studies and indicate that the deletion of the PKA phosphorylation sites and/or the STAS domain result in an inability to mediate iodide efflux. Deletions of carboxy-terminal sequences downstream of the PKA sites lead to decreased but detectable membrane insertion and a partially retained iodide efflux.
Conclusions: Pendrin translocates rapidly to the plasma membrane in response to activation of the PKA pathway resulting in increased efflux of iodide. Disruption of the PKA sites result in retention of pendrin in cytosolic compartments and a loss of iodide efflux. The presence of the STAS domain is also required for membrane insertion and it could play a role in the regulation of iodide efflux.

Nothing to Disclose: AB, TLC, SK, PK

P2-590

In Vivo Selective Expression of Thyroid Hormone Receptor Alpha 1 in Endothelial Cells Attenuates Myocardial Injury in Experimental Myocardial Infarction in Mice.

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Ischemic heart disease (IHD) is the single most common cause of death in the developed world. New approaches to enhance myocardial perfusion are needed. Thyroid hormones (TH) are known to increase blood flow; however, their use to increase perfusion in IHD has been limited because TH accelerates heart rate which can be detrimental. Therefore, selective activation of TH effects is desirable. We hypothesized that tissue specific thyroid hormone receptor expression can lead to a more selective TH action in the heart. Consequently, we generated a double transgenic mouse (DT) that selectively expresses thyroid hormone receptor (TR) alpha 1 in endothelial cells in a tetracycline inducible fashion. Our results showed that DT mice over-expressed TR alpha 1 by 32%. This increase in TR alpha 1 was accompanied by increased coronary blood flow by 77% (Wild type (Wt); 1.40±0.14 vs. DT; 2.48±0.34, ml/min/g Hw, $p<0.05$, n=4), coronary conductance by 60% in isolated perfused heart (Wt; 0.25±0.03 vs. DT; 0.40±0.04, ml/mmHg/g Hw, $p<0.05$, n=4), and coronary reserve by 47% estimated as the drop in perfusion pressure after an adenosine bolus (Wt; 2.55±0.39 vs. DT; 3.75±0.19, mmHg, $p<0.05$, n=5). These changes occurred in absence of changes in heart rate, blood pressure or body temperature. Furthermore, isolated perfused hearts from DT mice over-expressing TR alpha 1 submitted to 20 min ischemia/20 min reperfusion in a Langendorff system presented only a 20% decline in left ventricular pressure (LVP) (DT normoxic; 118.3+3.1 vs. hypoxic; 95.1+5.8, LVP (mmHg), $p<0.05$, n=4) compared with control mice which presented LVP decreased by 42% (Wt normoxic; 120.0±5.7 vs. hypoxic; 70.2±5.1, LVP (mmHg), $p<0.05$, n=4). Interestingly, in vivo studies using an infarction mouse model demonstrated that over-expression of TR alpha 1 in endothelial cells significantly decreased infarct size by 45% ($p<0.05$, n=4). These results demonstrate that selective expression of TR alpha 1 in endothelial cells protects the heart against injury after an ischemic insult.

Sources of Research Support: NIH Grant HL089938 awarded to WHD.

Nothing to Disclose: JS, HW, BTS, HL, JHB, ES, GS, WHD

P2-591

The Thyroid Hormone Receptor Alpha Locus and Brain: A Role for the Circadian Clock Gene Rev-Erbα.

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Objectives:
Thyroid hormone (TH) is essential for normal human brain development and function. TH action is mediated via the TH receptors alpha (TRα) and beta. TRα is the predominant receptor in brain. In mice, a mutation in TRα, leading to a 10-fold reduction in T3 affinity, results in signs of neurodevelopmental delay, reduced recognition memory and anxiety-like behavior, accompanied by histological abnormalities of the hippocampus (1,2).
Limited human data on the role of genetic variation in TRα and brain development and function are available. We therefore studied genetic variation in the TRα locus in relation to human brain imaging data.
Methods:
15 polymorphisms were selected to cover THRA and the 10 kb surrounding region, which includes Rev-Erbα, a circadian clock gene located on the opposite chromosomal strand partially overlapping TRα. 490 subjects (age: 73.4±7.9 yrs), of whom data on MRI-derived intracranial volume, hippocampal volume and white matter lesions (WML) were available, were studied. To reduce the risk of false-positive findings, multiple testing correction by permutation analysis was performed. Promising associations ($p<0.05$) were tested in a replication cohort of 895 persons (age: 67.5±5.5 yrs).
Results:
None of the polymorphisms were associated with intracranial or hippocampal volumes. However, Rev-Erbα-rs12941497-A allele homozygotes had a 47.5% higher WML volume compared to wildtype homozygotes ($p_{lin}=0.006$). Further analyses showed that this effect was driven in females by a haplotype formed with 2 adjacent polymorphisms (i.e., rs939347 and rs2071570). Absence of this haplotype resulted in a 38.7% higher WML volume ($p_{lin}=0.007$), which was replicated in the replication cohort ($p_{lin}=0.04$).
None of the studied polymorphisms were associated with serum TH levels.
Conclusions:
This is the first study on the effects of the TRα/Rev-Erbα locus on indicators of human brain development and function. Although genetic variation in this locus was not associated with intracranial or hippocampal volumes, variation in Rev-Erbα showed a significant association with higher WML volumes. WML, resulting from vascular damage to axons, increase the risk of various neurological diseases such as dementia and cognitive decline (3). Rev-Erbα has been shown to influence splicing and expression of TRα *in vitro*, suggesting that the effects of Rev-Erbα on brain might be mediated via TRα. However, the exact role of Rev-Erbα in WML development remains to be determined.

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Nothing to Disclose: MM, MAI, MV, AGU, MMB, TJV, RPP

P2-592

NCoR Regulates Thyroid Hormone Receptor Isoform-Dependent Adipogenesis.

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Thyroid hormone receptors (TRs) mediate the biological activities of the thyroid hormone T3. Two TR genes encode two major T3 binding isoforms (α1 and β1). We have shown that TRα1 and TRβ1 differentially regulate T3-stimulated adipogenesis in vivo. Since it is known that the nuclear receptor corepressor (NCoR) modulates the TR functions, we tested the hypothesis that differentially interaction of NCoR with the unliganded TR isoforms could underlie isoform-dependent adipogenesis. We prepared 3T3-L1 cells stably expressing an identical dominantly negative mutation (denoted PV) at the corresponding C-terminal region of TRα1 (L1-α1PV cells) or TRβ1 (L1-βPV cells). Adipogenesis was more strongly inhibited in L1-α1PV cells than in L1-βPV cells. We found that in control 3T3-L1 cells, the induction of adipogenesis in the presence of T3 was accompanied by progressive decrease of NCoR protein abundance. At day 6, when 3T3-L1 cells were differentiated into mature adipocytes, NCoR was barely detectable as compared to day 1. In contrast, ~80% and ~50% of NCoR was lost in L1-βPV cells and L1-α1PV cells, indicating the extent of loss of NCoR correlates with degree of adipogenesis. Retroviral-mediated siRNA knockdown of NCoR promoted the adipogenesis in control 3T3-L1 cells and a reversal of the inhibited adipogenesis in L1-βPV cells and L1-α1PV cells. These results indicated that de-repression of NCoR-mediated repression of TRs facilitates adipogenesis of 3T3-L1 cells. mSiah2, an ubiquitin ligase that targets NCoR for proteasome degradation, was up regulated on day 1 before the level of NCoR was degraded during adipogenesis. In addition, knockdown of NCoR induced a higher increase of mSiah2 protein in L1-α1PV than in L1-βPV cells. The correlation in the protein levels of NCoR and mSiah2 suggested NCoR was more resistant to degradation when associated with TRα1PV than with TRβPV in cells. Co-immunoprecipitation analyses showed the association of NCoR/mSiah2 complexes with TRs, TRα1PV, or TRβPV in control, L1-α1PV, or L1-βPV cells, respectively. Thus, de-repression of NCoR-mediated repression by protesasome degradation of NCoR was crucial for TR to stimulate adipogenesis. These results further show that differential association of NCoR with unliganded TRs could underlie the TR isoform-dependent regulation of adipogenesis and that regulation by NCoR is important in the pathogenesis of lipid abnormalities in hypothyroidism.

Nothing to Disclose: XGZ, SYC

P2-593

Novel *MCT8* Mutations in a Cohort of Patients with Unexplained Mental Retardation.

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Thyroid hormone (TH) is essential for normal brain development. Local cellular TH homeostasis in brain is controlled at different levels, including transport of TH across the plasma membrane. Among the TH transporters involved, monocarboxylate transporter 8 (MCT8) appears an essential protein as humans with mutations in *MCT8* suffer from a severe neurological and endocrine phenotype. The frequency of this syndrome is unknown.
We profiled TH parameters in a cohort of 946 people with unexplained mental retardation (TOP-R study). This strategy enabled us to screen for *MCT8* mutations in subjects with abnormal TH parameters, i.e. high T3 and low FT4 levels. In 8 subjects thus selected, we sequenced the coding parts of the *MCT8* gene and identified 3 mutations. Two were nonsynomymous mutations (c.1500_1502delCTT; p.F501del and a novel c.1475C>T; p.L492P) in the 10th transmembrane domain and one was a synonymous mutation (c.486C>T; p.T162T). In vitro studies were employed to investigate transport capacity of the mutants. The F501del and L492P mutants showed considerably diminished T3, T4 and rT3 transport. However, both mutants displayed significant residual capacity, which is in line with the mild phenotype in these patients, characterized by much better motor and communication skills. The pathogenic nature of the synonymous mutation is uncertain. We estimated that the frequency of MCT8 mutations in males with X-linked mental retardation is 3.9% (CI 0.4-7.4%).
In conclusion, we identified several (novel) *MCT8* mutations in a large cohort of subjects with unexplained mental retardation. The pathogenicity of two missense mutations was demonstrated by *in vitro* studies. The mild clinical phenotype corresponds with the marked residual transport capacity of the mutants. Our study indicates that mutations in *MCT8* are a frequent cause of X-linked mental retardation.

Nothing to Disclose: WEV, FEV, TJV

P2-594

Thyroid Hormone Signalling Is Suppressed in Progeroid and Normal Aging.

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DNA damage plays an important role in aging. This is underscored by human progeroid syndromes, resulting from defects in DNA repair pathways. Recently, a novel link between genome instability and age-related decline of endocrine signalling pathways was suggested in progeroid DNA-repair deficient mouse models. These mutants are defective in genes crucially involved in transcription-coupled repair and are used as models for accelerated and normal aging.

Since liver transcriptome analysis suggested a suppression of the TH signalling pathway in mice with extreme (*Csb*$^{m/m}$*/Xpa*$^{-/-}$) or severe (*Ercc*$^{-/\Delta-7}$) progeria phenotypes and naturally old mice, we assayed serum and tissue TH levels and deiodinase activities these animals at several ages. Thyroid glands were histologically normal. Serum T4 and T3 levels were decreased in *Csb*$^{m/m}$*/Xpa*$^{-/-}$ mice. Liver T3 levels significantly dropped, which was also reflected in a large reduction in D1 activity. A marked age-dependent up-regulation of liver D3 activity was seen in *Csb*$^{m/m}$*/Xpa*$^{-/-}$ mutants, explaining the preferential decrease in liver T3 levels. In naturally old animals (104 *vs* 52 wks) an increase in liver D3 activity mirrored a decreased D1 activity. Normal serum and liver TH levels contrasted the large decrease in liver D1 activity in *Ercc*$^{-/\Delta-7}$ mice. This paradox is explained if TH is not available for nuclear TH receptors, but sequestered in other cell compartments. TH levels in kidney paralleled serum levels in both mutants. Kidney D1 activities were decreased. In progeroid brains T3 levels were less affected than T4 levels. Furthermore, D2 and D3 activities did not differ compared with WT mice. Expression of most classic T3-target genes in brain did not differ between *Ercc*$^{-/\Delta-7}$ and WT mice. Since the GH/IGF1-axis is largely decreased in normal and premature ageing mice, we performed studies in mouse models with perturbed GH/IGF1 axis. These results indicated sex-dependent regulation of liver deiodinase activities by the GH/IGF1 system.Altogether, these results suggest a strong suppression of TH signalling in peripheral organs in premature and normal aging, probably lowering metabolism. Furthermore, it appeared that old brains aim to preserve energy expenditure. The induction of high D3 activity suggests that D3 plays a pivotal role in aging as it is normally undetectable in most adult tissues. We propose that regulation of deiodinase activities importantly contributes to the protective metabolic response in aging.

Nothing to Disclose: WEV, GAG, CB, IvdP, EK, RB, HvT, MCdW, RdK, JJK, EOL, VMD, MED, GTJvdH, JHJH , TJV

P2-595

Teratogenic Potential of Propylthiouracil and Methimazole.

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Context. Graves' disease (GD) is the most common cause of hyperthyroidism during pregnancy and is associated with maternal and fetal complications if poorly controlled(1). The use of antithyroid drugs (ATDs) propylthiouracil (PTU) and methimazole (MMI) is the preferred treatment option for GD during pregnancy(2,11). Because MMI use has been associated with congenital defects(3-8), PTU has been recommended as the drug of choice(9-11). However, there has not been a systematic analysis of the relative teratogenicity potential of PTU and MMI(12-13).
Objectives. To determine the teratogenic potential of PTU, MMI, and hyperthyroidism in an accepted model for studying birth defect causation.
Methods. Effects of ATDs on embryonic development were assessed in C57BL mice. PTU (25, 50, or 100 mg/kg), MMI (4.0, 10, or 20 mg/kg) or vehicle (saline) were administered orally by gavage once daily on embryonic days E7.5 through E9.5 to span critical periods of embryogenesis. At E10.5 dams were killed and embryos examined for viability, size, and morphology of live embryos. Levo-thyroxin (T4; 40 mcg/kg/d) was also administered over the same period to assess effects of hyperthyroidism.
Results. A total of 389 embryos from 45 dams were examined. There was no difference in embryo crown-rump length among the groups. PTU was more likely to be associated with morphological defects, including lack of cranial fusion and the presence of blood on pericardial sac that is indicative of abnormal cardiac function. A dose-response relationship was seen with PTU but not with MMI. Anomalies in the presence of induced hyperthyroidism did not exceed that of the vehicle-treated group.

Drug	Dose	Embryo	Crown-rump	Type of defect			Defects	Dead
	mg/Kg	(n)	(mm)	Head folds not fused	Pericardial blood	Other defects	(%)	(%)
PTU	25	44	4.24	1	4	1	2.1	20.5
	50	66	4.29	2	6	0	4.8	9.1
	100	76	4.61	7	6	6	14.06	2.6
MMI	4	60	4.37	1	2	0	1.29	28.3
	10	24	4.03	0	2	3	1.2	0.0
	20	40	4.48	2	3	0	1.6	20.0
T4	40	18	4.66	0	2	0	0.32	11.1
Vehicle	-	61	3.93	1	2	0	1.74	4.9

Conclusions. Studying pregnant mice, we observe that short-term PTU and MMI exposure during embryogenesis is associated with skull and cardiac defects. PTU appears to have greater teratogenic potential than MMI. Hyperthyroidism was not associated with increased birth defects. These observations show that the notion that PTU is not teratogenic is not correct. Further studies on the relative teratogenic potential of PTU, MMI and hyperthyroidism are warranted.

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Nothing to Disclose: VCB, SAR

P2-596

Physiological Response of the Thyroid Axis in Diet-Induced Obesity.

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The hypothalamic-pituitary-thyroid (HPT) axis plays a pivotal role in maintaining metabolic homeostasis in response to environmental and nutritional changes. One important stimulator of the HPT axis is the adipocyte hormone leptin, which activates the HPT axis by increasing thyrotropin-releasing hormone (TRH) levels. Leptin stimulates TRH in the paraventricular nucleus (PVN) of the hypothalamus through 2 pathways: (1) the direct pathway where leptin increases TRH directly in the PVN and (2) the indirect pathway where leptin stimulates the melanocortin system in the arcuate nucleus (ARC) which increases TRH levels in the PVN. The indirect pathway functions in the normal condition; however, during diet-induced obesity (DIO) the indirect pathway is blocked. Despite this leptin-resistance, the HPT axis remains functional in DIO animals. For example, we found that thyroid hormone (T3/4) levels were higher in DIO compared to control rats. Therefore, we tested two hypotheses: (1) that leptin activates TRH via the direct pathway in DIO and (2) that central and/or peripheral deiodinase enzymes that convert thyroid hormones to functional and non-functional states are altered in DIO.
In support of our first hypothesis, we found that leptin blockade in DIO animals fully abolished the diet-induced increase of (T3/4) levels. We also found that fasting in DIO rats (low leptin) decreased preproTRH mRNA, TRH, thyroid-stimulation hormone, and T3/4 levels indicating that these neurons remained sensitive to leptin. In addition, leptin partially overrode the T3 inhibition of the preproTRH promoter. We also found that deiodinase 3 (D3) protein expression was higher in the ARC/median eminence (ME) of DIO compared to control animals. D3 degrades T3 into biologically inactive metabolites; therefore, increased D3 may lower T3 levels reducing T3-mediated inhibition of TRH, which may in turn elevate HPT axis activity in DIO. In contrast, deiodinase 1 activity in liver and deiodinase 2 activity in ARC and brown adipose tissue were similar in control and DIO animals. Overall, our results suggest that HPT activity in DIO is centrally regulated by leptin activation of TRH via the direct pathway and by deiodinase 3 activity in ARC/ME rather than by peripheral deiodinase activity. These results may have important implications for understanding obesity-induced changes in the HPT axis of humans.

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Nothing to Disclose: NEC, MP, IC, AR, RCS, FC, ANH, EAN

P2-597

Levothyroxine Dose and the Risk of Fractures in Older Patients.

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Background: Older patients with hypothyroidism are at risk for overtreatment with excessive levothyroxine (L-thyroxine) replacement. Endogenous thyroid hormone levels impact bone turnover, but the effect of L-thyroxine replacement on bone mineral density and fracture risk is controversial. As well, the effect of L-thyroxine dose on fracture risk in an older population has not been adequately examined.
Methods: We designed a nested case-control study using population-based health databases in Ontario, Canada, to follow a cohort of persons aged 70 years or older who were prescribed L-thyroxine between April 1, 2002 to March 31, 2007. Patients were followed until March 31, 2008. Cases were defined as patients with an emergency room visit or hospital admission for any non-traumatic fracture or a non-traumatic hip fracture. Each case was matched with up to 5 controls sampled from the cohort. We compared the risk for fractures between current (taking L-thyroxine at time of fracture) and remote L-thyroxine users. We also compared fracture risk among current users prescribed a high cumulative L-thyroxine dose (above the 75th percentile, mean 0.122 mg/day) and medium cumulative dose (between the 25th and the 75th percentiles, mean 0.076 mg/day) to the risk in those prescribed a low cumulative L-thyroxine dose (below the 25th percentile, mean 0.047 mg/day).
Results: Of 211,160 patients studied, 20,599 (92%) were current L-thyroxine users who sustained fractures. Current L-thyroxine use was associated with a significantly higher risk of any fracture (adjusted rate ratio, (aRR) 1.96, 95% confidence interval (CI) 1.79 to 2.14) and hip fracture (aRR 1.63, 95% CI 1.42 to 1.86) compared to remote L-thyroxine use. Among current users, higher cumulative L-thyroxine dosages were associated with a significantly increased risk of any fracture compared to low cumulative dose (medium dose aRR 2.69, 95% CI 2.56 to 2.83; high dose aRR 3.54, 95% CI 3.35 to 3.75). Hip fracture risk was also significantly increased with higher cumulative L-thyroxine doses.
Conclusion: Among older patients prescribed L-thyroxine replacement, doses above a mean of 0.076 mg/day were associated with a significant increase in non-traumatic fractures. This suggests that a significant number of older patients may be overtreated beyond their needs for L-thyroxine replacement, putting them at risk for adverse events, such as fractures, and more careful monitoring of dosage in this population is warranted.

Nothing to Disclose: MRT, XC, HDF, PCA, GMA, CMK, PAR, LLL

P2-598

Levothyroxine Sensor for Rapid Quantitative Analysis of Levothyroxine in Pharmaceutical Formulation.

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Application of potentiometric electrodes in drug measurements, offer the advantages of speed and ease of preparation and procedures, relatively fast response, wide linear dynamic range, portability and low cost. The majority of drug sensors are based on ion pair complexes, to overcome the difficulties of synthesizing very differently shaped ionophores, which is an obligation caused by the very wide range of shapes of the drugs. The above-mentioned advantages are the result of the growing trend of development, and use of sensors in drug analysis.
Levothyroxine, also L-thyroxine, synthetic T4, or 3,5,3',5'-tetraiodo-L-thyronine, is a synthetic form of thyroxine (thyroid hormone). Levothyroxine is a replacement for a hormone that is normally produced by your thyroid gland to regulate the body's energy and metabolism. Levothyroxine is given when the thyroid does not produce enough of this hormone on its own.
Observation of the patients who are used levothyroxine tablets but still have problem with their thyroid, leads to analysis the pharmaceutical formulation used for the first step.
For this purpose, this research work introduces a novel levothyroxine potentiometric sensor for determination of levothyroxine in pharmaceutical formulation. This simple device can be easily used either by the pharmaceutical factories produce levothyroxine tablet in quality control unites or even by the physicians face to such patients.
The proposed sensor shows a fast (~30 s), stable and Nernstian response (58.6±0.3 mV/decade) over a relatively wide levothyroxine concentration range of 1×10^{-5} to 1×10^{-2} mol L^{-1}. Validation of the method shows suitability of the sensors for applies in the quality control analysis of levothyroxine in pharmaceutical formulation.

Nothing to Disclose: FARF, EN-E, BL, PN, MRG

P2-599

Serum Thyrotropin Concentration Is an Early Marker of Normalization of Low T3 Syndrome in Aged Hospitalized Patients after Discharge.

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Introduction: Natural history of thyroid dysfunction associated to hospitalization in aged patients who survive after discharge for acute illness is not known.
Objective: To assess short-term spontaneous evolution of alterations in thyroid function tests in aged hospitalized patients after discharge and establish whether there is any clinical or analytical parameter that could be related to the normalization of thyroid function in these patients.
Patients and methods: A group of 146 patients (mean age ± SD 85.9 ± 6.2 years) was studied. Serum concentrations of thyrotropin (TSH), free thyroxine (FT4), and free thriiodothyronine (FT3) were evaluated in every patient both after admission and one month after discharge.
Results: At entry, both serum TSH [median (interquartilic range), 2.19 mU/l (0.89-2.31)] and FT4 (mean ± SD, 16.7 ± 3.4 pmol/l) concentrations were into the normal range, whereas serum FT3 concentrations were low (3.3 ± 0.7 pmol/l). After discharge TSH and FT4 concentrations remained normal and FT3 low. However, both serum TSH [2.53 mU/l (1.24-3.33); $p<0.01$] and FT3 (3.7 ± 1.0 pmol/l; $p<0.001$) concentrations significantly increased. Most patients (n=124, 84.9%) showed the euthyroid sick syndrome (ESS). After discharge, ESS diminished to 76 (52.1%) subjects. Patients who normalized thyroid function tests showed significantly lower TSH values at entry compared with those who persisted with altered thyroid function tests [1.27 mU/l (0.69-1.89) vs 1.69 mU/l (0.96-2.91), $p<0.05$]. Logistic regression analysis showed that serum levels of TSH at admission was the only variable negatively related to normalization of thyroid function (OR 0.730; CI 95%, 0.567-0.940; $p=0.01$).
Conclusions: About 35% of aged patients hospitalized for acute illness spontaneously normalize their thyroid function tests one month after discharge, mainly due to the correction of ESS. Serum TSH levels at admission seem to be the only variable negatively related to normalization of thyroid function at this time.

Nothing to Disclose: PI, AM, FP, MTG, MCM, PT, CG-A, JJD

P2-600

Reference Intervals for Thyroid Hormone Function in the First Trimester in Pregnant Filipino Women.

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Introduction. Optimal thyroid funtion is essential in all life stages. This is more pronounced during the first trimester of pregnancy when the fetus is solely reliant on transplacental supply of thyroxine which is important in the normal neurological development.
Objective: To establish first-trimester specific reference intervals for thyroid function tests in pregnant Filipino women.
Design, setting and participants. This is a cross-sectional study. One hundred eighteen Filipino women with uncomplicated single intrauterine gestations in the first trimester (≤14 weeks) attending a tertiary center were recruited consecutively. Levels of serum thyrotropin (TSH) was measured using immunoradiometric assay while free thyroxine (fT4), free triiodothyronine (fT3) and thyroid peroxidase antibodies (TPOAb) were measured by radioimmunoassay method.
Main outcome measures: Reference intervals based on 2.5th and 97.5th percentiles for TSH, fT4 and fT3 among TPOAb negative pregnant patients.
Results: Derived reference intervals for thyroid function tests during the first trimester of pregnancy were: TSH 0.014-3.84 µIU/mL (NV 0.25-4 µIU/mL); fT4 10.44-21.58 pmol/L (NV 11.5-23 pmol/L); and fT3 2.4-5.82 pmol/L (NV 2.5-5.8 pmol/L). If the non-pregnant TSH reference range was applied to the study participants, 21 patients (18%) will be misclassified to subclinical hyperthyroidism.
Conclusions: Reference intervals for thyroid function tests in pregnancy differ from non-pregnant patients and will lead to misclassification of 18% of patients, hence pregnancy-specific reference intervals should be advocated.

Nothing to Disclose: PCP, JNH, MSL, MMN-H, CAJ, MAL-A

P2-601

Effects of Subclinical Hyperthyroidism and Variations in Thyroid Function within the Normal Range on Metabolic Function and Body Composition.

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Patients with overt hyperthyroidism have alterations in metabolic function, but it is not clear whether more mild (subclinical) hyperthyroidism or variations in thyroid function within the normal range affect metabolism. To address this, we report preliminary analysis of a cross-sectional study that recruited otherwise healthy women into three age and BMI-matched groups: 1. Healthy euthyroid controls (HC, n = 13); 2. Levothyroxine (L-T4) -treated hypothyroid women with normal TSH levels (T4Tx, n = 16), and 3. Women with low or suppressed TSH levels and normal free T4/T3 levels (SCHyper, n = 6) due to L-T4 suppression therapy (n = 5) or a functioning thyroid nodule (n = 1). Subjects underwent measurements of Resting Energy Expenditure (REE), respiratory quotient (RQ), and Thermic Effect of Food (TEF) by indirect calorimetry, and body composition by DEXA.

	HC	T4Tx	SCHyper
Age (yr)	40.4 ± 3.3	41.8 ± 2.8	43.0 ± 4.0
BMI (kg/m2)	26.5 ± 1.8	25.8 ± 1.3	24.3 ± 1.9
TSH (mU/L)	1.93 ± 0.28	1.84 ± 0.27	0.10 ± 0.04[a]
REE (kcal/kg LBM)	30.3 ± 1.0	28.9 ± 0.8	31.0 ± 1.4
RQ	0.85 ± 0.02	0.85 ± 0.01	0.82 ± 0.01
TEF (kcal/6h)	853 ± 98	837 ± 86	784 ± 114
LBM (lean body mass) (gm)	46,494 ± 1758	44,673 ± 1648	44,063 ± 2240
% fat mass	36.8 ±2.3[b]	30.9 ± 1.8	31.8 ± 2.2

Results are means ± S.E.M. [a]p < .0001, [b]p=0.09 compared to other two groups by ANOVA followed by t-tests to compare individual groups.
Groups differed in thyroid function, but no difference in measures of energy expenditure or nutrient oxidation were seen. There was a trend toward an inverse correlation between free T4 levels and % body fat by DEXA (p=.08).
In summary, utilizing sensitive and sophisticated techniques, women with SCHyper had similar energy metabolism compared to healthy euthyroid women and euthyroid women receiving L-T4. Likewise, metabolic parameters did not vary in association with variations in TSH levels within and near the normal range. However, trends toward reduced % fat mass in L-T4 treated women and associations between free T4 and % fat mass suggest that small alterations in thyroid function may affect body composition. These results are preliminary due to limited sample size, and further studies are needed in the complete cohort to confirm these findings.

Nothing to Disclose: KGS, IK, MAK, AS, DP, JQP, MHS

P2-602

The Relationship between Hyperthyroidism and Clot Structure: A Mechanism for Increased Thrombotic Risk in Hyperthyroid Individuals?.

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Hyperthyroidism is associated with increased risk of thrombotic events by mechanisms not fully understood. Fibrin clot structure has been shown to determine susceptibility to atherothrombosis and venous thrombotic events. Therefore, this study investigated the effects of hyperthyroidism on clot structure/fibrinolysis using a cross sectional and a longitudinal study.
In the observational study, blood samples were taken from 10 female patients with subclinical or overt hyperthyroidism (age 44.2±3.7 years) and 7 controls (age 48.0±2.1, 1 male) after obtaining informed consent. For the interventional study, 15 patients with hyperthyroidism were recruited (age 38.4±3.5, 4 males) and treated with antithyroid medications until euthyroid. Blood samples were collected at baseline and after attaining post-intervention euthyroidism. Clotting of plasma samples was triggered ex vivo and clot structure investigated using a validated turbidimetric assay, whereas fibrinolysis was assessed in the presence of tissue plasminogen activator. Three parameters were recorded: i) lag phase (LP): time from beginning of reaction to start of clot formation, an indicator of thrombotic tendency, ii) maximum absorbance (MA), a measure of clot density, iii) time from full clot formation to 50% lysis (LT), to assess fibrinolytic potential.
LP in hyperthyroid subjects and controls was 439±13 and 529±82 sec respectively (p=0.2). MA was higher in hyperthyroid individuals compared with controls (0.132±0.013 and 0.074±0.001 au respectively, p=0.02), whereas no difference was detected in LT (713±69 and 619±93 sec, respectively, p=0.47). In the interventional study, FT4 was 50.6±6.1 pmol/L at baseline, dropping to 18.0±1.7 pmol/L post intervention whilst initially suppressed TSH normalised to 2.1±1.1 mIU/L. LP was shorter during hyperthyroidism compared with the euthyroid phase (361±10 and 453±16 sec, p<0.01). MA at baseline and post-intervention was 0.226±0.017 and 0.148±0.019 au respectively (p<0.01), whereas LT was similar (500±17 and 495±40 sec, respectively, p=0.86).
In conclusion, hyperthyroid patients have a compact clot structure and increased tendency to clot formation ex vivo, which can be modulated by normalising thyroid hormone levels. Despite a denser fibrin network, clot lysis is unaffected, suggesting a compensatory capacity in the fibrinolytic system. The prothrombotic clot structure in hyperthyroidism may explain, in part, the increased thrombotic risk in such individuals.

Nothing to Disclose: JMWH, SMO, KFS, SHSP, SKAS, JUW, PJG, RAA

P2-603
TSH Secreting Pituitary Tumor in a Pediatric Patient.

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Background: A TSH secreting pituitary tumour is a rare cause of secondary hyperthyroidism but an important differential diagnosis of elevated thyroid hormone levels.
Clinical Case: A 15 year old girl with a 2 month history of tremor, diaphoresis, weight loss, headaches and secondary amenorrhea presented with persistently elevated free T4 levels of >65pmol/L (10-23) and TSH level of 10.5mIU/L (0.5-5). Tapazole and Propranolol therapy was initiated and further investigations were undertaken. TSH levels remained elevated and showed a blunted response to TRH stimulation. IGF1 levels and remainder of pituitary labs including prolactin (PRL) levels were normal; TSH antibodies were negative; α-subunit level was elevated 5.0ng/ml (0.04-0.38). On physical examination the patient was tachycardic, hyperreflexic, diaphoretic and had a large and firm thyroid gland, which on ultrasound was inhomogeneous. An MRI of the brain revealed a 1.0 x 0.8cm lesion in the anterior pituitary gland. The patient was diagnosed with a TSH secreting adenoma and, in conjunction with Tapazole, was started on subcutaneous Octreotide therapy, requiring up to 900mcg daily. Once thyroid function tests normalized she underwent transphenoidal resection of the adenoma. On pathological examination the adenoma had an unusual appearance, staining for multiple hormones including TSH and PRL. One year after surgery our patient remains euthyroid on no medications with no evidence of recurrence of the adenoma.
Conclusion: TSH secreting pituitary adenomas, a rare cause of secondary hyperthyroidism, represent <1% of all pituitary adenomas and have been infrequently described in the pediatric literature. Timely recognition and appropriate therapy is important to prevent potential complications of the growing lesion. The diagnosis should be considered when TSH levels are inappropriately nonsuppressed in the presence of high levels of free thyroid hormone, and can be confirmed by the presence of a pituitary lesion on MRI as well as supportive TRH stimulation test and elevated levels of α-subunit. Important differential diagnoses include thyroid hormone receptor gene defects or assay problems. The treatment of choice for TSH secreting adenoma is surgical excision. Of particular interest, our patient's tumor stained for PRL as well as TSH, making this a very unusual tumor. Furthermore, our patient had no physical or laboratory indication of hyperprolactinemia despite a prolactin secreting component of the tumor.

Nothing to Disclose: EB, DW

P2-604

Large Scale Profiling of Thyroid Hormone Parameters in a Large Mental Retardation Cohort: The TOP-R (Thyroid Hormone Origin of Psychomotor Retardation) Study.

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Abnormalities in thyroid state may affect development and function of many tissues. Despite the well-known importance of thyroid hormone for brain development and function, thyroid parameters in institutionalized subjects with mental retardation (MR) have not systematically been investigated. We carried out a nation-wide multi-center study (TOP-R study) to collect a cohort of 946 institutionalized subjects with unexplained MR. We extensively profiled thyroid parameters in this cohort.
Serum levels of TSH and T4 were similar, whereas serum fT4, T3 and rT3 levels were decreased compared with controls. After exclusion of subjects using antiepileptic drugs (AEDs), only serum T4 (101.8 ± 1.1 *vs* 93.8 ± 2.2 nmol/L, P < 0.001) and serum T3 (1.70 ± 0.02 *vs* 1.94 ± 0.03 nmol/L, $P < 0.0001$) differed in patients *vs* controls. The use of AEDs affected thyroid hormone measurements (T4: 102.1 ± 1.2 *vs* 83.9 ± 1.2 nmol/L, $P < 1 \times 10^{-24}$; fT4: 18.0 ± 0.2 *vs* 16.1 ± 0.2 pmol/L, $P < 1 \times 10^{-9}$; T3: 1.72 ± 0.02 *vs* 1.57 ± 0.02 nmol/L, $P < 1 \times 10^{-9}$ and rT3: 0.37 ± 0.01 *vs* 0.27 ± 0.01 nmol/L, $P < 1 \times 10^{-28}$ in subjects without *vs* with AEDs). AED polytherapy highly enhanced the effects on thyroid indices. The prevalence of unrecognized primary hypothyroidism and hyperthyroidism was 5.2% and 2.8%, respectively.
To our knowledge, the TOP-R study represents the largest cohort of institutionalized MR subjects in whom serum thyroid parameters have been profiled. In general, thyroid parameters in subjects without AED treatment are comparable with those in the general population, except for higher serum T4 and lower serum T3 concentrations. Our findings reiterate that subjects on AED treatment may have altered thyroid indices, which is important to interpret thyroid function tests. Since thyroid hormone abnormalities may be related to the cause of MR, future studies will investigate if mutations in key genes regulating thyroid hormone signalling in brain are associated with MR.

Nothing to Disclose: WEV, HvT, TJV

P2-605

Transfer of Hashimoto's Thyroiditis by Donor Immune Cells during a Stem Cell Transplant.

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Background: Thyroid dysfunction after hematopoietic stem cell transplantation (HSCT) has been previously reported but the pathogenesis and risks are unknown. We report a case of a newly developed Hashimoto's thyroiditis (HT) and hypothyroidism in a recipient of HSCT and discuss possible pathogenetic mechanisms.
Clinical case: A 54 year old Caucasian woman underwent allogenic HSCT donated by her HLA matched sister in May 2008 for treatment of anaplastic T cell lymphoma that initially presented as a right sided neck mass. Three months post-transplant, she developed graft-versus-host disease of the eyes, lung, skin and liver that required immunosuppressive treatment. A follow-up 18-FDG PET scan showed a significantly increased tracer uptake in the thyroid glands raising a concern of a lymphoma recurrence or a primary thyroid cancer. Prior to HSCT, TSH and FT4 had been within normal limits; anti-TPO and anti-thyroglobulin antibodies had been negative and thyroid ultrasound had shown a small diffuse goiter without discrete nodules. At six months post HSCT, thyroid glands became diffusely enlarged and firm, TSH was 64.3 mcIU/ml (ref. 0.4-4), FT4 0.4 ng/dl (ref. 0.8-1.9), T3 62 ng/ml (ref. 90-215), anti-thyroglobulin antibody was 16.5 index value (ref. 0-0.9) and anti-TPO antibody was 4.9 index value (ref. 0-0.9). Levothyroxine therapy caused normalization of TSH, decrease in the size of the thyroid, and decrease but not normalization of PET tracer uptake. A thyroid ultrasound showed a left lobe nodule. A fine needle aspiration yielded cytology consistent with chronic lymphocytic thyroiditis. Five months post HSCT, chimerism studies on the patient's peripheral blood revealed 100% of circulating lymphocytes to be of the donor origin. Additional history showed that her HLA matched sister donor has hypothyroidism for over 30 years, likely secondary to Hashimoto's thyroiditis.
Discussion: This case illustrates a possible transfer of Hashimoto's thyroiditis via primed immune cells (lymphocytes) from a HLA matched sibling. Alternative explanation could be a trigger of autoimmune attack in a predisposed individual by recovering own lymphocytes, similar to rebound immune response after HAART in AIDS patients. Of note is the high and diffuse 18-FDG PET tracer uptake in Hashimoto's thyroiditis.
Conclusion: Transfer of autoimmune thyroid disease from affected donor is a possibility and screening by history and thyroid antibody test should be performed.

Nothing to Disclose: WPL, VKB

P2-606
Ipilimumab and Graves Ophthalmopathy.

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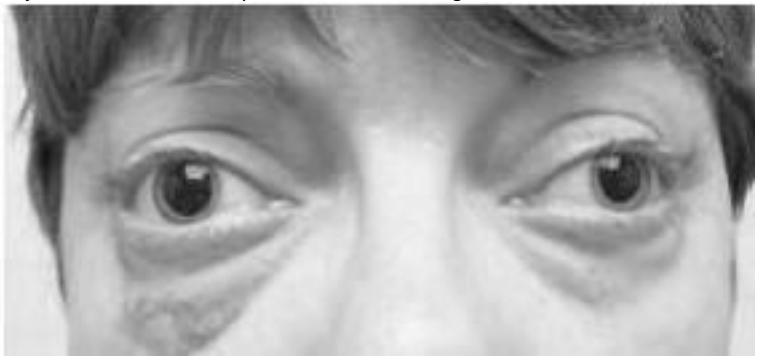
Background: Ipilimumab is a fully human monoclonal antibody against Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4). CTLA-4 counterbalances immune cell activation. In patients with metastatic melanoma, ipilimumab increases survival time and induces complete remission in some patients.
Clinical Case: A 51 year old female with 4 year history of stage IV melanoma on ipilimumab clinical trial was hospitalized for acute onset of severe eye pain, proptosis, and periorbital edema. Ipilimumab was started two months prior to hospitalization. She had no history of thyroid dysfunction before ipilimumab was initiated. After receiving four doses of ipilimumab at 10 mg/kg, she developed the presenting eye symptoms. Physical examination revealed proptosis, conjunctiva redness and periorbital edema (Fig.1).



Exophthalmometry showed OD 23, and OS 23 (normal: 12-22 mm) indicating proptosis. Intraocular pressures were slightly increased (OD 24 and OS 20, normal: 10-20 mmHg). Her laboratory studies revealed high anti-TPO antibody (662 IU/ml, n< 20) and thyroglobulin antibody (148.5 IU/ml, n< 3.9) though her thyroid function tests remained within the normal range with TSH 1.01 and free T4 index 2.5 (normal range: 1.1-4) . Imaging of the head with CT and orbital MRI showed bilateral thickening of extraocular muscles compatible with Graves ophthalmopathy. She received intravenous high dose Solu-Medrol 1 gm daily for 3 days, and subsequently prednisone taper . Her symptoms and ophthalmopathy resolved initially, but relapsed 2 months later as prednisone was tapered. High dose intravenous Solu-Medrol was again initiated. Five months later, her ocular symptoms persisted, although the levels of anti-TPO and thyroglobulin antibody decreased to 65 IU/ml and 57 IU/ml respectively. In contrast, her melanoma tumor burden has dramatically declined in response to ipilimumab therapy.
Conclusion: Anti-CTLA4 therapy has shown promising results in treating advanced malignancy such as melanoma and renal carcinoma. Immune-related adverse events, including endocrinopathies, have been reported. Here, we report the first case of iplimumab related Graves ophthalmopathy.

Nothing to Disclose: LM, AV, CB

P2-607

The Advantage of Orbital Surgery in Graves' Ophthalmopathy: Spectrum of the Disease.

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INTRODUCTION:
Graves' ophthalmopathy is an organ-specific autoimmune disorder that targets the extraocular muscles and retrobulbar tissues and is characterized by varying degree of inflammation and congestion. The swelling of the eye muscles can cause these muscles to become "stiff" or "tight," causing diplopia. The increase intraorbital pressure can cause proptosis severe enough to cause lagophthalmos and exposure keratopathy.
OBJECTIVE:
To present the role of orbital surgery in Graves' ophthalmopathy and its benefits
CASES: We are presenting 2 cases (Case 1: ER, 53/F and Case 2: CR 54/M, both were smokers) with Graves' ophthalmopathy aggravated after receiving radioactive iodine 131 treatment. Vision was associated with diplopia.
Case 1. ER was hyperthyroid controlled on propylthiouracil and propranolol for a year. Proptosis persisted thus was given prednisone. She received radioactive iodine then experienced vertical diplopia, which was temporarily remedied with patching then with eyeglasses with prism. She became hypothyroid with immediate replacement of thyroid hormone, however diplopia did not improved. Orbital surgery with recession of the recti muscles was performed and resulted in improvement in binocularity and self-image.
Case 2. Hyperthyroidism was associated with lagophthalmos in one eye and intermittent diplopia that developed over weeks to months. He was given radioactive iodine treatment. Few days later, blurred vision with worsening of chemosis and proptosis were related. Corneal ulceration and exposure keratopathy were noted in one eye with 20/60 vision. He was then hospitalized and medicated with methylprednisolone IV and antithyroid medications. Tarsorrhaphy was done on the lagophthalmic eye. His hyperthyroidism resolved and is on thyroxine replacement therapy. Visual acuity showed some improvement.
CONCLUSION:
Orbital surgery is indicated for patients with inactive and sight-threatening Graves' ophthalmopathy in which glucocorticoids failed to halt the disease process. It is also performed to minimize or prevent further damage of the cornea in events of perforation and ulceration. However, a successful surgery is dictated not so much with the technique of surgery but by knowledge of the disease process. It is important to recognize the individualization of management. The constructive surgery likewise significantly improved self-image of the patients.

Nothing to Disclose: ALC, SAK, LBM-A

P2-608

Unusual Clinical Course of a Patient with Euthyroid Infiltrative Ophthalmopathy Followed by Hypothyroidism and Euthyroidism.

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Background: The association of Graves' ophthalmopathy with autoimmune thyroiditis and primary hypothyroidism is an uncommon phenomenon. We report a case of a patient with infiltrative ophthalmopathy who initially presented as euthyroid, and whose clinical course evolved to a hypothyroid state then euthyroid state, accompanied by alterations in circulating thyroid antibody activities.
Case: A 42-year-old woman presented with rapidly progressive infiltrative ophthalmopathy and pretibial myxedema. She was euthyroid (TSH 3.8) and had no previous history of thyroid disease. Five months later, she developed symptoms and signs of hypothyroidism associated with elevated TSH of 30.8, low FT4 of 0.7. Her Tg antibodies were elevated at 1311 IU/ml (normal <20); TPO antibodies were 31 IU/ml (normal <35). Anti-TSH receptor antibodies were detected, including both blocking (TBII 57% inhibition; normal ≤16% inhibition) and stimulating (TSI 261% baseline; normal <125% baseline) type. Levothyroxine was initiated. After normalization of TSH, the infiltrative ophthalmopathy improved strikingly and pretibial myxedema resolved, associated with decreased circulating Tg, TBII, and TSI antibody activities to 265 IU/ml, 34% inhibition, and 200% baseline, respectively. TPO antibodies increased slightly to 34 IU/ml. Levothyroxine was tapered off after 5 months of therapy; she has remained euthyroid (TSH 2.15) in the ensuing 6 months.
Conclusion: Some of the potential explanations for the lack of sustained thyrotoxicosis in a Graves' patient with TSI include: 1. Fluctuating TSI levels, 2. Competing TSH-R stimulating and blocking/inhibitory antibodies in varying ratios, and 3. Coexisting Graves' disease and autoimmune thyroiditis with the latter limiting the biosynthetic response to TSI. Our patient's return to euthyroidism, the absence of anti-TPO antibodies, and the presence of TBII as well as TSI argue for explanation #2. In this case, ophthalmopathy improved as the circulating thyroid antibody activity decreased. It is controversial whether anti-TSH receptor antibodies are pathogenetic in Graves' ophthalmopathy and if there is a TSH-R-like orbital target for these antibodies. Nevertheless, in this case, the antibody activities appeared to be a serological marker for ophthalmopathy progression.

Nothing to Disclose: DAZ, ML

P2-609

A Case of Graves' Disease with Concomitant Moyamoya Disease.

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Background: We present a case of Graves' disease with concomitant Moyamoya disease.
Clinical Case: A 29 year old female, with history of Graves' disease diagnosed in 2006 and stroke (CVA) in 2007, presented in October 2008 with complaints of aphasia and right arm numbness. TSH was <0.004 (nl 0.3-3.7 units/ml. MRI of the head revealed multiple old CVAs in left medial cerebral artery distribution. CT angiogram revealed occlusions in both carotids with a Moyamoya-like revascularization. The patient declined revascularization and was non-adherenant to methimazole. She returned in March 2009 with complaints of aphasia, right arm numbness with a left middle cerebral artery distribution CVA. TSH was 0.009 with T4 2.03 (nl 0.8-1.7 ng/dl). While there was a history of sickle cell trait, there was no family history of Moyamoya disease (MMD). Work-up was negative for HIV, hematologic, vasculitic, cardiovascular or connective disorders and MMD was felt to be due to Graves' disease.
Cerebral artery occlusions and neurological symptoms did not improve with methimazole, blood pressure control, aspirin and steroids. Due to labile neurological symptoms and hypotension requiring pressor support, she was not offered revascularization and plasmapheresis was offered. Plasmapheresis reduced anti-thyroglobulin levels (29 to 2.2), microsomal antibodies (555.2 to 38.2) and TSI antibodies. This did not improve her neurological status and she continued to have more CVAs despite becoming euthyroid. The patient stabilized neurologically after having a revasularization bypass surgery.
Discussion: Moyamoya is a chronic progressive cerebrovascular disease that is characterized by bilateral stenosis of arteries around the circle of Willis with the development of collateral circulation, of unknown etiology. We present the 29th case of concomitant MMD and Graves' disease. A literature review revealed a case report where plasmapheresis led to improvement of neurologic symptoms by lowering thyroid antibodies. Unlike in this case, our patient did not show neurological improvement with antibody reduction. This suggests that antibody reduction may not improve neurological symptoms in some patients. Despite this result, there may still be a possible pathogenic link involving T-cell dysregulation affecting cellular proliferation and vascular dysregulation in Moyamoya and immunologic stimulation leading to Graves' disease. Further investigation is warranted.

Nothing to Disclose: PNS, SBM-S, SA, NK, JJ-K, TT

P2-610
Severe Cholestatic Jaundice Induced by Thyrotoxicosis.

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Intrahepatic cholestasis has rarely been observed in patients with thyrotoxicosis and generally occurs in association with coexistent congestive heart failure.
Case Report: We report the case of a 31 year old Native-American female with no significant past medical history, who presented with progressive weakness, palpitation, weight loss, diarrhea, heat intolerance, jaundice, of two months duration. She was found to have thyrotoxicosis and hyperbilrubinemia.
On arrival, she was afebrile, with a pulse of 116, blood pressure of 133/64. On physical exam , She had icteric sclera, very mild bilateral exophthalmus, jaundiced skin, a diffusely enlarged thyroid with no tenderness or nodules, and no myxedema.
Her initial labs: TSH 0.33, Free T4 5.8 (0.8-1.7); Free T3 12.8 (2.0-4.8); thyroid stimulating immunoglobulin 534 (< 125); T. bilirubin 16.4; D. bilirubin 9.6; PT 16.4; INR 1.3;AST69; ALT 76; Alkaline Phosphatase 103; LDH 173; Normal CXR; Celiac panel negative. Ultrasound of the abdomen was negative for obstruction or gallstones. An echocardiogram was with normal.
Her thyrotoxicosis was secondary to Graves' disease, which was confirmed by her high thyroid stimulating immunoglobulin and diffusely enlarged thyroid. A radioactive iodine uptake and scan study was performed, which showed increased uptake of 70% at 24 hours (normal 10-35%).
Clinical examination and laboratory investigations excluded viral hepatitis, autoimmune hepatitis, granulomatous disease, primary biliary disease, extrahepatic biliary obstruction, and heart failure. Liver biopsy showed severe centrilobular cholestasis with minimal inflammatory changes. She was started on prednisone to decrease the conversion of T4 to T3 and to also help with underlying autoimmune disease. Moreover, she was started on Cholestyramine to bind thyroid hormone in the intestine (1) and continued on beta blockers. Subsequently, she had a radioactive iodine ablation of her thyroid. She was continued on her beta blocker, with a tapering dose of prednisone and cholestyramine.
Discussion: Thyrotoxicosis can be an uncommon cause of profound cholestasis, A clinician should be aware of this rare cause of cholestatic jaundice, since treating hyperthyroidism will often lead to complete resolution of the liver injury. Though thionamide drugs are associated with hepatotoxicity, it has been suggested that the mere presence of hyperbilirubinemia should not delay the use of these drugs in treating thyrotoxicosis(2).

1. J Clin Endocrinol Metab. 1996 Sep;81(9):3191-3
2. Endocr. Pract. 2007 Sep;13(5):476-80

Nothing to Disclose: AN, EK, BS, MHH

P2-611

Lack of In Vitro Constitutive Activity for Three Previously Reported TSH-Receptor (TSHR)-Mutations Identified in Patients with Nonautoimmune Hyperthyroidism.

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The occurrence of functionally neutral TSHR mutations in families and the problems associated with the determination of constitutive activity in (CHO) cell lines recently demonstrated the caveats for the in-vitro determination of constitutive TSHR-receptor activity. Functional characterization after transient expression in COS cells is the standard for the determination of TSHR constitutivity. However, the mutations L677V and T620I identified in a hot Hürthle cell thyroid carcinoma and a hot follicular carcinoma were previously characterized in CHO and in 3T3-Vill cell lines, respectively, stably expressing the mutant without determination of TSHR expression. Moreover, I691F identified in a family with non autoimmune hyperthyroidism was only shown to segregate with the phenotype of nonautoimmune hyperthyroidism but was not characterized for it's in-vitro function. Therefore, we decided to reevaluate the in-vitro function of these three CAMs in COS-7 cells. We determined the cell surface expression, basal and TSH-mediated intracellular cAMP and IP levels and performed linear regression analyses (LRA) to determine the basal activity independently from the mutant's cell surface expression. T620I with 103% and I691F with 88% were well expressed on the cell surface (wt = 100%). L677V showed a reduced expression level of 66%. Interestingly, none of the characterized mutations exhibited increased basal cAMP levels. These results were confirmed by LRA studies showing a similar slope for all mutations and the wt. TSH-mediated signaling for T620I was unaltered. For L677V and I691F we found a slight decrease in cAMP production. The basal IP levels were not increased. A strong reduction after TSH stimulation was observed for L677V (21%) and T620I (53%) (wt = 100%). Basal activities correlated strongly with TSHR cell surface expression.
Taken together none of the three investigated mutations displays constitutive activity in COS cells. For cell lines stably expressing the respective construct, e.g. L677V and T620I determination of the receptor cell surface expression is a prerequisite for the determination of constitutive activity. Different receptor numbers in the wt cell lines as compared to the mutant cell lines is a likely explanation for the different results. Based on the lack of constitutive activity for L677V, I691F and T620I in COS-7 cells other molecular etiologies for the nonautoimmune hyperthyroidism in these 2 patients and one family should be elucidated.

Nothing to Disclose: HJ, SM, ME, RP

P2-612

Graves' Disease Presenting as Hypokalemic Thyrotoxic Periodic Paralysis in a Canadian First Nations Person.

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Background: Hypokalemic thyrotoxic periodic paralysis (HTPP) is a rare, potentially life-threatening complication of hyperthyroidism, characterized by episodes of transient flaccid paralysis. It is seen most commonly with Graves' Disease, but can occur with other causes of thyrotoxicosis. HTPP is more common in Asians, but has been described in other ethnicities. To our knowledge, there have been no published cases in a Canadian First Nations person.
Clinical Case: A 34-year old First Nations man was brought by paramedics to the emergency room after awakening with acute paralysis of his arms and legs. On presentation, serum potassium was 1.6 mmol/L (3.5-5), but nadir was <1.5 mmol/L. Other electrolytes were normal. TSH was <0.01 mIU/L (0.27-4.20), free T4 28 pmol/L (11-22), free T3 11.7 pmol/L (3.0-6.5). ECG showed QT interval prolongation and QRS widening. Potassium replacement resulted in resolution of his paralysis and ECG changes. He was admitted to hospital for investigation, and subsequently discharged home on oral potassium supplements.
Past medical history included sleep apnea, chronic bronchitis, and narcotic dependence, but no known history of hyperthyroidism or previous attacks. There was no family history of thyroid disease or periodic paralysis. He was a smoker. His medications were methadone, salbutamol and tiotropium.
On follow-up 1 week later, he felt well with no further paralytic episodes. Since hospital discharge, he noticed possible symptoms of hyperthyroidism, with heat intolerance, weight loss and irritability, as well as muscle weakness, but was not overtly hyperthyroid on exam. There were no extra-thyroidal manifestations of Graves' Disease. Laboratory investigations showed: TSH <0.01 mIU/L, free T4 33 pmol/L, free T3 20.7 pmol/L, anti-TPO 407 IU/mL (< 40), TBII 4.6 (<1.0). Repeated potassium levels remained normal while on potassium supplements. Thyroid iodine scintigraphy showed an enlarged thyroid with diffuse uptake of 44.8%. He was started on atenolol 25 mg od and methimazole 20 mg od. He is referred for radioactive iodine ablation as definitive treatment.
Conclusion: Hypokalemic thyrotoxic periodic paralysis is a rare complication of hyperthyroidism, and is more prevalent in Asians. However, it is becoming increasingly recognized in persons of non-Asian descent, and it is not clear if the frequency is increased in First Nation subjects. The diagnosis should be considered in any patient presenting with hypokalemia and paralysis.

Nothing to Disclose: SLL, BW, SVU

P2-613

Carbimazole-Induced Myositis in the Treatment of Graves' Disease – A Case Report and Review of the Literature.

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Background Patients with thyroid dysfunction may present with musculoskeletal complaints. However, the development of myositis after treatment of Graves' disease is rare.
Case report A 24 year old female presented with cramping and weakness in her limbs of 2 weeks duration. She was diagnosed with Graves' disease 2 months prior to presentation and had been commenced on carbimazole with improvement in her thyroid function. On admission she was clinically euthyroid and had symmetrical proximal myopathy. Her creatine kinase (CK) was 14203 u/l (38-164). FT4 was 10.4 pmol/l (9.6-19.1) and TSH was <0.006 mu/l (0.36-3.24). IV hydration was given and carbimazole was withheld as it was a possible cause for her rhabdomyolysis. Cessation of carbimazole resulted in resolution of symptoms and repeat CK showed rapid improvement. All autoimmune markers were negative. Electromyography of upper and lower limb muscles showed myopathic changes. PTU was started in place of carbimazole. 2 months later, CK was normal at 90 u/l, FT4 10.8 pmol/l and TSH <0.006 mu/l. She has since been on PTU treatment without further recurrence of myositis.
Conclusion Painless myopathy with normal CK may occur in patients with untreated hyperthyroidism but there are rare reports of myositis in patients undergoing treatment of hyperthyroidism. These reports include patients both treated with carbimazole, as in our patient, and any of the other thionamides. All the reports had patients who were started on standard high-dose antithyroid drugs (ATDs) with improvement of their thyroid function but development of symptomatic myopathy. Changing ATDs from one thionamide to another, decreasing the dose of the preexisting ATDs or the addition of levothyroxine, all appeared to have resulted in clinical improvement. It appears that myositis as a result of treatment of hyperthyroidism could be caused by various reasons and only in susceptible individuals. Possible postulations include direct effect of specific ATDs on myocytes, immune-related responses secondary to ATDs or rapid decrements of circulating thyroid hormone with ensuing myositis. Onset of symptomatic myopathy occurred anywhere from 1 week in other reports to 2 months (as in our case), suggesting that one should look out for musculoskeletal complaints during the early part of treatment in order to prevent potential complications.

Nothing to Disclose: AYYL, PCK, AWES

P2-614

The Association of Carotid Cavernous Fistula with Graves' Ophthalmopathy: A Case Report.

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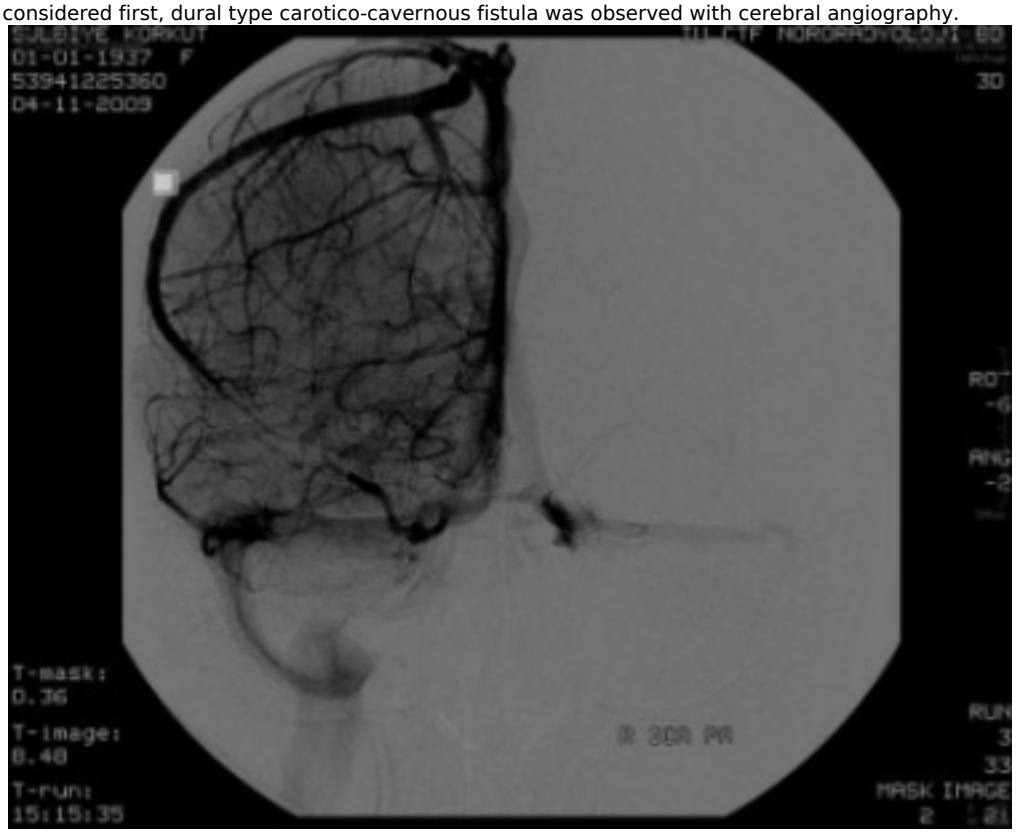
Background: Here we report a case with simultaneous occurrence of Graves' ophthalmopathy and carotid cavernous fistula. **Clinical Case:** A 72 year-old woman was addmited with swelling in both eyes, proptosis, decrease of vision, edema and chemosis in conjunctiva on the right. Steroid treatment was applied one year ago with the same complaint. She was referred to Istanbul University, Cerrahpasa Medical Faculty, Endocrinology and Metabolism outpatient clinic for the regulation of diabetes, planning of her radiotherapy and steroid treatment. 15 years ago she had propylthiouracil treatment for 1 year. Laboratory examination were as follows: fT4: 1.19 ng/dl (0.7-1.7), TSH: 2.24 mU/l (0.4-4), TRAb: 0.1U/L (0-1.1),HbA1c: 10.3. In the orbital MRI images, exophtalmos in the right eye, increase in thickness in the right extra ocular muscles, increase in the right retroorbital fatty tissue, enhancement in T1 and T2 signals were observed. The right optical nerve calibration was thinner than the left. Although asymmetrical thyroid ophthalmopathy was considered first, dural type carotico-cavernous fistula was observed with cerebral angiography.



The patient underwent endovascular treatment by transvenous embolization. After 2 months, in the orbital MRI control, it was seen that the symptoms were decreased and a full vision was obtained in the right eye of the patient. Symptoms associated with the Graves' ophthalmopathy are proptosis, ophthalmoplegia, periorbital edema, conjuctival chemosis and uncommonly compression of the optic nerve. Carotid cavernous fistulas develop between the cavernous sinus and carotid arteries as a result of abnormal connections. Due to similar findings, it is important in distinguishing diagnosis of the Graves' ophthalmopathy. **Conclusion:** To the best of our knowledge, the number of cases in which the carotid cavernous fistula and Graves' ophthalmopathy occur concurrently are quite few (1). Therefore carotid cavernous fistulas must be considered in differantial diagnosis of Graves'ophthalmopathy patients who are not responding to the treatment and who have especially unilateral and asymmetric eye symptoms.

(1) Lore F, Polito E, Cerase A, Bracco S, Loffredo A, Pichierri P, Talidis F. Carotid Cavernous Fistula in a patient with Graves' Ophtalmopathy. J Clin Endocrinol Metab. 2003; 88; 3487- 3490.

Nothing to Disclose: SG, OC, SS, HA, CI

P2-615

A 15-Year-Old Girl with Graves' Disease Associated with Moyamoya Disease.

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Background: Thyroid hormone excess in Graves' disease is harmful to arterial walls because it may alter vascular reactivity, and Graves' disease is often accompanied by many cardiovascular symptoms. There have been few reported cases of the coexistence of Graves' disease and moyamoya disease.
Clinical case: A 15-year-old girl who had suffered from Graves' disease for seven years visited our hospital with central type right facial palsy. She presented with a clinical picture of hyperthyroidism, goiter and ischemic attack consisting of right-side hemiparesis. Thyroid function tests demonstrated suppressed thyroid-stimulating hormone and elevated thyroid hormone levels. The patient was diagnosed with Moyamoya disease with typical stenosis of the ICA and MCA, based on MRI scans and cerebral angiography. Single photon emission computed tomographic (SPECT) scans revealed cerebral hypoperfusion of the left frontal lobe, left thalamus without vascular reserve. The patient underwent cranial revascularization by encephalo-duroarterio-synangiosis (EDAS). After surgery, the patient's symptoms improved, and the power of the right upper and lower extremities recovered.
Conclusion: To our knowledge, this is the first case of Moyamoya disease associated with thyrotoxicosis in a young girl in Korea. Moyamoya disease should be considered in the differential diagnosis of cerebrovascular events in patients with Graves's disease.

Nothing to Disclose: CKC, SUK, GMY, YJL

P2-616

Liver Transplantation Due to Medicine Interaction between Antithyroid Drug and Phytotherapics.

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Background: The most antithyroid drugs taken are propylthiouracil (PTU), carbimazole and methimazole . However, they are associated to hepatotoxicity which occurs in 0.1-0.2% of patients. PTU is related to hepatocellular injury and elevated aminotransferases level, besides submassive or massive hepatic necrosis. Meanwhile, methimazole causes intracanalicular cholestasis with preserved hepatocellular architecture. Symptoms are common on the first three to six months, generally presenting recovery after withdrawal.
Clinical case: A 47-year-old woman was admitted to the emergency presenting jaundice 3+/4, conscious, with normal vital signs, 38Kg/m2 of BMI, diffuse abdominal pain at palpation but no signs of peritonitis.
She reported hyperthyroidism by Grave´s disease for 4 years taking 40 mg/day of methimazole. She was also in treatment for obesity for two months with sibutramine 10mg plus phytotherapic formula containing aloine 40mg, chitosane 500mg, senne 100mg, G.camboja 380mg, faseolamine 250mg, passiflora 350mg twice a day.
Admission exams revealed total bilirubin 16,88mg/dL(normal range(NR):0,2-1,0mg/dL), direct bilirubin 14,87mg/dL(NR:0-0,2mg/dL), albumin 3,2g/dL(NR:3,5-5,2g/dL), alkaline phosphatase 135U/L(NR:35-104U/L), gama-glutamyltransferase 182U/L(NR:8-41U/L), AST 797 U/L and ALT 570 U/L(NR:till 31U/L), INR 1,47(NR:0,9-1,1), TSH 0,01 µU/mL(NR: 0,4-1,1 µU/mL), FT4 2,1µU/mL(NR:0,7-1,5 µU/mL). Abdominal ultrasound with signs of chronic hepatopathy, extended gallbladder suggesting cholestasis.Hepatic biopsy viewed acute cholestatic hepatitis, extensive areas of necrosis in “bridge” in 30% of the sample suggesting submassive hepatic necrosis due to methimazole. Search for auto-immune and viral hepatitis was negative.
The withdrawal of the drugs did not reset hyperthyroidism symptoms also did not stop progressive hepatic insufficiency requiring liver transplantation. The pathology started after sibutramine and phytotherapic intake, suggesting a possibility of medical interaction, considering reports of hepatotoxicity with senne and aloine with serious cholestatic hepatitis and centrolobular necrosis. Sibutramine has been safely used in hepatic steatosis degree III.
Conclusion: All patients in treatment with antithyroid drugs must be monitored for any hepatic change, mainly in the first six months being advised to these signs and symptoms, avoiding drug association, including phytotherapics, which increases the risk of hepatic injury.

Nothing to Disclose: DGF, GNA, DMN, LCS, FF, FZL

P2-617

Hyperthyroidism in a Patient Therapeutically Immunosuppressed after Successful Cadaveric Renal Transplantation.

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Rapidly progressive hyperthyroidism is often seen in the context of autoimmune thyroid disease such as Graves' disease. Although most patients are females and relatively younger, males with hyperthyroidism are seen increasingly and share many features with their female counterparts.On the other hand patients with non autoimmune etiology often have known presence of a goitre(uniform/nodular) antedating by months or years, any onset of overt thyroid dysfunction. Even in the context of so called non immune thyroid disease,immunological mechanisms are postulated to explain dyshormonogenesis.Given this context, it should be nearly impossible to develop Graves' disease or hyperthyroidism during succesful immunosuppresion.
We decsribe a 68 yr old male who had prevoiusly undergone a successful cadaveric renal transplantation nearly 18yrs ago and was maintained on immunosuppression with Cyclosporine, Imuran and Prednisone. Over the course following transplantaion he was diagnosed with recurrent basal cell carcinoma of skin and recently diagnosed with MALT non-hodgkins lymphoma following investigations for melanotic stools and anemia. Patient was referred to endocrinology service for evaluation of fatigue and abnormal thyroid indices. When seen in our clinic patient did not exhibit classic symptoms of hyperthyroidism. He weighed 177lbs, and his BP was 140/70mm Hg with a pulse of 60/minute(on beta blocker). He had palpable thyroid gland and no discernable nodularity. No thyroid bruit. His extraocular movements were intact with moderate impairment of convergence. Patient has a loud ejection systolic murmur in the aortic region consistent with aortic stenosis.Our presumptive diagnosis was apathetic thyrotoxicosis- possible autoimmune thyroid disease(Graves' disease). Initial laboratory indices revealed TSH 0.02mIU/ml;Free T4 2.1ng/dl;T4 13.5mcg/ml,TotalT3 189mg/ml. Negative thyroid peroxidase antibodies. TSI 101%; TBII 10%(<9%). Patient was started on Methimazole 30 mg once a daily and his thyroid indices improved significantly over the following three weeks and patient had gained 2.4 lbs.
Emergence of autoimmune thyroid disease under active immunesuppression is a rare occurence.Antibodies may develop that are not routinely measured or measurable masking the interplay of underlying immunological abberations.Previously 6 such cases have been reported in english literature including the previous one from our center that highlighted immune abberations in detail.

Nothing to Disclose: PJ, NS, SG, RK, CF

P2-618

A Case of Hyperthyroid Graves' Disease with Superimposed Post-Partum Thyroiditis Ultimately Resulting in Hypothyroidism.

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Postpartum thyroiditis consists of a group of autoimmune thyroid disorders, occurring in the first year after delivery. It presents as transient periods of thyrotoxicosis, hypothyroidism or both and results in permanent hypothyroidism in some patients (3). Serological evaluation for thyroid autoimmunity also indicates that thyroid-stimulating immunoglobulins are not a cause of hypothyroidism, but that destructive changes in the thyroid are probably responsible for hypothyroidism. The two most plausible patholophysiologic mechanisms for development of hypothyroidism in Grave's disease patients were progressive autoimmune-mediated destruction of the thyroid follicular epithelium and a predominance of blocking, non-stimulating antibodies to the thyroid stimulating hormone (TSH) receptor (1).
A 36 year-old hypertensive female was diagnosed with hyperthyroidism with TSH by chemiluminescence=0.05 mIU/L (0.35-5.50), total T3 by chemiluminescence=342 ng/dl (60-181), free T4 by chemiluminescence=2.6 ng/dl (0.89-1.80). After 2 years of being asymptomatic she then was treated based on a high radioiodine uptake and symptoms of agitation, tremulousness, palpitations and weight loss with methimazole 5 mg daily that was titrated to 10 mg daily. The patient had a miscarriage and 2 months later noticed an increase in the size of the thyroid. More than one month after self-discontinuation of methimazole TSH by chemiluminescence= 70.5 mIU/L (0.35-5.5), TSIG by bioassay= 140 % baseline (<125), TBG by RIA=22.3 (13. 5-30.9), free T4 by chemiluminescence= 0.87 ng/dl (0.89-1.76), T3 Uptake by spectrophotometry=36.2% (22-37), T4 by immunoassay=6.6 mcg/dl (4.5-10.9), anti-TPO by chemiluminescence=29.3 (<60), thyroglobulin ab by chemiluminescence= <20 (<20).The patient was started on l-thyroxine.
Patients with Graves' disease are generally hyperthyroid. However there are patients who spontaneously undergo changes in the thyroid function from hyperthyroidism to hypothyroidism during the evolution of the disease. These changes can be explained by different pathophysiologic mechanisms. The autoimmune tissue destruction by irreversible loss of a number of follicular cells with high functional capacity and an increase in the ratio of blocking to stimulating TSH receptor antibodies. This appears to be a very rare case of Graves' disease with superimposed post-partum (post-abortive) thyroiditis ultimately resulting in hypothyroidism (1), (2).

(1) Gonzalez-Gonzalez A et al.,Endocrinol Nutr. 2009; 56(5):273- 276
(2) V.C.De Moraes, A et al., Southern Medical Journal 2006; 99 (10):1068-1072
(3) Shahbazian HB et al., European Journal of Endocrinology 2001; 145:397-401

Nothing to Disclose: JDR, AS

P2-619

A Rare Case of Hypoglycemia and Liver Dysfunction in a Patient with Graves' Disease.

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[1]Tulane Univ New Orleans, LA.

Introduction: Hypoglycemia due to thyroid dysfunction is exceedingly rare. Only few cases are described in conjunction with thyroid storm and to our knowledge hypoglycemia with Graves' disease have not been described. Furthermore, liver dysfunction and hypercalcemia may occur in Graves' disease, the severity exhibited by our patient is uncommon.
Case: A 42 year old male was admitted to the Gastroenterology service with 5 week history of pruritus, dark colored urine, decreased appetite, diarrhea and a 50-lb weight loss over the past 2 months. Review of symptoms was otherwise negative. There was no history of alcohol intake, insulin injection, exposure to any hepatotoxic agents or medications. Examination was notable for heart rate of 100beats/min, scleral icterus, extensive excoriations and scratch marks diffusely over his body. The rest of the exam was within normal limits.
Laboratory results included: total bilirubin 37.6 mg/dl (0.1-1.2mg/dl), AST 61units/L (0-35units/L), ALT 58units/L (7-56units/L), alkaline phosphatase 1719unit/L (41-133units/L), calcium 12.5 mg/dl (8.5-10.5mg/dl), albumin 2.9 g/dl (3.4-4.7g/dl). Blood glucose level was in 42 mg/dl (60-110mg/dl), c-peptide level was 3ng/ml (0.8-4.0ng/ml), cortisol 35ug/dl (5-20ug/dl), ferritin was elevated at 5746 ng/ml (16-300ng/dl), serum iron was 47.3ug/dl (50-175ug/dl) and iron saturation was normal. Extensive GI work-up including viral, autoimmune, and malignancy etiologies was negative. Right upper quadrant ultrasound, and liver biopsy did not reveal any specific etiology. With no clear diagnosis additional testing was performed including thyroid function tests. TSH <0.01(0.4-6.0Uu/ml), free T4 5.91ng/ml (0.8-2.7ng/ml). Thyroid uptake scan was 73.4 %(0-30%) at 24 hours.
The patient was started on treatment for Grave's disease and within a few days hypoglycemia resolved, hypercalcemia and LFT'S also improved.
Discussion: Hypoglycemia due to thyroid dysfunction is rare. Kobayashi *et al*. described thyroid storm with hypoglycemia due to starvation. Here we present a rare case of hypoglycemia and liver function abnormalities in a patient with Graves' disease. Hypoglycemia should be considered part of a constellation of abnormalities associated with Graves' disease. It is also important to measure thyroid function tests in patients with liver dysfunction of unknown etiology

1)Kobayashi et all;2005. sever starvation hypoglycemia and congestive heart failure induced by thyroid crisis. Intern Med 44:234-239
2)Izumi K, Kondo S, Okada T.2009 A case of atypical thyroid storm with hypoglycemia and lactic acidosisEndocr J.Dec;56(6):747-52. Epub 2009 Jun 9.

Nothing to Disclose: SA, JW, JK, JM, VJ, JJK

P2-620

Pre and Post ACTH Analysis of Twenty Steroid Hormones in Adrenal Vein Samples.

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Background: Although the numerous steroid hormones produced by the adrenal gland play a critical role in human physiology, a detailed quantitative analysis of the steroid products has not been reported. This is the first study, which uses a single methodology (liquid chromatography-tandem mass spectrometry, LC-MS/MS) to quantify 20 steroids in adrenal vein (AV) samples pre and post adrenocorticotropic hormone (ACTH) stimulation. **Objective:** To define the adrenal steroid metabolome in women pre and post 15 min of ACTH infusion. **Design:** A retrospective study. **Patients:** Eight women, 51.3±18.1 years, with a suspected diagnosis of adrenal aldosterone producing tumor. **Methods:** Serum samples were collected from the iliac vein and the AV from adjacent normal and tumor containing adrenals, before and after administration of ACTH. Iliac vein and normal AV samples were analyzed by LC-MS/MS for concentrations of 20 unconjugated steroids previously reported as potential adrenal products. The net AV steroid concentration was calculated by subtracting the iliac vein concentration. The percentage of each steroid was defined as the percent of the molar net AV total of the 20 steroids measured. **Results:** Cortisol, 11β-hydroxyandrostenedione (11OHA), dehydroepiandrostenedione (DHEA) and corticosterone were the most abundant steroids in the AV samples pre and post ACTH stimulation. ACTH significantly increased the absolute adrenal output of 16 of the 20 steroids measured ($p<0.05$). ACTH increased the mean concentration of cortisol by 17-fold, 11OHA by 5-fold, DHEA by 18-fold and corticosterone by 82-fold. In the AV, cortisol was the most abundant of the steroids analyzed, representing 65±3% (588±262 nmol/L) prior to ACTH stimulation, and 57±2% (10,129 ± 2,497 nmol/L) post ACTH infusion. **Summary:** The current study defined the relative production of unconjugated steroids in the human adrenal and their precursors pre and post ACTH stimulation. We found that the human adrenal secretes mainly two glucocorticoids (cortisol and corticosterone) and defined 11OHA and DHEA as the primary adrenal androgens.

Nothing to Disclose: JR, FS, RM, HS, MRK, CA, SH, YN, WER

P2-621

Encapsulation Strategy: A Viable Opportunity for the Implantation of Adrenocortical Cells.

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Treatment for bilateral pheochromocytomas is adrenalectomy. Following surgery, patients require life-long glucocorticoid and mineralocorticoid replacement therapy. Adequate assessment of patients on their hormonal replacement therapy is of great importance to avoid the consequences of under or over treatment. However, it has been shown that patients with a primary adrenal insufficiency undergoing hormonal replacement therapy have a mortality rate two-fold greater than that of the general population.
To improve the quality of life and to diminish the risk of premature death of these patients, we have established a pre-clinical mouse model of subcutaneous cell transplantation. This study examined the engrafment and long-term function of human and bovine adrenocortical cells transplanted into adrenalectomized SCID mice using an implantable device consisting of a 2.5 cm-long cylindrical titanium mesh with a 0.6 cm diameter and two stoppers at the extremities. Twenty one days prior cell transplantation, the devices were implanted subcutaneously in the right dorsal region of the mice. Connective tissue rich in vascular structures embedded the device through the pores of the mesh. In order to prevent complete occlusion of the device lumen by the mouse's tissue, a polytetrafluoroethylene plunger was inserted filling the lumen at the time of implant. At the time of cell transplantation, mice were totally adrenalectomized, the plunger was removed and the cells were implanted into the lumen of the device in a small volume of culture medium. The device was then sealed by placing a titanium stopper.
Animals that received two million primary human or bovine adrenocortical cells survive indefinitely. Most animals were sacrificed 35 days after cell transplantation. The tissues formed inside the device resembled that of normal human or bovine adrenal cortex. The assessment of graft function was validated by the presence of cortisol in the plasma. Abundant vascular structures were observed throughout the sections suggesting that neovascularization occurs through vessels arising from the wall of the device. Finally, the proliferation rate was very similar to the rate observed in the adrenal gland.
Taken together, our results describe a potentially clinically cell therapy alternative to hormonal replacement therapy by transplanting the patient's own adrenal cells.

Sources of Research Support: Fondation de l'Avenir.

Nothing to Disclose: PC, BT, OC, MT

P2-622

Corticosteroid-Binding Globulin (CBG) as an Important Modulator of Cortisol Pharmacokinetics.

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Cortisol is extensively protein bound in the circulation (>80% to CBG and 10-15% to albumin) which creates a circulating pool of potentially available steroid. This protein binding is predicted to affect cortisol bioavailability and duration of biological action.
We studied cortisol pharmacokinetics (PK) using serum (total and free) and saliva in n=6 healthy volunteers (HV), n=6 women on the oral contraceptive pill (OCP) and n=2 patients homozygous for a CBG mutation that prevents cortisol binding (null). Salivary cortisol (SalF) was measured by a previously published liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay and an ultrafiltration/LC-MS/MS method was used for free serum cortisol (FreeF). Total serum cortisol (SerF) was measured by a routine electrochemiluminescent immunoassay (Roche Diagnostics GmbH). Subjects were studied after dexamethasone suppression.
Cortisol concentrations peaked following IV hydrocortisone and then showed an exponential decay. Peak concentration, area under the concentration curve (AUC) and half-life (t1/2) of SerF were significantly higher in the OCP group and lower in the CBG null (Table). Cortisol clearance was significantly lower in the OCP group and about 2-fold increased in the CBG null, compared to HV.

PK parameters in all groups

SerF parameters	HV	OCP	CBG null
peak (nmol/L)	1141(960-1429)	1573(1158-2368)	854(686-1022)
AUC (nmol.hr/L)	3129(2616-4714)	7353(4297-9605)	1515(1139-1891)
t1/2 (mins)	118(95-133)	203(132-262)	66(63-69)
clearance (L/hr)	17.6(11.7-21.1)	7.6(5.7-12.8)	38.8(29.2-48.4)

All variables were significantly ($P<0.05$) different between groups

When FreeF and SalF were measured PK parameters were not different between the CBG null and HV, although OCP patients still had higher AUC (nmol.hr/L) compared to HV [475.5(300.2-711.5) vs 251.8(184.5-305.9), P=0.009] and prolonged t1/2 (mins) [90.2(77.7-109.5) vs 68.9(54-76.6), P=0.004]. In the CBG null FreeF and SalF half-life was similar to SerF half-life, as opposed to being lower in HV and OCP.
We have demonstrated a clear effect of CBG on cortisol PK. When binding to CBG is disrupted, SerF kinetics are defined by FreeF, which retains normal PK characteristics. The decrease in SerF clearance observed in women on OCP causes an increase in FreeF AUC, as bound and unbound cortisol are always in equilibrium, which may be clinically significant. SalF can be a surrogate for FreeF in cortisol PK studies.

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Nothing to Disclose: IP, LJO, BGK, DWR

P2-623

A Novel Unequal Cross-Over Mutation between CYP11B1 and CYP11B2 Genes Causes Familial Hyperaldosteronism Type I.

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Background: Familial Hyperaldosteronism type I (FH-I) is an autosomal dominant disorder caused by an unequal cross-over of the gene encoding steroid 11ß-hydroxylase (CYP11B1) and aldosterone synthase (CYP11B2), giving rise to a chimeric CYP11B1/CYP11B2 gene that displays aldosterone synthase activity regulated by adrenocorticotrophin hormone instead of angiotensin II. **Aim**: To report the case of a *de novo* unequal cross-over mutation between CYP11B1 and CYP11B2 genes causing FH-I and to study a possible association with a parental polymorphism. **Patients and Methods**: The index case is a 45-year old Chilean male diagnosed with primary aldosteronism (PA). All family members were also studied; consist of his biological parents, one brother, six sisters, two daughters and one son. Plasma renin activity, serum aldosterone and its ratio were measured in all patients. Genetic analyses were performed using long-extension PCR (XL-PCR), DNA sequencing and Southern blot methods. **Results**: PA was diagnosed for the index case, one of his daughters and his son, but not for his parents or siblings. XL-PCR and Southern blotting demonstrated the presence of the chimeric CYP11B1/CYP11B2 gene solely in PA-affected subjects, thus suggesting a case of a *de novo* mutation. Sequence analysis showed the unequal cross-over CYP11B1/CYP11B2 at intron 2 (IVS2-273 CYP11B2 gene). We also identified a polymorphism at the same intron (IVS2-145 C/A) in the genome of the index case's father. **Conclusion**: We describe an unprecedented case of a novel unequal cross-over mutation for the chimeric CYP11B1/CYP11B2 gene causing FH-I, which is may linked to a polymorphism in the index case's father germ line.

Sources of Research Support: Chilean “FONDECYT 1070876, 1100356” (CC, CF), “FONDECYT 1070352” (AK), and “Millennium Nucleus of Immunology and Immunotherapy (P04/030-F)” grants (PG, ER, CF, AK).

Nothing to Disclose: CAC, CBS, PAG, EMR, TDM, MJS, AMK, CEF

P2-624

Clinical, Genetic, and Functional Characterization of Patients with Non-Classic Congenital Lipoid Adrenal Hyperplasia (NCLCAH) and Partial Loss-of-Function Mutations in the Steroidogenic Acute Regulatory Protein (StAR).

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NCLCAH is a recently recognized disorder caused by StAR mutations that retain partial activity (1). Affected individuals have presented with adrenal insufficiency and mildly disordered sex development. We describe three NCLCAH. Pt 1: a 4 yo Thai male with glandular hypospadias presented with pigmentation, low basal cortisol (0.1 µg/dl), elevated ACTH (>1250 pg/ml), and no cortisol response to ACTH. PRA was mildly elevated, but electrolytes and aldosterone were normal. He was homozygous for StAR R188C (known). Pt 2: a 27 yo male born with ambiguous genitalia had adrenal insufficiency since infancy; he was a compound heterozygote for L260P (known) and F267S (novel). Pt 3: a 15 yo Nepalese 46,XX girl presented with adrenal crisis at 11 mo but had normal puberty and regular menses; she was homozygous for G221D (novel). We also studied R192C recently identified by others (2). We built expression vectors for each mutant and co-transfected COS-1 cells with wild-type (wt) or mutant StAR and the F2 vector expressing the cholesterol side chain cleavage system, and measured pregnenolone production by EIA. Each mutant was also expressed as N-62 StAR in *E. coli*, purified, added to mitochondria from mouse Leydig MA-10 cells, and pregnenolone synthesis was measured. Cholesterol binding was measured by combining each purified mutant protein with fluorescent NBD-cholesterol. Activities for R192C were half of normal, for R188C, G221D and F267S low but measureable, and for L260P insignificant (Table 1). L260 & F267 are conserved hydrophobic residues in α-helix 4, forming part of the cholesterol-binding site. R188, R192 lie in sheet β6 and G221 is in the loop joining sheets β8 and β9, contributing to the sterol-binding pocket. Our study illustrates the broad clinical spectrum of StAR mutations, and shows that *in vitro* StAR activities correlate well with clinical phenotypes. Partial loss-of-function StAR mutations should be considered in patients presenting with atypical Addison's disease; comprehensive assessment of adrenal and gonadal function and mutation analysis are essential for accurate diagnosis and management.

Table 1. The summary of StAR activities in cells, in vitro, and cholesterol binding

	% of wild-type activity		
Mutant	Cellular activity	Mitochondrial activity	Cholesterol binding
R188C	8.0%	12.8%	6.7%
R192C	39.4%	54.8%	55.3%
G221D	2.4%	6.3%	10.2%
L260P	3.1%	1.8%	4.6%
F267S	6.1%	9.5%	20.9%
Wild-type	100%	100%	100%

(1)Baker BY et al.,JCEM 2006;91:4781
(2)Metherell LA et al.,JCEM 2009;94:3865

Nothing to Disclose: TS, RES, JCA, WLM

P2-625

Competitive Inhibition of Adrenal 3-Beta Hydroxysteroid Dehydrogenase Type II by Cortisol: A Possible Mechanism for Human Adrenarche.

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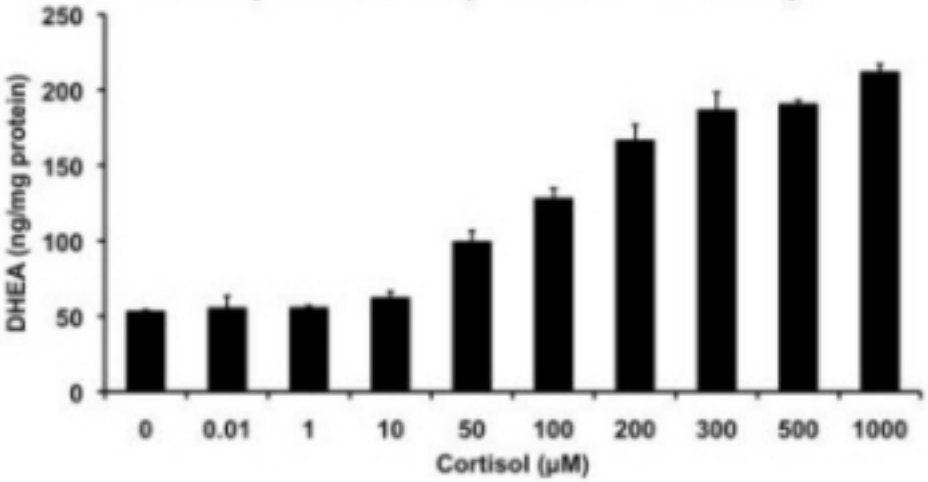
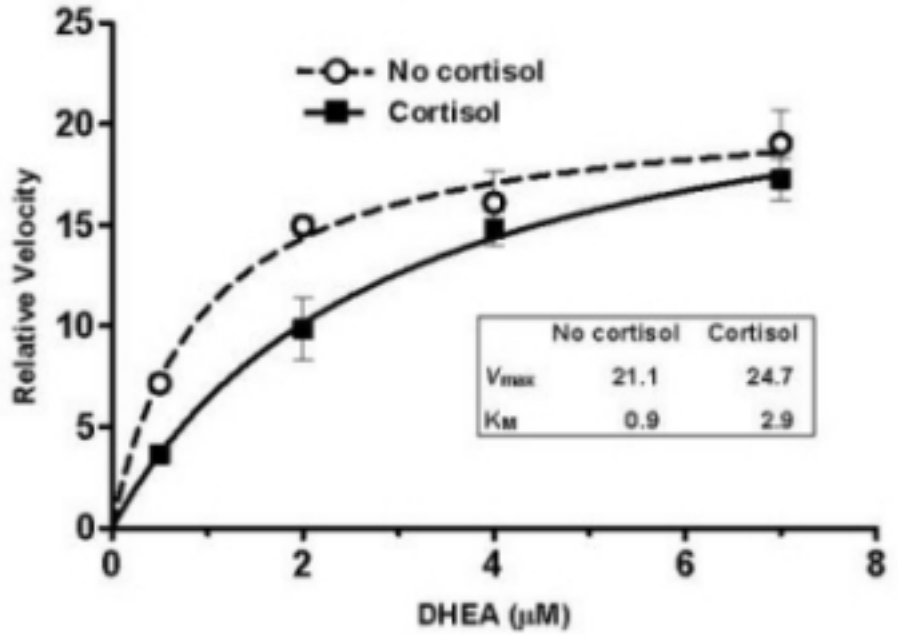
BACKGROUND
Adrenarche, the onset of adrenal DHEA secretion that normally occurs in mid-childhood, manifests with the appearance of pubic hair and apocrine odor. Premature adrenarche is a common pediatric disorder, usually benign but occasionally the first sign of an adrenal tumor or enzyme deficiency. To date, the factors that initiate adrenarche remain unknown. Since cortisol production rises during childhood growth, we asked whether cortisol could be responsible for the initiation of adrenarche.
METHODS
Cortisol concentrations were measured in human adrenals. Human adrenal NCI-H295R cells were treated with physiologic concentrations of cortisol, and DHEA secretion was measured by immunoassay, tandem mass spectrometry, and thin layer chromatography. The effects of cortisol upon the adrenal enzymes, 17, 20 lyase and 3βHSD2, were measured in adrenal NCI-H295R cells and in COS-7 cells transfected with either human *CYP17A1* or *HSD3B2*.
RESULTS
Cortisol in physiological concentrations stimulated a marked dose-dependent rise in DHEA secretion from NCI-H295R cells.

Figure 1: Cortisol stimulates DHEA from NCI-H295R cells in a dose-dependent fashion (P<0.001 for cortisol effect).



This finding was reproduced using cortisol analogs with minimal or no transcriptional activity, but not by glucocorticoids that are transcriptional regulators, suggesting that cortisol inhibits 3βHSD2 activity by direct enzyme interaction. Studies in COS-7 cells engineered to express *HSD3B2* demonstrated that cortisol competitively inhibits 3βHSD2 activity.

Figure 2: Relative velocity of 3βHSD2 with and without cortisol (50 μM)



CONCLUSIONS
Cortisol stimulates DHEA production in adrenal cells through inhibition of 3βHSD2 activity. A rise in cortisol production during mid-childhood growth may lead to increased intra-adrenal cortisol concentrations and trigger adrenarche. Further clinical studies in children are needed to determine if an early or more marked rise in intra-adrenal cortisol is responsible for premature adrenarche.

Sources of Research Support: Grants from the NIH (JAM and WB), the Clinical Investigator Training Program: Harvard/MIT Health Sciences and Technology - Beth Israel Deaconess Medical Center, in collaboration with Pfizer Inc .and Merck & Co. (LMS), and the Timothy Murphy Fund.

Nothing to Disclose: LMS, MA, WB, JD, MJ, JAM

P2-626

Therapeutic Concentrations of Mitotane Inhibit Thyrotroph Cell Viability, TSH Secretion and Expression in a Mouse Cell Line Model.

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Mitotane therapy is associated with many side effects, including thyroid function perturbations mimicking central hypothyroidism, possibly due to laboratory test interference or pituitary direct effects of mitotane. Therefore, we aimed at investigating whether increasing concentrations of mitotane in the therapeutic range might interfere with thyroid hormone assays and evaluate the effects of mitotane on a mouse TSH- producing pituitary cell line. TSH, FT4 and FT3 levels do not significantly change in sera from hypo-, hyper- or euthyroid patients after addition of mitotane at concentrations in the therapeutic window. In the mouse TaT1 cell line mitotane inhibits both TSH expression and secretion, blocks TSH response to TRH and reduces cell viability, inducing apoptosis at concentrations in the therapeutic window. TRH is not capable of rescuing TaT1 cells from the inhibitory effects of mitotane on TSH expression and secretion, that appear after short time treatment and persist over time. Our results demonstrate that mitotane does not interfere with thyroid hormone laboratory tests but directly reduces both secretory activity and cell viability on pituitary TSH-secreting mouse cells. These data represent a possible explanation of the biochemical picture consistent with central hypothyroidism in patients undergoing mitotane therapy, and open new perspectives on the direct pituitary effects of this drug.

Nothing to Disclose: EG, FD, FT, GR, GC, MRA, MT, ECDU, MCZ

P2-627

Altered Expression of Members in the Wnt/ß-Catenin Signaling Pathway in Benign and Malignant Human Adrenocortical Tumors.

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Background: The molecular pathogenesis of benign and malignant adrenocortical tumors (ACT) is incompletely clarified. Alterations in the Wnt/beta-catenin signaling pathway are common in human tumors, and mutations in the beta-catenin gene has been described in both adrenocortical adenomas (ACA) and carcinomas (ACC). Other members of this pathway, however, have not been systematically studied in ACT.
Methods: Using specific antibodies and immunohistochemistry, the protein expression of beta-catenin, c-myc, APC, DKK1, DKK4 and the ligands Wnt1 and Wnt3, were examined in 10 normal adrenal cortex specimen and 80 ACT (20 aldosterone-producing ACA, 20 cortisol-producing ACA, 20 non-functioning ACA and 20 ACC). mRNA expression was determined by quantitative RT-PCR for c-myc, DKK1, DKK4, Wnt1 and Wnt3, and standardized to GAPDH, in the same cohort. Data are expressed as mean ± SD for triplicate experiments.
Results: Significant differences in both gene and protein expression was noted between normal adrenal cortex, benign and malignant ACT. Protein expression of beta-catenin, c-myc, and Wnt1 was higher in ACC vs. ACA, whereas no difference was noted in APC, DKK1, DKK4 and Wnt3. Functioning ACA displayed higher levels of beta-catenin, but reduced expression of DKK1 vs. non-functioning ACA. mRNA expression of DKK1 and c-myc was upregulated (10.5±2.6 fold, $p<0.005$, and 28±4.3 fold, $p<0.05$, respectively) and DKK4 downregulated (13.8±1.3 fold, $p<0.05$) in ACC vs. ACA, whereas Wnt1 and Wnt3 displayed similar expression levels. Functioning ACA exhibited higher DKK1 and Wnt3 (23.4±4.7 fold; $p<0.05$, and 18±0.98 fold; $p<0.005$, respectively), but lower DKK4 mRNA levels (8.6±1.7 -fold, $p<0.05$)
Conclusions: Significant differences in both mRNA and protein expression of members in the Wnt/beta-catenin signaling pathway was identified in human ACT. Altered expression patterns were noted both between benign and malignant as well as functioning and non-functioning ACT. Given the increasing evidence of the role of this pathway in the development of adrenal tumors, these molecules may present potential diagnostic and therapeutic targets in ACT.

Nothing to Disclose: PB, PH, GA, GW, TC

P2-628

Abundant Expression of Ovary-Specific Acidic Protein in the Midgestation Human Fetal Adrenal Gland: Implications for Fetal Adrenal Androgen Biosynthesis.

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Ovary-specific acidic protein (OSAP) is a novel molecule discovered in a project searching for ovary-selective genes in mice (1). We have recently investigated the expression profile of OSAP in human and murine adult tissues, and found out that OSAP is almost selectively expressed in steroidogenic tissues (2). However, its expression in fetal tissues remains unknown. In this study, we investigated the expression profile of OSAP in midgestation human fetal tissues. Real-time quantitative RT-PCR (qPCR) demonstrated that OSAP expression was almost limited to steroidogenic fetal organs (values expressed as ratio to ß-glucuronidase [%]: adrenal, 258; testis, 122; ovary, 24; other tissues, < 2), consistent with our previous results for human adult organs (2). As the highest OSAP expression is observed in the human fetal adrenal gland (HFA) that consists primarily of the outer, definitive zone (DZ) and inner, fetal zone (FZ), we examined zonal expression of OSAP by laser-capture microdissection coupled with qPCR. The results revealed that OSAP mRNA is more abundant in the steroidogenic FZ of the HFA. As FZ cells produce copious amounts of dehydroepiandrosterone sulfate (DHEAS), OSAP may be implicated in adrenal androgen synthesis. To explore this possibility, OSAP expression was transiently silenced in the human steroidogenic adrenocortical NCI-H295A cells that express OSAP mRNA at levels comparable to those in the human adult and fetal adrenals. Morpholino antisense oligonucleotides were used to suppress OSAP expression. OSAP knockdown in NCI-H295A cells resulted in a decrease in DHEAS secretion by 4-fold at 24 h. Our studies, therefore, indicate that human OSAP, a mitochondrial protein, is expressed almost exclusively in steroidogenic organs of the human fetus, with highest expression in the adrenal gland. The more abundant OSAP expression in the inner, steroidogenic FZ of the HFA suggests that OSAP is likely implicated in fetal adrenal androgen synthesis. This assumption was supported by OSAP knockdown experiments using human adrenocortical cells. OSAP may play a role in fetal adrenal androgen biosynthesis, very likely through involvement in the mitochondria-associated processes of the steroidogenic pathway.

(1) Hennebold JD et al., Endocrinology 2000; 141: 2725
(2) Matsumoto T et al., Endocrinology 2009; 150: 3353

Sources of Research Support: Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science (No. 21591423) awarded to HI.

Nothing to Disclose: HI, KM, TM, AK, KD, MM, MT, RBJ

P2-629
Genetic Variants in the Glucocorticoid Pathway.

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Cortisol is an unique glucocorticoid (GC) essential for maintaining human life. There are several steps from the biosynthesis to the action of cortisol. 17α-hydroxylase (encoded by *CYP17*) is essential for cortisol production in adrenal zona fasticulata, and 11β-hydroxylase (*CYP11B1*) mediates the final step of cortisol production. After secreting into general circulation, local modulation of cortisol regeneration at a tissue level is employed by 11β-hydroxysteroid dehydrogenase (*HSD11B1*) and NADPH supplying hexose-6-phosphate dehydrogenase (*H6PD*). Following this prereceptor enhancing mechanism, cortisol reaches the GC receptor (*NR3C1*). Variations in the GC action have been implicated in the etiology of common diseases such as hypertension, diabetes, obesity, etc. Here we investigated common polymorphisms in a series of genes on the GC pathway in 1442 subjects including 486 hypertensives and 368 type 2 diabetes, and analyzed their associations with diseases and clinical measures.
No allelic difference of polymorphisms with disease status was detected. The cc genotype of *CYP17* His46c/t was frequent in hypertensives (P= .025), and the gg genotype of *HSD11B1* Int4_1345t/g was less frequent in type 2 diabetes (P= .021). Plasma cortisol was higher in the cc genotype of *HSD11B1* Int4_1705c/t. As regards intermediate or clinical phenotype, the QQ genotype of *CYP11B1* R43Q had higher urinary cortisol excretion and lower plasma insulin, but this genotype had lower visceral versus subcutaneous fat volume. The gg genotype of *HSD11B1* Int4_1345t/g had higher HDL cholesterol and lower visceral versus subcutaneous fat volume, suggesting this minor genotype having anti-metabolic syndrome property. Plasma ACTH and HbA1c was higher in the QQ genotype of *H6PD* R453Q. Total cholesterol was higher in the aa genotype of *H6PD* Ala212g/a.
Although the association of polymorphisms with diseases were not significant, common variations in the genes involved in the synthesis and the metabolism of cortisol are associated with blood pressure homeostasis, glucose tolerance and lipid metabolism.

Nothing to Disclose: TM, TS, JT, MM, KK

P2-630

The Role of TLR4 in Tumorigenesis of the Human Adrenal Cortex.

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Adrenocortical carcinoma (ACC) is a rare tumour associated with poor prognosis. Toll-like receptor 4 (TLR4) together with CD14 and MD2 molecules are cellular sensors for bacterial endotoxin (LPS), and are normally expressed in cells of the innate and adaptive immune system. Recently, the expression of a functional TLR4 has been found in various human tumours and has been correlated with increased or decreased proliferation, apoptosis and resistance to chemotherapeutical agents.
Since functional TLR4 is also expressed in the human adrenocortical cells the aim of the present study was to investigate its expression, signalling and function in the process of tumorigenesis in the human adrenal cortex.
First, the expression of TLR4, CD14 and MD2 was analyzed in several adrenocortical carcinomas (n=8), adenomas (n=8) and in permanent adrenal tumour cell lines using real-time PCR, immunohistochemistry and Western blot techniques. Second, the functionality of TLR4 was assessed by performing a comprehensive analysis of TLR-related signalling pathways after stimulation with bacterial endotoxin. Finally, the effect of TLR4 and CD14 overexpression on apoptosis and viability in adrenocortical carcinoma cells was evaluated using TUNEL- and MTS- based assays.
Real-time PCR as well as immunohistochemical analysis of human adrenocortical tumour samples and SW13, NCI-H295R and HAC15 cell lines, revealed significant downregulation of TLR4, MD2 as well as CD14 on mRNA and protein level, respectively. Moreover, Western blot analysis has shown that LPS stimulation did not activate any known TLR-related signalling pathways such as: MAPKs, AKT and NF-κB. This suggests that TLR4 system is not only downregulated but also inactive in adrenocortical carcinoma cells. An overexpression of TLR4 and CD14 in the NCI-H295R cells partially restored the LPS signalling but also decreased viability of NCI-H295R cells which correlated with an increased basal apoptotic rate as compared to MOCK transfected cells.
Taken together, our results demonstrate that significant reduction in the TLR4 expression and signalling is a novel feature of adrenocortical carcinomas which may be an important step in the process of adrenal cortex tumorigenesis.

Nothing to Disclose: WK, PT, ME-B, SRB

P2-631

microRNA Expression Profiling of Non-Tumorous Adrenal Tissue and Modulation of 11β-Hydroxylase (CYP11B1) & Aldosterone Synthase (CYP11B2) Genes by miR-24.

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The CYP11B1 and CYP11B2 genes encode 11β-hydroxylase and aldosterone synthase which catalyse the production of cortisol and aldosterone, respectively, and have been implicated in the development of hypertension. For this study we wished to investigate the role of microRNAs (miRNAs), a novel class of post transcriptional gene regulators. We propose a regulatory role for miRNA and have generated a profile of human adrenal miRNAs to study. We have now investigated the effect of one adrenal miRNA, miR-24, on the CYP11B1 and CYP11B2 genes in vitro.
miRNA expression was measured in four non-tumorous human adrenal glands using µParaflo® technology microarray and confirmed by qRT-PCR. Putative miRNA-binding sites located in the 3' UTR of the CYP11B1 and CYP11B2 genes were identified using four bioinformatic databases (microCosm, microRNA.org, TargetScan and miRanda). The human adrenocortical cell line, H295R, was transfected with miR-24 pre-miR and a control, scrambled pre-miR. 48h post-transfection mature miR-24, CYP11B1 and CYP11B2 mRNA was measured by qRT-PCR and steroid secretion quantified by liquid chromatography with tandem mass spectrometry.
Cross-referencing of microarray expression and bioinformatic data identified 23 adrenal miRNAs predicted to bind putative sites in CYP11B1 and 16 predicted to bind CYP11B2; 13 of these miRNAs were common to both genes. Transfection of H295R cells with miR-24 pre-miR produced a significant increase in cellular levels of mature miR-24, in a concentration-dependent manner ($p<0.001$). CYP11B1 mRNA levels were reduced by 36.1 ± 5.6% ($p<0.05$) and CYP11B2 mRNA by 36.4 ± 7.3% ($p<0.05$), relative to scrambled pre-miR controls. The level of secreted cortisol was reduced by 19.6 ± 4.7% ($p=0.07$) and aldosterone by 15.9 ± 3.1% ($p<0.05$).
Our microarray and bioinformatic data have identified several candidate miRNAs that may be involved in the regulation of the CYP11B1 and CYP11B2 genes, including miR-24. This study is the first to demonstrate that CYP11B1 and CYP11B2 are subject to regulation by miRNAs and, furthermore, can be modulated by a single miRNA species. Given the pivotal role of these genes in adrenal pathophysiology and hypertension, such regulation may prove to be an important therapeutic target.

Nothing to Disclose: SW, SMM, PMS, MI, RF, JMCC, ED

P2-632

Hyperaldosteronism Does Not Account for Renal Mineralocorticoid Receptor Down Regulation at Birth: Lessons from Aldosterone Synthase Deficient Mice.

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Sodium wasting is a major problem during the neonatal period, most notably in preterm infants, responsible for failure to thrive and dehydration. We have previously demonstrated that this impaired sodium reabsorption relies on a physiological renal aldosterone resistance at birth, with functional signs of hypoaldosteronism contrasting with high plasma levels of aldosterone and renin in newborns (Martinerie, Pediatric Res, 2009, 66:323). This hormonal unresponsiveness is related to low renal mineralocorticoid receptor (MR) expression at birth, both in humans and mice, which increases in the postnatal period (Martinerie, Endocrinology, 2009, 150:4414). To investigate whether neonatal hyperaldosteronism is responsible for renal MR down regulation, we analyzed MR expression in the kidneys of newborn (D0) and eight postnatal day (D8) mice, generated from aldosterone synthase deficient (AS-/-) or heterozygous mice, in order to compare AS-/- mice exposed or non exposed in utero to trans-placental aldosterone, with wild type mice. Analysis of MR mRNA expression, using quantitative PCR, showed a significant renal MR down regulation at birth at variance to D8, both in AS-/- and wild type mice. However, no statistical difference was observed at birth between AS-/- and wild type mice, thus demonstrating that MR regulation is independent of aldosterone in the neonatal period. In sharp constrast, aldosterone was fully required for optimal renal induction of the alpha subunit of the epithelial sodium channel, one of the main MR target genes, crucial for renal tubular sodium reabsorption. These results are in accordance with sodium wasting observed in AS deficient children or infants carrying heterozygous inactivating MR mutations, and underscore the importance of both the ligand and the receptor for a functional mineralocorticoid signaling pathway to maintain sodium homeostasis in the postnatal period.
In sum, we have demonstrated that aldosterone has no significant impact on renal MR expression at birth but is rather pivotal for appropriate hydroelectrolytic balance in the postnatal period. We are currently investigating the molecular mechanisms involved in MR expression by using primary cultures of newborn mouse kidney cells and the differentiated cortical collecting duct KC3AC1 cells. Understanding of MR regulatory mechanisms could ultimately lead to new therapeutic strategies for the management of sodium loss in preterms and neonates.

Sources of Research Support: Inserm, University Paris-Sud,and fellowship from Assistance Publique-Hopitaux de Paris (to LM).

Nothing to Disclose: LM, SV, HSK, JML, ML

P2-633

Combined Transcriptome and Pathological Analysis Reveals Subgroup Structure in Aldosterone Producing Adenoma.

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Primary aldosteronism (PAL) is the most common form of secondary hypertension (HT), with a prevalence of ~6% of all hypertensive patients; the prevalence increases to 20% in resistant hypertension. Among subtypes of PAL, aldosterone producing adenoma (APA) and bilateral hyperplasia (HP) are of equivalent prevalence, together accounting for 95% of diagnosed cases. Yet, the molecular etiology of PAL remains elusive.
In order to study the mechanisms involved in the development of PAL and to investigate their genetic determinants, we have developed an integrated genomic and genetic approach.
We have established the transcriptional profiles of 138 APA (COMETE network). Analysis of the expression of markers of aldosterone production demonstrated that 89% of APA express high levels of Cyp11B2 compared to 11 control adrenals. Unsupervised clustering of transcriptional profiles revealed two major clusters of PAL [adenoma group A (Ad_A), adenoma group B (Ad_B)]. Subtraction analysis identified 1125 and 2918 differentially expressed genes in Ad_A and Ad_B, respectively, compared to controls; 90% of them were underexpressed in both adenoma groups. An additional subgroup structure in Ad_A was identified by candidate signaling pathway analysis including G-protein coupled receptors, Ca2+ signaling, steroid biosynthesis and angiogenesis.
Immunohistochemical and in situ hybridization in tumoral and extratumoral tissues demonstrated that: i) 68% of APA samples contained multiple nodules; ii) adrenals from Ad_A expressed high levels of Cyp11B2 in the principal nodule, associated with functional HP of the adjacent zona glomerulosa (ZG) (continuous or focal) in 93% of cases; iii) adrenals from Ad_B presented unique atypical large nodules (>3cm), with no expression of Cyp11B2, but functional HP of the adjacent ZG.
Our results demonstrate that different subgroups of APA are distinguished by their transcriptional profiles. In Ad_B, increased aldosterone production is due to functional HP of the ZG, suggesting unilateral adrenal HP rather than APA. Further analysis should allow identifying dominant transcriptional changes between different subsets, opening new perspectives for the development of new diagnostic tools and therapeutic targets.

Nothing to Disclose: SB, JFGD, BS-C, TM, EL, HL, LA, PFP, XJ, AB, MCZ

P2-634

Studies of Adrenal Gland Volume and Aldosterone Production in Normal Volunteers.

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Hypertension is a major cardiovascular risk factor which accounts for 7 million premature deaths per annum. The significant role of the renin-angiotensin-aldosterone system (RAAS) in hypertension and cardiovascular disease highlights the importance of the adrenal gland in these disorders. The adrenal cortex is a major target organ for angiotensin II and the site of production of aldosterone. Measurement of adrenal gland volume could therefore provide a new method of assessing activity of the RAAS as well as a novel marker of hypertension and a method of assessing the effectiveness of anti-hypertensive treatment. The aim of this study was to explore the role of MRI in measuring adrenal volume and investigate whether adrenal volume is related to mineralocorticoid status.

Twenty healthy male subjects underwent non-contrast MRI scanning of abdomen using a 1.5T whole body scanner. Axial T1-weighted images of abdomen were recorded with a slice thickness of 1.2mm. Volume of the left adrenal was calculated using Argus application software by one operator on 2 occasions separated by at least 1 month. Subjects underwent measurement of plasma aldosterone and cortisol (analysed using LCMS technology) after 15 minutes of supine rest as well as blood pressure evaluation (mean of 3 readings).

The mean (+/- SEM) left adrenal volume (calculated from mean of 2 readings) for 20 normotensive subjects was 4.9ml (+/-0.2). The intraobserver variation was low; limit of agreement (mean difference +/- 2SD) was 0.06ml +/-1.4, a t-test of difference showed no evidence of bias ($p=0.713$) and the 2 sets of readings showed strong correlation ($r=0.8$, $p<0.0001$). Mean adrenal volume correlated with plasma aldosterone ($r=0.504$, $p<0.03$) and diastolic blood pressure ($r=0.5$, $p<0.03$) but not plasma cortisol.

These preliminary data demonstrate that MRI provides a reliable and precise method of measuring adrenal volume and adrenal volume may relate to blood pressure and/or mineralocorticoid status. Further studies, in a larger population, are underway to validate these data, explore other methods of adrenal volume analysis as well as explore the association of blood pressure and variation in adrenal corticosteroidogenic genes with adrenal volume.

Nothing to Disclose: EMF, NM, MI, RF, JMC

P2-635

Genetic Profiling of Omental Adipose Tissue in Patients with Primary Aldosteronism and Candidate Genes for Cardiometabolic Complications.

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Primary aldosteronism (PA) is associated with a higher prevalence of metabolic syndrome and of glycemic abnormalities which can be improved by removing aldosterone excess. Previous studies have shown that aldosterone levels positively correlate with visceral obesity and inversely with insulin sensitivity but the exact relationship between aldosterone, glucose metabolism and insulin action remains mostly unresolved. Aim of the study was to evaluate the expression of genes involved in glucose metabolism, insulin action and inflammatory cytokines in omental adipose tissue from patients with aldosterone producing adenoma (APA) compared with control subject. We collected omental adipose tissue during surgery for adrenalectomy in patients with APA (n=17) and during adrenalectomy for non functioning adrenal adenoma (n=10) or in healthy subjects undergoing colecystectomy (n=4). We first performed a microarray analysis in a subset of patient, using Affymetrix Human Genome U133 Plus 2.0 array, and then validated the results by real time PCR, analyzing few selected genes: hexose-kinase 1, IL-1R1, IL-6, cholesterol-25-hydroxylase, lipoprotein lipase, omentin, visfatin. Gene expression studies showed that omental adipose tissue samples of patients with PA have a significantly higher expression of IL-6, a proinflammatory cytokine involved in insulin resistance and vasculopathic disease states. Interleukin-6 mRNA expression was indeed 10-fold higher in patients with PA compared with control subjects ($p=0.010$). Although not statistically significant, a linear relationship has been observed between BMI and IL-6 expression. Moreover, IL-6 expression values were positively correlated with total cholesterol values ($r^2=0.414$, $p<0.05$). As for insulin sensitizing adipokines omentin and visfatin, we found no differences in terms of gene expression between patients with PA and patients with non functioning adrenal adenoma. However, regression analysis showed significant correlations between both, omentin and visfatin, and HDL cholesterol levels ($r^2=0.381$, $p<0.05$ and $r^2=0.326$, $p=0.05$, respectively). In conclusion our data suggest that the significantly higher expression of IL-6 in patients with PA can contribute, at least in part, to the pathogenesis of the cardiometabolic syndrome observed in these subjects.

Nothing to Disclose: VR, FT, VdT, GA, MB, GG

P2-636

Steroidogenic Enzyme Transcript Levels in Aldosterone-Producing Adenomas and Adjacent Normal Adrenal Tissue.

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Background: Primary aldosteronism (PA), as a result of the inappropriate aldosterone levels, has profound adverse effects on the cardiovascular and renal systems. Aldosterone production in PA occurs under low-renin conditions and the mechanism that maintains production of aldosterone in PA remains unknown.
Objective: This study was designed to compare the expression profiles of enzymes involved in adrenal steroid hormone production between aldosterone-producing adenomas (APA, a common cause of PA) and their adjacent normal adrenal tissues (AN).
Design: A retrospective study.
Patients: Eight APA patients, diagnosed by adrenal vein sampling were included in this study.
Methods: Total RNA was extracted from APA and AN for cDNA generation respectively. Then the cDNA was analyzed by microarray and real time PCR (qPCR). The microarray data was analyzed by GeneSpring GX 11 with paired t-test and fold change calculation for 11 genes involved in the production of all classes of adrenal steroids.
Results: Microarray analysis indicated 10.92±5.61 fold greater expression of CYP11B2 (aldosterone synthase) in APA compared to AN ($P=0.0001$). AKR1C3 (17-beta-hydroxysteroid dehydrogenase type 5), CYP17 (steroid 17 alpha-hydroxylase), and CYB5 (Cytochrome b5) showed significantly lower expression in APA ($P<0.05$). These results were further confirmed by qPCR. No significant differences were observed for the expression of cholesterol side-chain cleavage, steroidogenic acute regulatory protein, 21 hydroxylase, 11 beta-hydroxylase, 3 beta-hydroxysteroid dehydrogenase, cytochrome P450 reductase or steroid sulfotransferase.
Conclusion: Current data suggests that elevated production of aldosterone in APA is associated with increased expression of CYP11B2, and reduced expression of other steroidogenic enzymes normally associated with adrenal androgen production including AKR1C3, CYP17, and CYB5.

Nothing to Disclose: TW, FS, RM, YN, HS, WER

P2-637

Polymorphisms in the 5' Untranslated Region of the Aldosterone Synthase Gene Can Alter Transcription Factor Binding.

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Background: The final step in the production of aldosterone is undertaken by the enzyme aldosterone synthase, encoded by the gene CYP11B2. The CYP11B2 gene is polymorphic and variants within the gene and the regulatory region have been associated with hypertension and a phenotype of relatively higher level of aldosterone and its metabolites. However despite their association with hypertension, to date none of the polymorphisms in the regulatory region of CYP11B2 have been shown to produce a functional alteration in transcriptional activity. A number of novel polymorphisms in the regulatory region of CYP11B2 have been identified. The aim of this study was to identify polymorphisms that may cause a change in binding of transcription factors, leading to altered gene expression and the phenotype of hypertension with an increased aldosterone to renin ratio.
Aims and Methods: An initial study to confirm a high degree of linkage disequilibrium in the regulatory region of the CYP11B2 gene was undertaken by analysis of sequencing data from 200 individuals. Polymorphisms were screened for putative transcription factor binding sites using a bioinformatics database (Transfac©). Based on this data, a polymorphism at position -1651 (C/T) was selected for initial binding studies. Electromobility shift assays performed using nuclear extracts from human adrenal cell line (H295R). The C (wild type) and T (mutant) showed different patterns of binding and nuclear extracts were incubated with 5'biotinylated double-stranded DNA probes and streptavidin-agarose beads in order to identify the bound proteins. The protein-DNA complexes were separated on SDS-PAGE gel and following trypsin digestion peptides were analysed by tandem mass spectrometry. Two peptides were identified which bound to the T oligo only; Ape1 which functions as a redox factor, maintaining transcription factors in an active reduced state, and HNRNPK, which interacts with RNA polymerase II transcription machinery, and stimulates transcription.
Conclusion: We have confirmed differential binding of adrenal nuclear protein extracts to contrasting alleles of a polymorphism at position -1651 in the promoter of CYP11B2 and identified candidate proteins. This suggests a mechanism by which polymorphisms in the regulatory region of CYP11B2 may produce a phenotype of relative aldosterone excess and hypertension.

Sources of Research Support: MRC Clinical Training Fellowship (FMcM). MRC Programme Grant (ED, WS, JMC).

Nothing to Disclose: FM, WS, ED, JMC

P2-638

Immunohistochemical Characterization of Mast Cells in Aldosterone-Producing Adenoma.

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We have previously shown that, in the normal adrenal, 5-hydroxytryptamine (5-HT) locally produced by mast cells, stimulates aldosterone production in a dose-dependent manner through a paracrine mechanism involving activation of 5-HT4 receptor (5HT4R). In agreement with this data obtained in vitro, administration of the 5-HT4R agonist cisapride to healthy volunteers was found to induce an important increase in plasma aldosterone levels. Cisapride also stimulates aldosterone production in patients with primary aldosteronism. Moreover, 5-HT-positive cells were observed in aldosterone-producing adenoma (APA) tissues and 5-HT4R mRNAs were markedly overexpressed in APAs in comparison with the normal adrenal cortex. All these results suggest that 5-HT produced by adrenal mast cells could be involved in the physiopathology of APAs. The aim of this work was to investigate the presence of mast cells in APAs. Mast cells were characterized by specific histological and immunohistochemical stainings using RAL555, anti-tryptase, anti-chymase and anti-CD117 antibodies. Quantification of mast cells in APAs revealed a high density of mast cells of MCT type in most of the tissues. Tryptase-positive mast cells were mainly observed in peritumoral adrenal tissue surrounding adenomas. Tryptase labeling was also detected in some steroidogenic cells in the vicinity of mast cells, suggesting interactions between these two cell types. In order to explore this hypothesis, adrenocortical cells will be cultured in the presence of the mast cell lines HMC-1 and LAD2. In most organs, mast cells establish contacts with sympathetic nervous fibers. Using double immunostaining of APA tissues with tryptase- and protein S100, substance P or CGRP-antibodies, we showed: i) the presence of protein S100-positive-fibers in peritumoral adrenal tissue; ii) interactions between nervous fibers and mast cells suggesting that the secretory activity of mast cells is controlled by neural inputs. Altogether, our results show the presence of numerous mast cells in adrenal tissues from patients with APAs. These cells are mainly localized in peritumoral adrenal tissue surrounding adenoma. APAs also contain nervous fibers which could interact with mast cells. In conclusion, adrenal mast cells, which physiologically produce 5-HT, could be involved in the physiopathology of primary aldosteronism.

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Nothing to Disclose: CD, EL, VP, FG, LM, TM, PFP, MCZ, HL

P2-639

Hyperuricemia May Mediate the Increase in Intra-Renal Renin-Angiotensin System Activity in Obesity.

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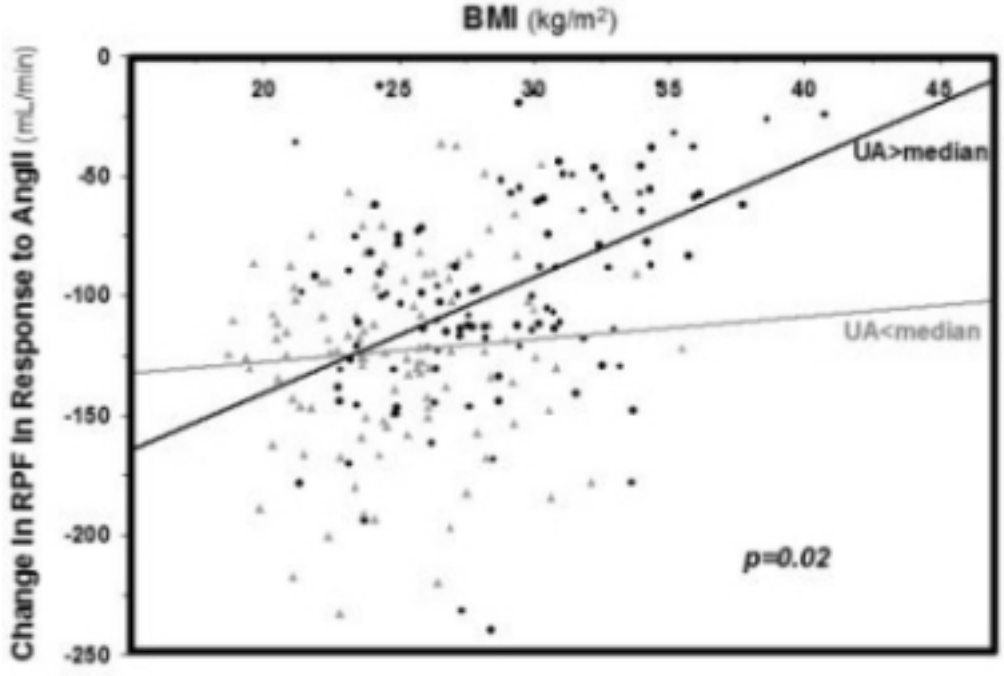
BACKGROUND: Increased activity of the renin-angiotensin system (RAS), particularly the renal RAS, has been implicated in obesity-related hypertension and renal disease. Hyperuricemia is common in obesity, and we have previously shown that hyperuricemia is associated with increased activity of the intra-renal RAS (1). We hypothesized that uric acid may mediate intra-renal RAS activity in obesity. METHODS: We retrospectively analyzed data of 228 subjects, studied in high sodium balance, in whom the renal plasma flow (RPF) response to angiotensin II (AngII) was measured. This response is known to be *inversely* proportional to endogenous intra-renal RAS activity. RPF, measured via para-aminohippurate clearance, was measured before and after an infusion of AngII (3 ng/kg/min). Subjects were characterized as having a serum uric acid above or below the median (5.1 mg/dL). RESULTS: Increasing BMI predicted a blunted RPF response to AngII (r= -0.40, β= -4.1, p<0.0001), even after multivariable adjustment (β= -1.2, p=0.02), consistent with increased intra-renal RAS activity in obesity. In stratified analyses, obesity was associated with a blunted RPF response to AngII only in subjects with uric acid levels above the median (r= -0.41, p<0.0001), in contrast to subjects with uric acid below the median (r= -0.10, p=0.39). An adjusted interaction model confirmed that this disparity in responses mediated by uric acid levels was significantly different (p=0.02) (**Figure**). DISCUSSION: These findings suggest increased intra-renal RAS activity in obesity in the setting of hyperuricemia; consistent with our hypothesis that uric acid may mediate intra-renal RAS activity in obesity. Whether uric acid lowering therapy in obesity dampens RAS activity and reduces the risk for vascular and renal disease warrants investigation.



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Nothing to Disclose: AV, JSW, TSP

P2-640

Effect of DNA Methylation on Aldosterone Synthase Gene.

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Aldosterone production from hypertrophic or failing hearts is increased and has critical roles in the development of cardiac hypertrophy or failure. Aldosterone synthase encoded by CYP11B2 catalyzes final two steps from corticosterone to aldosterone. The Ad1(-71/-64) and Ad5(-129/-114) *cis*-acting elements are required for the transcriptional regulation of CYP11B2. These elements contain CpG dinucleotides, which are target sites for DNA methylation. CpG methylation ratios of the Ad1 and Ad5 in aldosterone-producing adenoma (APA) (n=9) were about half those of adrenal tumor (n=5), normal adrenal (n=4) or normal artery (n=3). Inverse significant nonparametric correlations were found between CpG methylation ratios and the CYP11B2 gene expression levels. Interestingly, the Ad1 and Ad5 in hypertrophic cardiomyopathy were even more hypomethylated than those in APA. Analysis of the CYP11B2 promoter fused to a reporter gene showed that CpG methylation within its promoter completely abolished CYP11B2 promoter activities. Promoter constructs with incomplete CpG methylation weakly responded to stimulation by angiotensin II, KCL or cAMP, suggesting that the CYP11B2 promoter activity was dependent upon CpG methylation. NoShift transcriptional factor assays demonstrated that CpG methylation significantly decreased CREB binding to the Ad1 by 90% and NURR1 binding to the Ad5 by half in nuclear extracts from adrenocortical H295R cells. Likewise, CpG methylation significantly increased methyl-CpG binding protein 2 (MECP2) binding to the Ad1 *in vitro*. Chromatin-immunoprecipitation-quantitative PCR showed that MECP2 interacted strongly with methylated Ad1 and weakly with methylated Ad5. Other methyl-CpG binding repressors including MBD1 and 2 appeared to bind to neither Ad1 nor Ad5. Taken together, CpG methylation repressed human CYP11B2 promoter activity by decreasing binding of activators as CREB and NURR1 and increasing binding of a repressor as MECP2. Hypomethylation of those CpG dinucleotides may explain increase in aldosterone production from hypertrophic hearts.

Sources of Research Support: Grant-in-Aid for Young Scientists (B) (#21790889) from JSPS.

Nothing to Disclose: MD, FW, TY, SK, SM, MK, MO, YC, KU, MY, YM, MN, YT

P2-641

Regulation of the Late Pathway of Aldosterone Biosynthesis.

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Aldosterone (aldo) is synthesized in the adrenal zona glomerulosa by a series of enzymatic steps. Regulation occurs at two steps of the pathway: early, with the regulation of cholesterol transport into the mitochondria by StAR protein, and late, with the entry of deoxycorticosterone (DOC) into the mitochondria where it is converted to aldo by the CYP11B2, or aldo synthase, enzyme. It is known that angiotensin II (A-II), potassium and sodium depletion stimulate the late pathway by increasing CYP11B2 transcription and aldo synthase expression. We present data indicating that in addition to transcriptional regulation of aldo synthase, the rate of transfer of DOC into the mitochondria is also regulated.
H295R cells were incubated with or without 10-8M A-II for 24 hrs. Cells were homogenized and centrifuged to separate mitochondria and cytosol. Mitochondria were incubated for 20 min with 10 µM DOC and a system to generate NADPH in the presence of buffer, cytosol from control cells, or cytosol from A-II stimulated H295R. Cytosol from stimulated cells contained aldo below the sensitivity of the assay. There was a significantly greater conversion of DOC to aldo by mitochondria incubated with cytosol from A-II stimulated cells compared to those incubated with cytosol from control H295R cells.
We also infected H295R cells with lentivirus carrying the human CYP11B2 cDNA and CMV promoter to produce cells that stably and constitutively expressed aldo synthase. H295R or H295R-hAS cells were incubated for 3 hrs with or without 10-7M A-II in the presence of 0, 3, 10 and 30 µM DOC and 30 µM cyanoketone (inhibitor of the 3α-hydroxysteroid dehydrogenase enzyme). After 3 h H295R cells incubated with various amounts of DOC, with and without A-II, produced barely detectable amounts of aldo. H295R-hAS cells incubated with A-II produced significantly more aldo than H295R-hAS not incubated with A-II. We previously showed that detectable increases in aldo synthesis by H295R after A-II stimulation occurs after 6 h. In the H295R-hAS cells in which the enzyme was not the limiting factor, the increase occurred in half the time. These results suggest that the regulation of the late pathway of aldo biosynthesis involves the facilitated transfer of DOC into the mitochondria, as well as transcriptional regulation of aldo synthase. The nature of the factor involved in the transfer of DOC into the mitochondria is unknown.

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Nothing to Disclose: CVM, MP, EPG-S, CEG-S

P2-642

Adrenal Venous Sampling in Primary Aldosteronism: The Value of Bilateral Simultaneous Sampling before and after Bolus Stimulation with ACTH.

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Background: The evaluation of primary aldosteronism (PA) includes adrenal CT to detect large masses and bilateral adrenal venous sampling (AVS) to establish the unilateral or bilateral etiology of PA, defining surgical or pharmacological management (1). Although AVS is the gold standard for lateralization of aldosterone secretion (2, 3), many technical aspects are not standardized. Current protocols for AVS differ in terms of ACTH stimulation (basal or stimulated sampling) and how it is performed (bolus or continuous infusion of ACTH; simultaneous bilateral or sequential sampling) (1, 4, 5).
Objective: To compare the interpretation of bilateral simultaneous AVS between basal state and following bolus administration of ACTH.
Patients and Methods: Bilateral simultaneous AVS for aldosterone and cortisol were performed 5 and 0 min before and 5, 10 and 15 min after ACTH (250 µg iv, bolus) in 92 consecutive patients. The sequential technique was simulated by analyzing basal data from the right AVS performed 5 min before the left AVS. Selectivity of adrenal vein catheterization was evaluated by the adrenal/peripheral cortisol ratio (Ca/Cp). Lateralization of aldosterone hypersecretion was defined as an adrenal aldosterone/cortisol ratio (A/C) >4 the opposite side.
Results: Failure of adrenal vein catheterization occurred in 12 (13%) AVS, however 8 of these were successfully repeated. Basal catheterization was not selective (Ca/Cp <2) in 30 AVS (34%) for simultaneous and 34 (38%) for sequential analysis. Stimulated simultaneous AVS was considered not selective in 12 (13%) or 16 (18%) patients respectively for Ca/Cp >2 or >10. The analysis of 77 selective AVS revealed that sequential and simultaneous basal AVS had discordant lateralization in 18 (23%) patients. After ACTH stimulation, the basal AVS lateralization did not change in 55 (71%), was modified to bilateral in 8 (10%), left lateralization in 11 (14%) and right lateralization in 3 (4%) patients. When cortisol-corrected aldosterone ratio was > 16 before ACTH, all AVS had basal ratio concordant with its respective stimulated ratios in terms of laterality.
Conclusions: Sequential AVS for PA may miss lateralization but does not affect selectivity. ACTH stimulation is helpful to determine selectivity, but can modify the assessment of lateralization of the aldosterone source. The combination of basal and ACTH-stimulated levels during AVS contributes to the determination of the subtype of PA.

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Sources of Research Support: Grant MT-13189 from the Canadian Institutes of Health Research.

Nothing to Disclose: TLM, PC, LMM, SG, IB, MD, GG, ET, AL

P2-643

Can We Differentiate Bilateral Adrenal Hyperplasia from Bilateral Adenomas in Primary Aldosteronism by Adrenal Venous Sampling?.

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Objective: The differential diagnosis between aldosterone-producing adenoma (APA) and idiopathic hyperaldosteronism (IHA) is critical to decide how to treat them in patients with primary aldosteronism (PA). It is well known that adrenal venous sampling (AVS) can only differentiate the unilateral disorder from the bilateral adrenal lesions. On the other hand, AVS, detecting the laterality of hypersecretion of aldosterone, can not differentiate bilateral hypersecretion of bilateral APA (Blt-APAs) from that of bilateral hyperplasia of IHA. To solve the problem, we try to develop a new method of supper-selective ACTH-stimulated adrenal venous sampling (SS-ACTH-AVS).
Methods: We performed SS-ACTH-AVS in 50 patients by using a strip-tip type 2.2 Fr micro-catheter (Koshin Medical Inc. Japan). Adrenal effluents were sampled at the central veins and at one or two tributaries of adrenal veins in each side.
Results: 29 patients with unilateral APA, 17 with IHA and 4 with Blt-APAs were clinically diagnosed by SS-ACTH-AVS. Unilateral adrenalectomy was performed in 19 patients with unilateral APA and 3 patients with Blt-APAs, and the diagnosis of APA were pathologically confirmed. In patients with unilateral APA, aldosterone concentrations in effluents sampled at the central and one tributary veins in the affected side of the adrenal were more than 1400ng/dl. In patients with IHA, aldosterone concentrations in effluents sampled at the central and all tributaries veins in each adrenal were more than 1400ng/dl. On the other hand, aldosterone concentrations 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.
Conclusion: The present data clearly demonstrated that SS-ACTH-AVS could definitely differentiate Blt-APAs from IHA. Thus, we should perform SS-ACTH-AVS especially in patients demonstrating bilateral adrenal lesions such as bilateral tumors and hypersecretion of aldosterone from bilateral adrenal glands.

Nothing to Disclose: NT, OM, SJ, MY, MK, SH

P2-644

Adrenal Venous Sampling Criteria for Surgically Remediable Primary Hyperaldosteronism.

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Background. Primary hyperaldosteronism (PHA) patients undergo simultaneous bilateral adrenal venous sampling (AVS) to identify surgically remediable hyperaldosteronism (SRA). Different AVS criteria may predict SRA. According to the Endocrine Society clinical practice guidelines (ESCPG), SRA is correctly predicted by a serum aldosterone concentration/cortisol concentration ratio (A/C) of the dominant adrenal vein (a.v.) to non-dominant a.v. (D:ND) > 4 following $ACTH_{1-24}$ stimulation (ACTHStim) (1). However, this criterion is validated in the largest studies by pathologic endpoints, not biochemical cure (2). Using an endpoint of biochemical cure, we test the hypothesis that AVS sensitivity can be increased by performing AVS pre- and post-ACTHStim and by using the criterion of a peripheral vein A/C to non-dominant a.v. A/C (P:ND) > 1. **Methods.** We retrospectively analyzed 57 consecutive PHA patients with successful bilateral AVS and duplicate sampling both before and 15-20 minutes after iv 250 mcg ACTHStim. Adrenalectomy had been offered to all patients with a mean P:ND > 1.0 either pre- or post-ACTHStim and to all patients with a mean D:ND ≥ 5.0 post-ACTHStim. The primary endpoint was biochemical cure defined as upright serum aldosterone concentration < 7 ng/dl or plasma renin activity > 2 ng/ml/hr, measured over 2 months post-adrenalectomy. **Results.** Of the 34 PHA patients predicted to have SRA, 26 elected adrenalectomy. In the 23 that returned for post-adrenalectomy testing, we correlated biochemical cure with the predictive criteria. There were 6 patients that did not meet the ESCPG adrenalectomy criterion; the D:ND was < 3.5 post-ACTHStim, but adrenalectomy had been performed since P:ND was > 1 either pre- or post-ACTHStim. Adrenalectomy cured 5 of 6 (83% specificity). Of the 19 patients with D:ND ≥ 5 plus P:ND > 1 post-ACTHStim, 16 were tested post-adrenalectomy; all were cured (100% specificity). One patient had a D:ND > 5, but with P:ND < 1.0 post-ACTHStim; this patient was cured. Two patients were identified only by AVS pre-ACTHStim; both were cured. The specificities of the different SRA predictive criteria were not statistically different. AVS pre-ACTHStim increased the SRA yield by 12%, and the criterion of P:ND > 1 pre- or post-ACTHStim increased the SRA yield by 29%. **Conclusions.** AVS sensitivity is increased with a non-significant decrease in specificity by performing AVS pre- and post-ACTHStim and by using the additional criterion of P:ND > 1.

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Nothing to Disclose: SK, MA, BET, SS, DCM, CDM

P2-645

Unilateral Adrenal Hyperplasia Is Not a Rare Cause of Curable Hypertension.

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Background The existence of unilateral hyperplasia (UAH) has been considered a rare cause of primary hyperaldosteronism (PA). Our objective was after defining diagnostic cut-off limits for aldosterone-renin ratio (ARR) and serum aldosterone to prospectively screen for PA in a non-selected (NSP) and selected hypertensive population (SP), to define the definitive cause of PA.
Methods We included 353 consecutive patients with hypertension; age 20 to 88 years, 165 women and 188 men from a university-based Hypertension and Nephrology Outpatient clinics, (123 SP patients) and two primary care centres, (230 NSP patients) from the same catch-up area.
S-aldosterone and plasma renin activity were measured and ARR calculated. Verifying diagnostic procedure was performed in patients with both elevated aldosterone and ARR. Patients diagnosed with PA were invited for CT-adrenals, 131I-chol-scintigraph and adrenal venous sampling (AVS) and offered endoscopic adrenalectomy (EA) when AVS found the disease to be unilateral.
Results After screening, 46 patients or 13% of the screened population (22.8% SP and 7.8% NSP) had serum aldosterone and ARR above the locally defined cut-off limits (0.43 mmol/l and 1.28 respectively). Twenty eight of these 46 patients were studied further with verifying diagnostic procedure and 18 patients were excluded due to concommitant illnesses or denied further investigations. After diagnostic verification, 20 patients (6%) had PA, (14.5% SP and 1.4% NSP).Nine patients had positive findings on CT-adrenals, 9 patients had negative finding. Three patients had positive findings on 131I-chol-scintigraphy and 14 negative. AVS, performed in 15 patients verified bilateral disease in 4 and unilateral disease in 10 patients. One AVS failed. aldosterone producing adenoma was found in 4 and unilateral adrenal hyperplasia (UAS) in 5 patients. One patient denied operation.
Conclusion The overall prevalence of PA was in agreement with previous studies. Prevalence was 3-fold in specialised hypertensive clinics as compared with primary care centers. The finding that UAH is a common cause of PA might be due to the fact that all patients were evaluated by AVS. CT and 131I-chol-scintigraphy were not helpful in the definition of lateralization of PA and to differentiate adenoma from AH. The study indicates the importance of AVS in the evaluation of PA.

Nothing to Disclose: HAS, MG, OA, RE, HH, AS, BW, GJ

P2-646

In Vivo Systematic Screening for Hormone Receptors in Primary Aldosteronism Reveals Frequent Aberrant Hormone Responsiveness.

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Background: 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) in unilateral tumors and bilateral macronodular adrenal hyperplasia (1). By analogy, some recent studies identified over-expression or function of several GPCRs as a potential cause for excess aldosterone production in some aldosterone producing adenomas (APA) and in bilateral idiopathic hyperaldosteronism (IH) (2-8).
Objective: To investigate patients with PA for aberrant regulation of aldosterone secretion in vivo.
Patients and Methods: 21 patients (8 F, 13 M) with PA (11 APA, 10 IH) were studied. An in vivo screening protocol performed under dexamethasone suppression included measurements of plasma aldosterone concentration (PAC), renin activity, cortisol, ACTH during upright posture, mixed meal or stimulation with ACTH, GnRH, TRH, vasopressin and metoclopramide or tegaserod. Additional tests (isoproterenol, LH, GIP, oral glucose) were performed in specific cases. PAC or cortisol increase over baseline > 50% was defined as a positive response.
Results: Data were analysed for 18 patients in whom ACTH was adequately suppressed by dexamethasone. 83% of the patients had at least one aberrant response of aldosterone. In ten patients, variable degree of ACTH-independent secretion of cortisol was present. Nine of 13 patients had a renin-independent PAC increase and 3/10 showed cortisol increase as well during upright posture. Following administration of 10 U of vasopressin, PAC increased in 10/16 patients and cortisol in 6. Seven of 15 patients increased PAC after GnRH, and one of them with LH. One patient had a positive response of PAC and cortisol to isoproterenol infusion. One patient had an increased in PAC following mixed meal, oral glucose and GIP infusion. TRH increased PAC in 1/14 patients. In addition to the expected increase in PAC, administration of metoclopramide or tegaserod produced aberrant response of cortisol in 3/9 subjects.
Conclusions: This study confirms that aberrant regulation of aldosterone is frequent in PA secondary to IH or APA; aberrant regulation of cortisol in addition to aldosterone can occur in some cases. The identification of aberrant hormone receptors could provide novel therapeutic strategies to treat PA with specific receptor antagonists.

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Nothing to Disclose: SG, TLM, LMM, IB, AL

P2-647

Correlation between Aldosterone to Renin Ratio (ARR) and Clinical Demographics in Primary Aldosteronism.

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Aim of the study: Primary aldosteronism (PA) is one of the most frequent causes of secondary hypertension. The widespread use of the aldosterone renin ratio (ARR) as a screening test led to a marked increase in the detection rate of PA. Dynamic endocrine testing is however recommended to confirm the autonomic hypersecretion of aldosterone in the ARR-positive patients. The aim of the study was to investigate whether all ARR-positive patients should be subjected to the confirmatory testing. We compared the clinical demographics and positive rate of confirmatory tests with ARR in patients with PA. Methods: Hypertensive patients were defined as screening positive if ARR was larger than 200 with plasma aldosterone concentration (PAC) in pg/mL and plasma renin activity (PRA) in ng/mL/h. Patients positive for ARR were subjected confirmatory tests including captopril challenge test (cut-off: ARR after 60min or 90min >200), furosemide upright test (cut-off: PRA after 2hr <2.0ng/ml/hr), and saline infusion test (cut-off: PAC after 4hr >85pg/ml). Total 30 patients showed positive results for at least two of the confirmatory tests and were subjected to the following analysis. The patients were divided into 2 groups with ARR less than 1000 (n=17) and equal to and greater than 1000 (n=13). We compared the clinical demographics including prevalence of hypokalemia, number of medication, PAC, PRA, tumor size and prevalence of positive ratio of confirmatory tests. Results: Prevalence of hypokalemia (76.9% vs. 17.6%) and positive ratio of all 3 confirmatory tests (77.8% vs. 10.0%) were significantly higher and PRA was significantly lower in the patient group with ARR>1000 than those with ARR<1000 (P<0.01). Number of medication, PAC and tumor size showed no significant difference between the 2 patient groups. Conclusions: The present study clearly demonstrated that ARR is definitely a useful marker for the extent of hyperaldosteronism. In addition, time consuming confirmatory testing could be skipped in those patients with ARR higher than 1000.

Nothing to Disclose: KN, TT, YU, SS, HN, TU, TT, AS, MN

P2-648

Clinical Outcome of Unilateral Adrenalectomy and Medical Therapy in Primary Aldosteronism.

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[Aim] Since primary aldosteronism (PA) shows many cardiovascular complications, early diagnosis and treatment is crucial. Treatment of PA consists of unilateral adrenalectomy (ADEX) for aldosterone-producing adenoma (APA) and medical therapy (MED) for idiopathic hyperaldosteronism (IHA). This study investigated prognosis of blood pressure (BP) and renal function by ADEX and MED in PA.
[Patients] Seventy-one patients with PA (20 APA and 51 IHA patients) were included in this study. The diagnosis of PA was performed based on the clinical practice guideline by the Japan Endocrine Society. Lateralization was performed by adrenal venous sampling. The patients were categorized into three groups according to their urinary aldosterone excretion under oral salt loading (low-aldo: <12μg/day, moderate-aldo: 12-20μg/day, high-aldo: >20μg/day). Unilateral ADEX was performed for 20 cases of APA, whereas MED was performed for 51 cases of IHA (eplerenone, n=30; spironolactone, n=17). BP and renal functions were compared during 6 months period.
[Results] **1) BP outcome:** Pretreatment systolic and diastolic BP levels were comparable between ADEX and MED groups (systolic 138±15 vs. 139±12, diastolic 85±8 vs. 83±10 mmHg). The systolic BP levels were effectively and similarly reduced after ADEX or MED in the low- and moderate-aldo groups (low: 134±2 vs. 130±14mmHg, moderate: 127±8 vs. 125±12 mmHg, respectively). In the high-aldo group, the systolic BP levels were reduced more effectively by ADEX than MED (125±13 vs. 136±11mmHg, respectively). **2) Renal function outcome:** Proportions of proteinuria were decreased at 6 months in all treatments (ADEX: 33% to 13%; MED: eplerenone 12% to 4%, spironolactone 33% to 20%). Pretreatment eGFR levels were comparable between ADEX and MED groups (70±18 vs. 72±12ml/min/1.73m^2). The eGFR levels of ADEX and spironolactone groups were significantly reduced (57±19 and 61±14 ml/min/1.73m^2, respectively), whereas those of eplerenone group was maintained (69±12ml/min/1.73m^2). [Conclusion] 1) Both ADEX and MED were similarly effective to control BP levels and proteinuria in PA patients with low-to-moderate urinary aldosterone excretion less than 20μg/day. 2) ADEX and spironolactone treatment reduced the eGFR levels at 6 months which may reflect pretreatment “masked chronic kidney disease” due to aldosterone-induced glomerular hyperfiltration. The beneficial effects of eplerenone treatment on eGFR levels remain to be clarified.

Nothing to Disclose: YM, HS, IK, KM, AM-T, YM, TH, RJ, HI

P2-649

Measurements of Aldosterone and Cortisol in Human Plasma by Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS): Comparison to Radioimmunoassays or Immunoassays.

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Introduction: Plasma aldosterone concentration (PAC) to plasma rennin activity (PRA) ratio (ARR) is an established screening test for primary aldosteronism (PA), and dexamethasone suppression test (DST) is widely used in confirming a diagnosis of Cushing's syndrome. Accordingly, reliable measurement of aldosterone and cortisol are desirable and necessary for exact diagnosis of these diseases. Our aim of this study is to compare the PAC and serum cortisol measured by Liquid Chromatography-electrospray ionization tandem mass spectrometry(LC-ESI-MS/MS) with the results by Radioimmunoassays (RIA) or Immunoassays used prevalently.
Methods: We prepared 100 EDTA plasma samples (from the 95patients who were examined high or low-dose DST. 23/95 patients diagnosed PA were selected for adrenal venous sampling, and finally, 13/95 patients were underwent adrenalectomy as PA or Cushing's syndrome). Successively, we measured aldosterone and cortisol in each sample by LC-ESI-MS/MS. Differently, we also measured aldosterone by 3 RIA kits: SPAC-S Aldosterone Kit(SPA), DPC Aldosterone Kit(DPCA), RIA Aldosterone(RIAA), and cortisol by 1 RIA kit and 4 Immunoassay kits: Cortisol kit TFB(TFB RIA), ECLusys 2010 cortisol assay(ECL), Cortisol EIA Kit(Oxford), AxSYM TM Corisol Abbott(Abbott), Cortisol AIA-2000(Tosoh).
Results: The results of PAC measured by each RIA kits were consistently 1.5- to 2-times higher than those measured by LC-ESI-MS/MS. Otherwise, the cortisol levels were different among the kits, especially in the lower range. Cortisol levels measured by TFB RIA, ECL, and Abbott were higher, but those by Oxford and Tosoh were lower than those.
Conclusion: It is suggested that the PAC, ARR and the cortisol levels examined DST may be influenced by which kits we chose during diagnosis of PA and/or Cushing's syndrome. We should recognize the characteristic of each aldosterone and cortisol kits, and the worldwide standardization of aldosterone and cortisol measurement may be required in the near future.

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Nothing to Disclose: MK, FS, RM, OM, AS, AU, KY, MN, SI

P2-650

Primary Familial Hyperaldosteronism Type 1 in Hypertensive Children: Prevalence, Clinical and Biochemical Characteristics.

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Background: Familial hyperaldosteronism type I (FH-1) or glucocorticoid-remediable aldosteronism (OMIM; #103900) is an autosomal dominant disorder caused by a chimeric gene (CG) CYP11B1/CYP11B2. In adults FH-1 represents 0.5–1.0% of primary aldosteronism, but the prevalence in pediatric population has not been established and their clinical presentation is not well defined. The FH-1 diagnosis is clinically relevant because it can be treated specifically by suppressing ACTH with glucocorticoids. Aim: To report the prevalence of FH-1 in hypertensive children and to describe their clinical and biochemical characteristics. Patients and methods: We studied 120 untreated hypertensive children aged 4 to 15 years recruited from the nephrology and endocrinology outpatients programs of our institution. Hypertension diagnosis was made according the 2004 Task Force guidelines. In all of them we measured plasma potassium (K), Plasma Renin activity (PRA), serum aldosterone (SA) and we calculated the aldosterone to renin ratio (ARR). In patients with ARR ratio >25, the detection of CG was performed using long-extension PCR. Results: We found 4/120 (3.3%) children with ARR>25 which belong to 4 unrelated families. After genetic study we confirmed a CG gene in all of them. The same study was conducted in 20 first degree relatives, 8 of whom were affected, making a total of 12 patients. Severe hypertension was present in 8/12 of them (the youngest child was 4 years old and he presented a stroke), pre-hypertension was present in 3/12 and normotension in 1/12 CG patients. The PRA was suppressed (< 0.3 ng/mL/h) in 6/12 and hypokalemia (K <3.5 mEq/L) was present only in 3/12 patients. A high SA levels (>16 ng/dL) was present in 10/12 patients (range 8.1-69.4 ng/dL). The ARR >25 was observed in 11/12 affected subjects (range of 16.2 to 161.3), the lower value corresponds to a woman 20 years old who had pre-hypertension. Conclusion: The prevalence of FH-1 in pediatric hypertensive population was surprisingly high when a ARR >25 was used as screening. Because all hypertensive children with ARR >25 were positive for a CG, we suggest that a lower ARR cut off needs to be used and shoud be determined in pediatric population. We found a high variability in the clinical and biochemical characterics of the affected patients, suggesting that FH-1 is an heterogenous disease with a wide spectrum of presentation even in a same family group.

Sources of Research Support: Chilean Grant Fondecyt 1100356 and 1070876.

Nothing to Disclose: HG, MA, AM, CC, CC, LB, CA, RB, CL, KB, VG, VM, AR, CF

P2-651

The Analysis of 42 Cases of Primary Aldosteronism in Our Hospital.

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The primary aldosteronism (PA) has become more common disease, and the prevalence of those patients was reported as high as 11.2%. We screened hypertensive patients by aldosterone-renin retio (ARR) > 20 (or 200 with aldosterone in pg/ml). We, then, performed the confirmatory tests, such as rapid ACTH stimulating, captopril challenge and upright furosemide tests, to those ARR positive patients to definitively confirm or exclude the diagnosis. All patients with PA undergo an adrenal CT scan as the initial study in subtype testing and to exclude adrenal cancer. When surgical treatment is practicable and desired by the patient, the distinction between unilateral (UHA) and bilateral hyper aldosteronism (BHA) is made by ACTH-loading adrenal venous sampling (AVS). We, then, finally perform unilateral laparoscopic adrenalectomy upon AVS documented unilateral aldosterone producing adenoma. If a patient is unable or unwilling to undergo surgery, or those with BHA, we administrated a MR antagonist.
Since patients with PA have become more frequent since our opening in April 2007, to seek any difference which may predict UHA or BHA, we analyzed the clinical features and the usefulness of confirmatory tests in our 42 cases of PA. The mean age was 56.5 ± 13.2 years old including 16 males and 26 females, and the ARR was 76.1 ± 84.1. The positive ratios of those three confirmatory tests were 90.1%, 45.2%, 83.3%, respectively, and the rapid ACTH stimulating test showed the highest sensitivity among these tests. Then, AVS was performed to 33 patients, and 30 cases showed plasma aldosterone concentration ≥ 1,400 ng/dl in either (14 cases) or both (16 cases) side, which we consider the AVS positive. We, then, analyzed the positive ratios of those three confirmatory tests again among those cases. It is revealed the mean ARR of UHA was significantly higher than one of BHA, 104.9 ± 145.2 vs. 77.7 ± 95.8, respectively, and the each positive ratio of confirmatory tests in UHA was higher than those in BHA, 100 vs. 87.5%, 64.2 vs. 43.8%, 92.9 vs. 75.0%, respectively. Moreover, it also revealed that the reproducibility of ARR positive by measuring 4 times in different days was significantly higher in UHA than BHA, 3.3 ± 0.9 vs. 2.4 ± 1.3 times, respectively.
In conclusion, our results confirm the usefulness of the confirmatory tests and ACTH loaded AVS, and multiple measurement of ARR with confirmatory tests may give us some prospect of the distinction between UHA and BHA before AVS.

Nothing to Disclose: TI, KY, EY, SU, YA, HO, NH, MH

P2-652

Detection of Undiagnosed Primary Hyperaldosteronism through Use of the Veterans Affairs Computer Database.

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Background: Based on reports in the literature, the estimated prevalence of primary hyperaldosteronism is approximately 10-20% in patients with poorly controlled hypertension.
Purpose: To estimate the prevalence of primary hyperaldosteronism in outpatients with resistant hypertension using a pharmacy database.
Methods: Retrospective review of computerized data was used for this analysis. Patients were included if they had: 1) uncontrolled hypertension and use of 3 antihypertensive medications or 4 or more antihypertensive medications regardless of blood pressure, 2) low or normal potassium levels, and 3) received continuous health care from 10/1/08 to 2/28/09. Exclusion criteria were: 1) Past or current use of an aldosterone antagonist, 2) medication possession ratio <80% for any antihypertensive, and 3) elevated potassium level. Patient lists were sent to primary care providers suggesting they evaluate their patients for hyperaldosteronism using the angiotensin/renin ratio (ARR) >30. For patients in whom these data were available, we determined the utility of computer screening.
Results: 5,516 patients were on three or more antihypertensive medications. 746 patients remained after applying inclusion and exclusion criteria using computer analysis. Manual chart review was conducted two months following computer analysis for each patient to verify and update the inclusion and exclusion criteria. 333 patients remained for analysis; their providers were sent letters suggesting they obtain ARR. In the 184 individuals in whom ARR was obtained, 39 (21.2%) had an elevated value. There was a statistical but not clinically significant difference in potassium when comparing patients with elevated ARR vs. those with normal ARR (4.0 vs. 4.2, respectively; p=0.023). There were no statistically significant differences in class of antihypertensive medication use, age, blood pressure, or serum creatinine.
Conclusions: The VA computer database is a useful tool to screen for patients with undiagnosed primary aldosteronism.

Nothing to Disclose: ALMS, EAG, JLM, JRL, DS

P2-653

Careful Treatment Selection Following AVS and CT Results in Excellent Blood Pressure Control: A Review of 100 Patients with Primary Hyperaldosteronism.

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It is recommended that all patients with primary hyperaldosteronis (PA) who are suitable for surgery should undergo adrenal CT and AVS unless there is a large unilateral adenoma with a completely normal contralateral gland. AVS is however a technically difficult procedure and it is often difficult to cannulate both adrenal veins. We reviewed 100 sequential patients diagnosed with PA. AVS was performed in 93. We reviewed the outcomes of adrenalectomy and medical therapy in patients in whom AVS was bilaterally successful and in those with less than ideal sampling. We also evaluated which pre-operative characteristics predicted cure of hypertension in patients referred for unilateral adrenalectomy.

Successful cannulation was defined as a 1.2-fold increase in cortisol from IVC to adrenal. Bilateral cannulation was successful in 67%. Our policy is to refer patients for surgery if they have clear lateralisation on AVS and concordant CT results. In patients with less than ideal sampling data, decisions regarding surgery are based upon the AVS data available, CT imaging and blood pressure control of each patient.

43 of 46 patients referred for surgery had confirmed unilateral disease, 3 had what appears now to be bilateral disease. Of the 43, 39 had long-term outcome data. Cure rate for blood pressure was 38.5% with improvement in another 59.0%. 53 patients with PA were treated with medical therapy after AVS. Outcome data was available for 46. Blood pressure improved to a target of <140/90mmHg in 80.4% and remained above target in 19.6%. Potassium normalised in all patients.

Using univariate analysis to investigate blood pressure outcome post adrenalectomy, significant (p=<0.05) predictors of persistent post-operative hypertension were raised creatinine, LVH and male sex. On multivariate analysis, male sex and higher pre-operative systolic BP remained predictive.

Our studies demonstrate that AVS contributes significantly to decision making for adrenalectomy even when bilateral results are not available (data not shown). When patients were carefully selected for surgery based on available AVS and CT imaging, 97% had cure or improvement in blood pressure control.

Nothing to Disclose: UMG, KRM, SJH, HL, PKE, ABA

P2-654

Aldosterone and the Metabolic Syndrome in the Study of Health in Pomerania (SHIP-1).

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Objective:
Recent data suggest that circulating aldosterone promotes the development of the metabolic syndrome (MetS) (1,2). While it is unquestioned that excessive aldosterone levels cause hypertension (3,4), current studies found associations to insulin resistance (5) and dyslipidemia (6) as well. The aim of this study was to identify possible associations between the plasma aldosterone concentration (PAC) and MetS or its single components in a population-based cohort.
Methods:
3,086 participants of the first follow-up of the Study of Health in Pomerania (SHIP-1) were eligible for analyses. MetS was defined as the presence of three out of five components in women (w) or men (m), respectively: waist circumference ≥94 (m), ≥80 cm (w); plasma glucose ≥8 mmol/l or antidiabetic medication; HDL-cholesterol <1.03 (m), <1.29 mmol/l (w); triglycerides ≥2.3 mmol/l or lipid modifying medication; blood pressure ≥130/85 mmHg or antihypertensive medication. We categorised PAC in age-specific quintiles and calculated multiple logistic regression models separated by sex.
Results:
530 women (33.5%) and 762 men (50.7%) presented with MetS. Mean PAC was 44.1 ng/l (1SD 33.4) in women without MetS, 50.6 ng/l (1SD 36.9) in women with MetS, 53.4 ng/l (1SD 36.5) in men without MetS, and 55.3 ng/l (1SD 36.1) in men with MetS. Subjects with the highest PACs had higher odds of MetS (w/m), larger waist circumference (w/m), higher triglycerides (w), lower HDL-cholesterol (w) and more frequently hypertension (w/m) than subjects with low PACs.
Conclusion:
We conclude that high PAC is associated with the MetS, increased waist circumference and hypertension in both sexes, as well as with dyslipidemia in women.

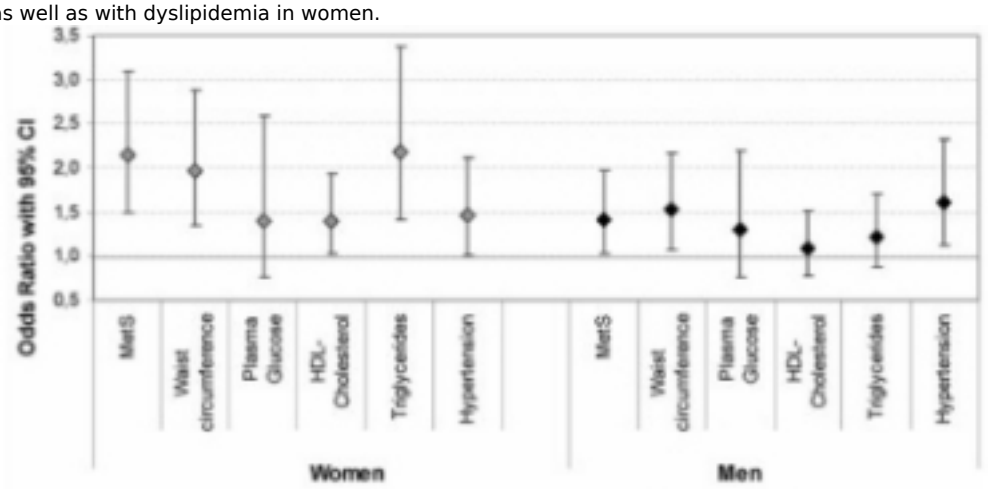

Figure 1: Associations between high aldosterone levels (PAC) and the Metabolic Syndrome (MetS) and its components. PAC levels were divided in 5 groups by age- and sex-specific quintiles. Odds ratios with 95% confidence interval (CI) for the highest vs. lowest PAC groups are displayed. Multiple logistic regression models were constructed with MetS and its components as outcome, comparing the highest vs. lowest PAC group as exposure. The sex-specific models were adjusted for age (w/m), intake of estrogens (w), and weighted to the population of west Pomerania.

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Nothing to Disclose: AH, NF, SS, JL, MN, RR, JP, HV, MR, HW

P2-655

Effect of Renin Angiotensin System Inhibition on Cardiovascular Sequelae in Elderly Hypertensive Patients with the Metabolic Syndrome.

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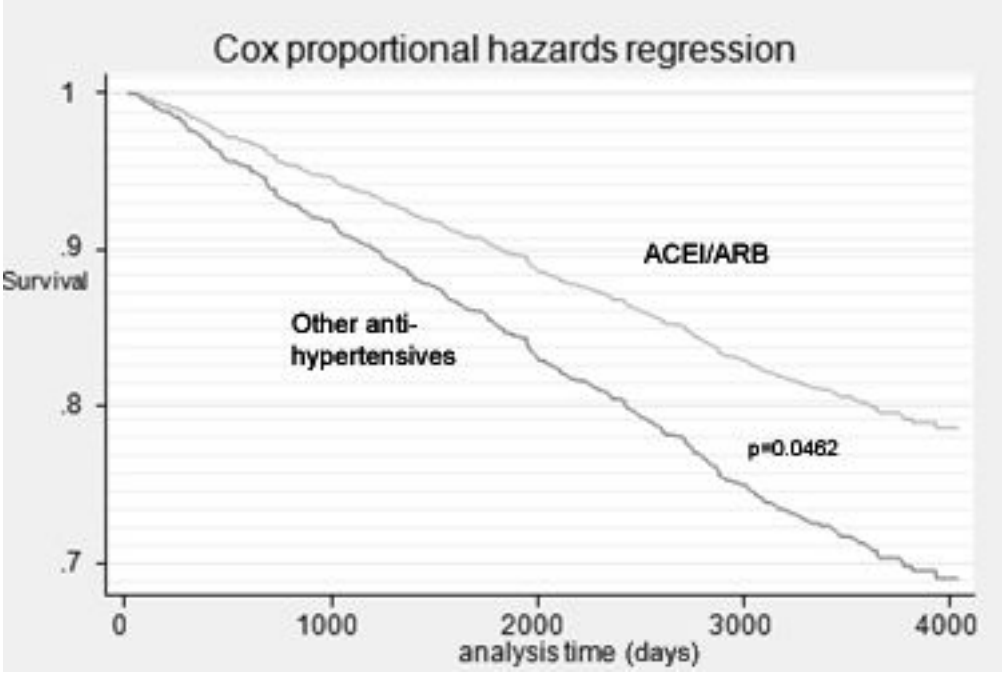
Background The metabolic syndrome (MBS) has been associated with cardiovascular disease (CVD). Insulin resistance has been hypothesized as the underlying pathophysiologic feature of MBS. Current management of MBS focuses on the specific individual risk factors without targeting the underlying insulin resistance. Angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB) are widely used antihypertensives that may improve insulin sensitivity. We hypothesize that they can be used to reduce long term CVD events in elderly hypertensive patients with MBS. **Methods** Our project utilized the Cardiovascular Health Study (CHS) dataset. This dataset is an observational study where individuals > 65 years of age were followed up for up to 15 years and the time to cardiovascular events was recorded. We included hypertensive, non-diabetic subjects with MBS (as defined by ATPIII criteria), but free of CVD at baseline. Cox regression model was used to evaluate the effect of ACEI/ARB on the time to the first cardiovascular event compared to the other antihypertensives adjusting for possible confounders (age, race, gender, smoking status, triglycerides, LDL, blood pressure, development of diabetes, congestive heart failure and the number of antihypertensives). **Results** In elderly hypertensive non-diabetic subjects with MBS, the hazard ratio for CVD associated with the use of ACEI/ARB was 0.65 (95 % C.I. [0.45, 0.99]) compared to other antihypertensives (see figure).



Use of ACEI/ARB did not significantly decrease individual CVD endpoints (MI, stroke, TIA, CABG, claudication and ECG MI). However, use of ACEI/ARB was associated with lower rates of angioplasty and coronary disease (HR of 0.13 and 0.56 respectively with 95 % CI [0.02, 0.95] and [0.34, 0.93]). C**onclusions** Elderly hypertensive patients with MBS seem to have lower risk of CVD as an aggregate with ACEI/ARB. The significant protective effect with ACEI/ARB on the incidence of coronary heart disease suggests a difference in the effect between coronary and non-coronary events.

Nothing to Disclose: HHZ, SEH, PWS, DPM, PAE, KIC

P2-656

Metabolic Abnormalities Are Associated with Blood Pressure Control.

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Background: It is known that both the rate of knowledge, such as rates of hypertension control are low and get influenced by several factors, including age and educational level. However, little is known about the biochemical factors associated with these conditions.
Objectives: To identify biochemical factors associated with inadequate blood pressure control in 24-hour period in hypertensive subjects.
Methods: We conducted a cross-sectional observational study in which 353 hypertensive patients were analyzed for mean 24-h blood pressure (BP) levels obtained by ambulatory blood pressure (ABPM), and divided into groups with and without BP control (24-h BP ≤130/80 mmHg in ABPM). We used Fisher's exact test for analysis of categorical variables and Student t tests for continuous.
Results: Individuals with uncontrolled BP (n=236, 66.8%) had higher fasting glucose (118.0±55.1 vs. 107.7±30.1 mg/dL, P=0.012), triglycerides (154.6±91.6 vs. 127.7±66.0 mg/dL, P<0.001), creatinine (1.4±1.2 vs. 1.2±0.4 mg/dL, P=0.003) and lower HDL-C (52.3±14.4 vs. 55.6±12.4 mg/dL, P=0.018) than subjects with BP within the recommended values. Furthermore, this uncontrolled BP group had higher left ventricular mass (117.7±32.8 vs. 108.7±27.9 g/m2, P=0.007) and albuminuria (90.8±66.7 vs. 34.6±51.3 µg/min, P=0.002) than those with BP under control. The groups did not differ (P>0.05) regarding age, gender, body mass index, LDL-cholesterol, use of hypoglycemic or lipid-lowering drugs, history of diabetes, stroke or myocardial infarction.
Conclusion: Metabolic alterations such as hyperglycemia and atherogenic dyslipidemia (hypertriglyceridemia and low HDL-C) may contribute to endothelial dysfunction and, consequently, worse blood pressure control. This inadequate control of BP may be observed in higher incidence of target-organ damage that these patients develop (left ventricular hypertrophy and microalbuminuria).

Sources of Research Support: Medical School of Sao Jose do Rio Preto (FAMERP).

Nothing to Disclose: LNC-M, JFV-M, ROV-M, JCY-T, DRS, HM-J, MAD, JPC

P2-657

Beta Blockers, ACE-I and ARB Are Related to Body Weight Change, Hospitalization Rate and Survival in Patients with Non Small Cell Lung Cancer.

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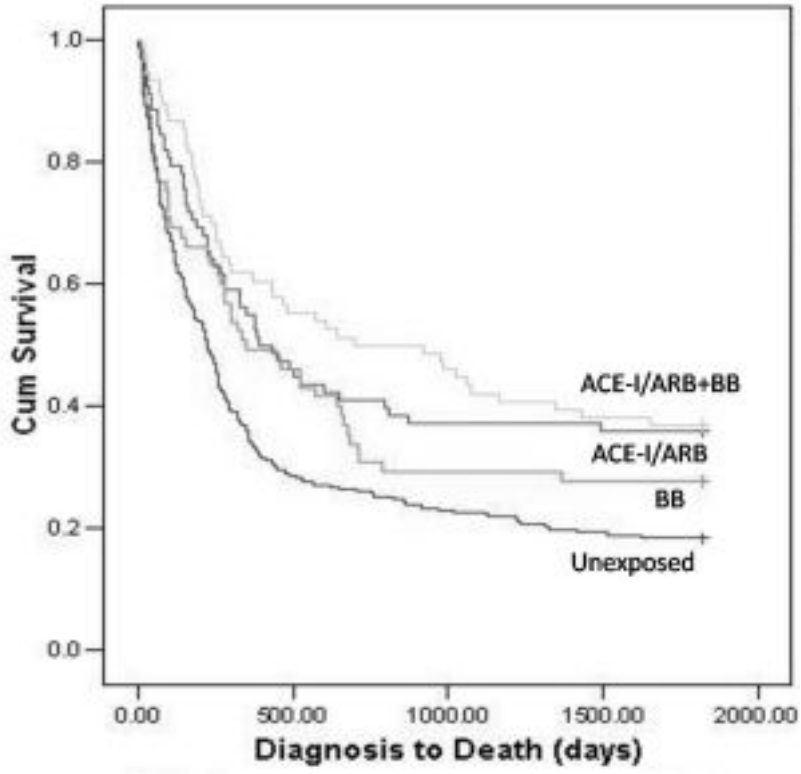
Background: Patients with non small cell lung cancer (NSCLC) often suffer from cachexia, a condition characterized by increased energy expenditure (EE) and inflammation that leads to a decrease in body weight. Beta blockers (BB) may interfere with the development of cachexia by decreasing EE, while angiotensin converting enzyme inhibitors (ACE-I), and angiotensin II receptor blockers (ARB) may interfere with cachexia development by decreasing inflammation. Since cachexia is associated with increased hospitalizations and decreased survival, these outcomes may also be improved by ACE-I/ARB and BB.
Design: Seven hundred records were retrospectively reviewed from NSCLC patients, and 487 were included in the final analyses. Subjects were excluded if they had severe COPD, ≥2+edema, class 3-4 CHF, liver failure, other reasons for weight loss, or research or anabolic medications. Data including medication history, body weight, hospitalizations and death were collected from one year pre-diagnosis until three years post-diagnosis. Drug exposure for ACE-I/ARB and BB was affirmed if a patient was exposed to the drug ≥50% of any given year in the observation period. Statistical analyses employed Kaplan-Meier survival, univariate ANOVA with Games-Howell or Tukey post hoc tests, and Kruskal-Wallis.
Results: ACEI/ARB exposed patients had significantly more weight loss than those exposed to BB (-11.5% vs.-4.7%, p= .019). 50%-survival rate was significantly improved by ACE-I/ARB and the combination of ACE-I/ARB+BB when compared to unexposed subjects (394, 696, 227 days respectively, p<.05). BB were not different from any other group for survival. Unexposed subjects showed a significantly higher hospitalization rate group for both cancer-related hospitalizations (p=.035) and total hospitalizations (p<.0005) compared to the other groups.



Conclusion: Both ACE-I/ARB and ACE-I/ARB+BB improved survival over patients exposed to neither drug class. However, ACE-I/ARB patients experienced more weight loss than BB. Patients who were not exposed to either drug class had poorer survival and more hospitalizations, but did not differ from the other groups in weight loss.

Sources of Research Support: Department of Veterans Affairs.

Nothing to Disclose: AEU, DLW, LHI, AS, JB, CPS, ET, BK, JMG

P2-658

Aldosterone Blocking Agents Improved Visceral Adiposity in Patients with Primary Aldosteronism.

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Purpose:Mineralocorticoid resecptor is expressed not only in the kidney but aldo in the adipose tissue. Patients with primary aldosteronism (PA) was found to be associated with impairment of insulin sensitivity and frequent metabolic syndrome. We examined the effects of aldosterone blockade on visceral adiposity in patients with PA.
Methods:Thirty patients with PA were treated with eplerenone or spironolactone for 18 months. A single slice CT scan was taken at L4 and visceral and abdominal subcutaneous adipose tissue (VAT and ASAT respectively) quantified using Fat Scan image analysis software. BMI, waist circumferences, HOMA-IR, serum potassium and lipids were measured before and after the treatment.
Results:Aldosterone blocking agents normalized blood pressure and increased serum potassium, but did not affect HOMA-IR and serum lipids. VAT was significantly decreased from 96 cm^2 to 87 cm^2 ($p<0.05$), however, ASAT and waist circumferences were not affected by the treatment with aldosterone blockers.Conclusions:These results suggest that aldosterone blockade improves visceral adiposity in PA and anti-metabolic effects of aldosterone blockers should be further clarified in obese-related hypertension.

Nothing to Disclose: YT, MD, SK, TY, MK, MO, SM

P2-659

Validation of Association of Hypertension at the *CYP11B1/B2* Locus in Caucasians.

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The genes encoding 11-beta hydroxylase (*CYP11B1)* and aldosterone synthase (*CYP11B2*) are strong candidate genes in human essential hypertension. However, the association studies in this locus have been inconsistent.
Previously, we showed in the discovery phase an association with hypertension at the *CYP11B1/CYP11B2* locus in a Caucasian case-control population (BRIGHT Study). Here, we were able to validate these findings in an independent population of the same ancestry. In the discovery phase, 8 SNPs were genotyped and 29 SNPs imputed in 1643 hypertensive cases and 1697 controls. The SNPs significantly associated with hypertension (rs6410, rs4471016(-1859A/G), rs43131369(-1889G/T), rs6471581, rs4546, intron conversion and rs1799998(-344T/C)) were tested for validation in 1612 hypertensive cases (NORDIL Study) and 1317 controls (MDC Study). Using single SNP analysis, only the intron conversion showed significant association; subjects with the conversion allele in intron 2 of *CYP11B2* had a higher risk of hypertension (OR=1.19 [1.10-1.29], $p=1.06 \times 10^{-5}$). Using three-marker sliding window haplotype analysis, three haplotypes showed significant association with hypertension in a combined meta-analysis of the two studies:

				Combined meta-analysis n=6269	
Gene	**Haplotype SNPs**	**Haplotype**	**Frequency (BRIGHT)**	**OR [95% CI]**	**p**
CYP11B1	rs6410,rs4471016,rs4313136	TAG	0.02	3.14 [2.27-4.32]	2.99×10^{-12}
CYP11B1/CYP11B2	rs4471016,rs4313136,rs6414	AGG	0.02	2.64 [1.87-3.74]	3.90×10^{-8}
CYP11B2	rs6414,rs4546, intron conversion	ATConv	0.02	4.64 [2.63-8.18]	1.11×10^{-7}

In conclusion, we have validated our original observations in the BRIGHT Study, using another Caucasian population. Thus, the regions most strongly associated with hypertension in Caucasians – one between introns 2 and 3 of *CYP11B2* and another between the promoter and exon 1 of *CYP11B1* – have been validated. The variability in the strengths of these associations suggests the locus is involved in hypertension in a complex manner. This is the first time a positive association of a locus implicated in a rodent model of hypertension and in rare autosomal human disorders is validated in human essential hypertension. Additional functional studies are required to elucidate the mechanism by which these genetic variations lead to alterations in aldosterone and cortisol production and to subsequent hypertension.

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Nothing to Disclose: SA-M, SP, EF, SMM, MJB, MJC, PBM, MF, JW, NJS, AFD, OM, ED, JMC

P2-660

High Dietary Sodium Is Associated with Increased Flow-Mediated Dilatation in Healthy Men.

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Background: Among several environmental factors that could potentially account for the development of hypertension, such as smoking and exercise, dietary sodium has been explored extensively. However, the mechanisms underlying dietary sodium manipulation and hypertension are not clearly understood. We have chosen to examine the impact of dietary sodium on vascular function as evidenced by endothelial function.
Objectives: To test the hypothesis that high dietary sodium will lead to impairment in endothelial function and low dietary sodium will improve endothelial function.
Participants/Interventions: We chose to examine the impact of dietary sodium on the endothelium through measurement of brachial artery flow mediated vasodilatation (FMD). We studied 16 normotensive, normal weight (BMI, mean SD, 24.1 ± 2.2 kg/m^2) men ages 24.2 ± 4.8 years who each underwent both a high (250 mEq/day) and low (10 mEq/day) sodium diet for 7 days. A 24-hr urine was obtained for sodium and creatinine to evaluate salt balance; a one-hour para-aminohippurate infusion was performed to evaluate renal plasma flow (RPF) and the endothelium-dependent FMD of the right brachial artery was determined.
Results: Sodium balance was achieved on both diets: urinary sodium on high sodium (mean SD) 247.5 ± 54.3 mmol/TV and on low sodium 14.1 ± 11.4 mmol/TV. The RPF was greater on the high compared to the low sodium diet (655.19 ±133.85 vs. 580.07 ± 139.76 ml/min/1.73 m^2; p = 0.01). Similarly, the endothelium-dependent percent change in FMD was greater on the high compared to the low sodium diet (13.5 ± 6.3 vs.10.2 ± 4.8%; p = 0.03), although they were both still within the expected normal range for FMD. Additionally, there was no significant difference in the brachial artery baseline diameters between the two diets (3.6 ± 0.4 vs. 3.7 ± 0.4%; p= 0.6).
Conclusion: Renal plasma flow is sensitive to changes in dietary sodium intake and has been responsive to manipulaton of the renin-angiotensin-aldosterone system (RAAS). Endothelial function, as reflected in brachial artery FMD, is similarly modulated by dietary sodium, such that a high sodium diet was associated with a greater percent change in FMD than a low sodium diet. This finding suggests a potential role for the RAAS in mediating endothelial function in a young, normotensive, normal-weight male population exposed to acute dietary sodium manipulation.

Sources of Research Support: Dr. Bentley-Lewis was supported in part by grants from the NIH 1K23RR023333 and the Harold Amos Medical Faculty Development Program, a national program of the Robert Wood Johnson Foundation. In addition, this work was supported by General Clinical Research Centers NIH grants M01RR02635, M01RR00095, M01RR00064.

Nothing to Disclose: RB-L, DK, GS, GHW, ES

P2-661

Successful Treatment of Postural Orthostatic Tachycardia Syndrome (POTS) with High Dose Fludrocortisone.

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Postural orthostatic tachycardia syndrome (POTS) is an increasingly recognized syndrome characterized by symptomatic orthostatic tachycardia without concomitant hypotension. A uniform treatment strategy has not been well defined, and a variety of treatments have been reported, many in single case reports. We report our experience using escalating high dose fludrocortisone.
Methods: Patients with POTS were identified based on a clinical presentation of fatigue, shortness of breath, palpitations or near-syncope after standing. Further criteria included an increase in heart rate of 30 beats per minute (bpm) or a heart rate >120 bpm following 10 minutes of standing without a decrease in blood pressure. Secondary causes of orthostatic tachycardia such as structural heart disease, adrenal insufficiency, pheochromocytoma, and volume depletion were excluded. Fludrocortisone was initiated at a dose 0.2 mg daily and was titrated at weekly intervals by 0.1-0.2 mg daily. 20 mEq of KCl was given daily with each 0.1 mg of fludrocortisone. Clinical improvement of POTS was based on resolution of symptoms and normalization of standing heart rate.
Results: Ten patients (7 female/3 male) were identified with a mean age of 23.1±12.5 years. The most common presenting symptoms were fatigue (60%), dizziness (40%), and palpitations (20%). The mean increase in heart rate from supine to standing was 49±17 bpm. Patients were followed for a mean of 4 years. Complete resolution of symptoms in all patients occurred after a mean of 3.4 months. Fludrocortisone and KCl were well tolerated, edema was not seen. Serum K+ was checked monthly and no patient had a level < 3.5 mEq/L. 3 patients went into remission after a mean of 4 years and 4 patients were lost to follow-up. Initial mean fludrocortisone dose was 0.5 mg/day (0.3-0.8), and mean maintenance dose of fludrocortisone was 0.34 mg/day. 2 patients required low dose atenolol (12.5-25 mg/day)
Conclusion: We successfully treated 10 patients with POTS with high dose fludrocortisone and KCl. Fludrocortisone monotherapy alleviated signs and symptoms of orthostasis in all but 2 patients who also received low dose beta blockade. Fludrocortisone was safe and effective therapy even when given for prolonged periods of time.

Nothing to Disclose: JM, DB

P2-662

Adrenocortical Cancer: Use of New ENSAT Staging System and Re-Evaluation of Old and New Markers of Prognosis.

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A modification of the TNM classification (WHO-UICC 2004) for adrenocortical cancer (ACC) has been recently proposed in order to improve the prognostic value as to the disease-free and disease-specific survival.(1) So far an increasing number of molecular markers has been proposed for early detection, confirmation of malignancy and/or outcome prediction, but none of them is considered as reliable as the Weiss score morphological system.(2,3) **Aim**: to compare the prognostic value of WHO-UICC 2004 and new ENSAT TNM staging classification in a single Institution ACC series as to the disease-free and disease-specific survival and to compare these findings with tumor Weiss score and ki67 expression. **Methods**: Clinical and pathological data of 26 pts (19F, 7M, median age 52 yrs, range 25-78) surgically treated for ACC, were retrospectively reviewed; the follow up period was 26 months (median, range 6-192). ACC staging was performed according to both WHO-UICC 2004 and ENSAT classification, tumor malignancy was assessed according to Weiss system. A multivariate analysis of disease-free and overall survival in ACC according to Weiss score and ki67 (IHC, monoclonal Mib1 Ab) was performed. **Results**: 70% of tumors were hypersecreting ; mean size was 10,7cm (range 50-2500), mean weight was 512g (range 50-2500). WHO-UICC identified 2 stage I, 11 stage II, 4 stage III and 9 stage IV disease (Kaplan-Meyer, log-rank test: p=0,0004). Two pts with stage IV WHO-UICC and good prognosis switched to stage III ENSAT (Kaplan-Meyer, log-rank test: p=0,0002). In our series ki67>7% (sensitivity 85,7%, specificity 62,5%, ROC analysis) and Weiss score>4 (sensitivity 100%, specificity 50%) were the best cut-off values suggestive for a poor prognosis (Kaplan-Meyer, p=0,06 and p=0,04 respectively); taken together the two risk factors showed a significant effect on survival probability (Cox-regression, p= 0,04). Ki67>7% and Weiss score>4 showed a slight correlation also with disease-free survival (Kaplan-Meyer, p=0,06 and p=0,04 respectively); when both risk factors were concomitantly present, the correlation with probability of disease recurrence was higher (Cox-regression, p=0,02). **Conclusions**: The outcome of pts with ACC is strictly related to the stage of the disease, being new ENSAT proposal a valuable tool to improve the power of the stage-related prognostic value. Ki67 expression and Weiss score combined may play a role of interest, but more reliable molecular markers are needed.

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Nothing to Disclose: PDC, DP, EMG, EG, MV, MG, PL

P2-663

Mitotane Induces a Concentration-Dependent Impairment of Platelet Aggregation in Patients with Adrenocortical Carcinoma.

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Standard treatment of adrenocortical carcinoma (ACC) comprises adrenolytic therapy with mitotane. Prolongation of bleeding time has previously been observed based on a series of 7 patients (Haak et al. 1991). As patients with ACC frequently undergo surgery for local recurrence or metastases, we have studied the effect of mitotane on coagulation in 67 patients with ACC before and/or during treatment with mitotane (total sample size n=113).
Platelet aggregation was performed with standard light transmission aggregometry using platelet rich plasma. Major components of the extrinsic and intrinsic coagulation cascade pathways were determined by routine coagulation testing using the Dade Behring BCS analyzer system or manual ELISA testing. In vitro bleeding time was detected as PFA-100 measurement.
During mitotane therapy, a successive decrease in platelet aggregation occurred that was closely correlated with mitotane levels. This effect was most pronounced for ADP-induced platelet aggregation (Pearson r= -0.76; p<0.001) and induction with collagen (r=-0.47; p<0.001), and also for challenge with epinephrine (r=-0.28; p=0.02), and ristocetin (r=-0.28; p=0.04). Platelet counts, in-vitro bleeding time, global plasmatic coagulation and von Willebrand parameters remained unaffected. With mitotane levels above 10 mg/l, almost all patients had pathologic ADP- and collagen-induced platelet aggregation. In conclusion, in a large series of patients with ACC we show impaired agonist-induced platelet aggregation strongly dependent on plasma mitotane levels. Routine in vitro bleeding time is not suitable to detect this platelet defect and to assess bleeding risk. ADP-induced platelet aggregometry testing prior to surgery is recommended to determine mitotane-induced bleeding risk. Further studies should investigate the potential role of prophylactic administration of DDAVP to improve platelet function.

Nothing to Disclose: SH, ME, DW, BA, MF

P2-664
Hormonal Profile Heterogeneity in Anorexia Nervosa.

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Background. The relevance of hormonal assessment in anorexia nervosa (AN) management is still unclear. The short term physical risk during undernutrition period of the disease is partially predicted by anthropometric and electrolytic parameters. The aim of the study to evaluate hormonal profiles in a large cohort of AN and their relationship with critical states.
Methods. Hormonal parameters: thyroid hormones, GH, IGF-I, cortisol, oestradiol, FSH, LH, SHBG, DHEAS, plasma metanephrine, bone markers were assessed in 210 young female subjects with restrictive-type AN and 42 age and sex matched controls. Their relation with registered short term evolution after assessment was evaluated.
Results. Except for metanephrines and DHEAS, most of the hormonal abnormalities previously reported in AN were confirmed. Each of these abnormalities was identified below a specific critical BMI ranging between 17 and 15 kg/m². Even tough, an important percentage of normal values for every parameter was still noticed for very low BMIs. All patients that developed critical states during the three months following the hormonal assessment presented with BMI < 15 kg/m² and very increased level of cortisol, GH and increased values of metanephrines.
Conclusions. The hormonal response to undernutrtion is heterogeneous in a large population with restrictive AN. In clinical practice, metanephrines, GH and/or cortisol data could be used as important predictors for severe short term outcome.

Nothing to Disclose: BE, NG, GC, FL, BG

P2-665

Adrenal Is Likely the Major Source of Progesterone Secretion in Women during the Normal Follicular Phase and after Menopause.

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This study aimed to investigate the source of progesterone (PRG) secretion during follicular phase (FOL) and after menopause. Plasma hormonal levels were repeatedly measured over a 24-h period to explore possible relations between diurnal variations of PRG and of several hormones of gonadotropic and corticotropic axes. Cross-correlations analyses (Spearman rank test) were performed to assess temporal coupling between simultaneous and lagged hormonal values in individual time series. In 8 normal postmenopausal women, blood samples were obtained at 11, 15, 19, 22, 23, 01, 02, 03 and 07 h. The 24-h PRG levels averaged (± SEM) 0.18 ± 0.05 µg/l. Highly significant correlations were found between simultaneous concentrations of ACTH, cortisol and PRG, but no relation was detected between PRG and LH, FSH or estradiol (E2) (see Table). Ten normal young women were investigated, once at early-mid-FOL (24-h PRG levels 0.51 ± 0.01 µg/l) and once at late-luteal phase (LUT) (24-h PRG levels 7.4 ± 1.1 µg/l). On each occasion, blood was sampled at 20-min intervals for 24 hours. In LUT, as expected, highly significant correlations were evidenced between PRG and FSH, LH or E2, but not between PRG and cortisol. In contrast, in FOL, highly significant correlations were evidenced between PRG and cortisol, only a trend was detectable between PRG and FSH, and no relation was found between PRG and LH or E2. (see Table).

CORRELATION	MENOPAUSE		FOLLICULAR		LUTEAL	
	median R	p	median R	p	median R	p
cortisol-ACTH	0.78	0.02				
PRG-ACTH	0.79	0.02				
PRG-cortisol	0.86	0.02	0.45	0.0002	-0.03	0.80
PRG-LH	0.46	0.19	0.17°	0.15	0.42°	0.0005
PRG-FSH	0.26	0.46	0.22°	0.07	0.51°	0.0001
PRG-E2	0.23	0.50	-0.13	0.29	0.34	0.005

°PRG lag of 20 min. All other correlations performed between simultaneous hormonal concentrations. Menopause: 8 subjects, 9 samples per subject; FOL and LUT: 10 subjects, 73 samples per subject.

These results strongly suggest that in FOL and after menopause, the major -if not the only- source of PRG secretion is the adrenal rather than the ovary. This is consistent with the results of two previous studies that used different experimental approaches (1,2). To our knowledge, this is the first report on the origin of PRG secretion after menopause.

Judd S et al, J Clin Endocr Metab 1992; 74:299
Eldar-Geva T, Hum Reprod 1998; 13:9

Nothing to Disclose: AC, RL, ML-B, GC

P2-666

Biochemical Presentation of Primary Adrenal Insufficiency in Childhood.

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Background. Primary adrenal insufficiency is usually thought to present with both hyponatremia and hyperkalemia, but most affected individuals present in infancy or adulthood, and cases presenting in childhood have not been systematically reviewed.
Methods. We reviewed medical records of inpatients admitted to Children's Medical Center Dallas between January 1999 to December 2009, identifying 41 patients with newly diagnosed primary adrenal insufficiency.
Results. 25 patients (61%) with median (interquartile ranges, IQR) age of 9 days (3-19) had congenital adrenal hyperplasia (CAH) and were not reviewed further. 16 (39%; 7M/9F) were diagnosed with other etiologies. Of these non-CAH cases, 10 were of autoimmune etiology of which 2 had autoimmune polyendocrinopathy syndrome type 1 (APS1). 3 had adrenoleukodystrophy (ALD) and 2 had no etiology identified. The median (IQR) age of these patients was 11.8 (5.8-15.0) y. Ethnic distribution was 11 Caucasian, 3 Hispanic, 1 Asian and 1 African American. 2 patients (1 APS1, 1 ALD) were being closely monitored when adrenal insufficiency developed and were not included in the analysis of presenting signs and symptoms.
10/14 patients were hypotensive with a median (IQR) systolic and diastolic blood pressure z scores of -1.03 (-3.53-0.08) and -1.62 (-2.56--0.11) respectively.
Hyponatremia (<135 mEq/l) occurred in 13 patients; median (IQR) [Na+] was 127 (118-132). However, frank hyperkalemia (>5.0 mEq/l)) was noted in only 7 patients; median (IQR) [K+] was 4.6 (4.0-6.1).
Hypoglycemia and ketosis were documented in 4/12 and 3/3 patients in whom these data were available. 11 patients underwent cosyntropin stimulation testing with median (IQR) baseline cortisol of 1.1 (0-3.6) mcg/dL and stimulated values of 1.2 (0-3.8) mcg/dL. 3 patients were diagnosed with median random cortisol levels of 10.3 (6.8-10.6). The median (IQR) ACTH was 892 (265-1744) pg/mL and the median renin was 3892 (323-20000) ng/dL/hr. 9 patients had documented hyperpigmentation.
Conclusions. Hyperkalemia is usually not a presenting sign of primary adrenal insufficiency in childhood, and its absence cannot rule out this condition. A varying combination of hypotension, hyponatremia and clinical symptoms should alert the clinician to be suspicious of adrenal insufficiency.

Nothing to Disclose: SYH, PW

P2-667

Assessment of the Hyperglycemic Effect of Glucocorticoids during an Exacerbation of Chronic Obstructive Pulmonary Disease with a Continuous Glucose Monitoring System.

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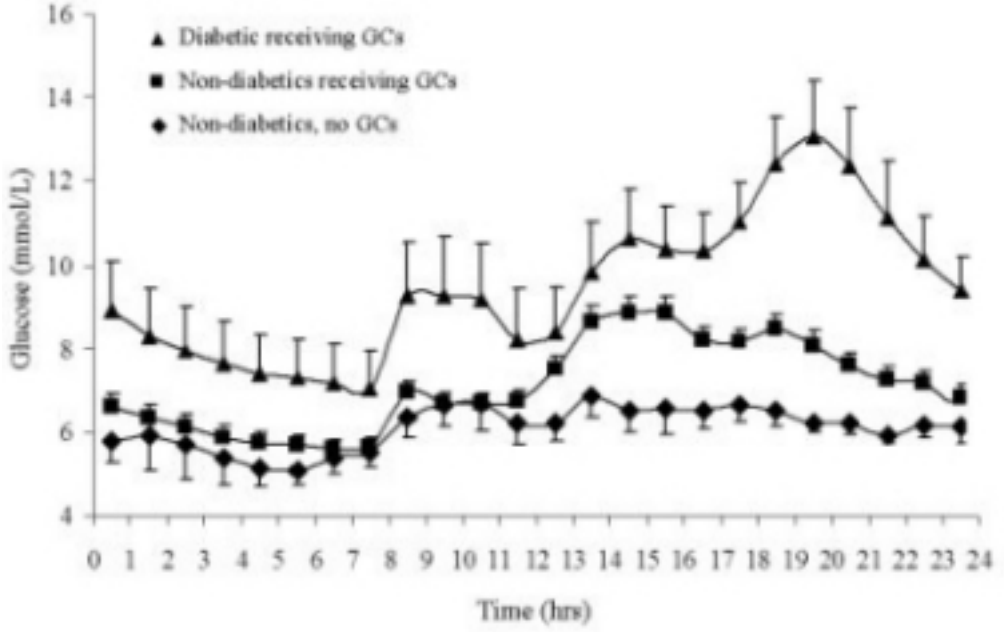
Glucocorticoids (GC) are frequently prescribed to treat acute exacerbations of inflammatory disease, including chronic obstructive pulmonary disease (COPD). While Cushing's syndrome predominantly causes postprandial hyperglycemia[1], the pattern of hyperglycemia induced by GCs in hospitalized patients has not been well described. The aim was to produce a detailed assessment of the effect of GCs on glucose concentration to optimize management of GC-induced hyperglycemia.

Forty consecutive consenting patients without known diabetes admitted to hospital with an exacerbation of COPD and treated acutely with GCs (Group 1, 25 men, age = 77±14 yrs, weight = 72±17 kg, prednisolone = 30±6 mg/day), 9 controls with COPD admitted for other indications and not treated with GCs (Group 2, 8 men, age = 75±11 yrs, weight = 65±13 kg) and 7 diabetic COPD patients (Group 3, 5 men, age = 84±9 yrs, weight = 87±23 kg, prednisolone = 26±9 mg/day) treated with GCs and usual diabetes therapy were studied. A continuous glucose monitoring system (CGMS, Medtronic Gold, Medtronic Minimed, Northridge, CA) was connected for up to 72 hours. The area under the curves (AUC) for glucose from 0.00 to 12.00 hrs (period A) and 12.00 to 24.00 hrs (period B) were calculated.

There were no significant differences in age, weight or HbA1c between Group 1 and 2. A higher proportion of subjects in Group 1 recorded a glucose ≥ 11.1 mmol/L during CGMS than Group 2 (20/40 vs 1/9, p=0.03). Age, weight, HbA1c and prednisolone dose were not significantly different in normo- and hyperglycemic subjects in Group 1. Group 1 had a higher AUC for period B than Group 2 (AUC = 564±138 vs 456±87 mmol/L/min, p=0.03), whereas the AUC for period A was not significantly different (Figure). Group 3 had a significantly higher AUC than Group 2 for period A and B ($p<0.005$) and a higher AUC than Group 1 for period A (p=0.001) (Figure).



In summary, GCs frequently cause hyperglycemia in hospitalized patients, predominantly in the afternoon and evening. We conclude that treatment of GC-induced hyperglycemia should be targeted at this time of the day.

1. Arnaldi G et al. J Clin Endocrinol Metab 2003; 88:5593-601

Sources of Research Support: Grants from Foundation Daw Park, Faculty of Health Sciences Flinders University and the Novo Nordisk Regional Diabetes Scheme. CGMS were supplied by Medtonic Minimed.

Nothing to Disclose: MGB, GR, NA-L, SS

P2-668

A Longitudinal Study of Plasma and Urinary Cortisol in Pregnancy and the Post-Partum Period.

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Previous studies reporting plasma cortisol (PC) and 24-h urinary free cortisol (UFC) levels during pregnancy are based primarily on cross-sectional data. There is a paucity of longitudinal pregnancy data using modern immunoassays. Furthermore, conflicting data exist as to the effect of exogenous estrogen, with some studies reporting that combined oral contraceptive pill (OCP) containing low-dose estrogen (≤35mcg ethinylestradiol or equivalent) has no effect on PC. We conducted a prospective longitudinal study on PC and cortisol binding globulin (CBG) (measured between 8-9am) and UFC levels in 20 pregnant women during the first (between 8-14 weeks' gestation), second (18-24 weeks), third (30-36 weeks) trimesters and 2-3 months post-partum, compared to 12 subjects on low-dose OCP and 15 non-pregnant subjects not taking the OCP (control group).
The results (mean ± SEM) are shown in the table. A progressive rise in PC was demonstrated during pregnancy, peaking during the third trimester (mean 2.3-fold rise compared to controls), and a similar increase in PC was seen in the OCP group. The mean UFC levels increased 3-fold by the third trimester compared to controls, whereas the mean UFC in the OCP group was not statistically different to controls. CBG levels are pending.
Our study demonstrated elevations in PC levels during pregnancy and with low-dose OCP use, presumably due to the estrogen-stimulated increase in CBG levels. Importantly, pregnancy was also associated with significant increases in UFC levels, suggesting upregulation of the maternal hypothalamic-pituitary-adrenal axis. Given our findings, we recommend that pregnancy-specific reference ranges for PC and UFC need to consider gestational age at time of testing, and the interpretation of PC levels in non-pregnant women need to consider whether patients are taking exogenous estrogens.

Group (n)	Plasma cortisol mean ± SEM (nmol/L)	24-h UFC mean ± SEM (nmol/day)
Control group (15)	332 ± 20	108 ± 15
Oral contraceptive pill group (12)	738 ± 41 *	112 ± 24
Pregnancy (20): 1st trimester	498 ± 25 *	187 ± 10 *
Pregnancy (20): 2nd trimester	672 ± 21 *	260 ± 17 *
Pregnancy (20): 3rd trimester	778 ± 32 *	323 ±19 *
Pregnancy (20): post-partum	429 ± 28 *	114 ± 12

* P <0.05 vs. control group.

Sources of Research Support: Dr C Jung was awarded a scholarship by the University of Melbourne and a research grant by St Vincent's Hospital, Melbourne.

Nothing to Disclose: CJ, JTH, DJT, JGL, WJI

P2-669

Grapefruit and Liquorice Increase Bioavailable Cortisol in Addison's Disease.

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Background and aims
Patients with Addison's disease (AD) have impaired subjective health status, and increased all-cause mortality, and overtreatment may cause osteoporosis and cardiovascular morbidity. The conventional therapies with hydrocortisone or cortisone acetate 2-3 times a day are unsuccessful in mimicking the diurnal cortisol rhythm, rendering the patient both over- and undertreated. Furanocoumarins in grapefruit juice (GFJ) are potent inhibitors of CYP3A4 and P-glycoprotein, which are involved in metabolism and membrane transport of cortisol. Liquorice derivatives, glycyrrhizic acid and glycyrrheteninc acid, are potent inhibitors of 11ß-HSD type 1 and 2. The aim of this study was to determine if liquorice and GFJ influence the absorption and metabolism of cortisone acetate.
Design
17 patients with AD on cortisone acetate replacement therapy were enrolled in an open-labelled clinical study. After baseline assessment the participants took either 200 ml GFJ 3 times a day or liquorice containing 150 mg glycyrrheteninc acid per day; they were assessed after 3 days in each protocol with serum and saliva absorption profiles and 24-hour urine collection.
Results
Compared to baseline liquorice significantly increased urinary cortisol(F):cortisone(E)-ratio (mean 0.26 vs. 0.44, $p<$ 0.01). The serum absorption profile showed significantly increased F $C_{t=160}$ (mean 202 vs. 271 nM, $p<0.05$), F $AUC_{t=0-160}$ (mean 53226 vs. 60953, $p<0.05$), and decreased E C_{max} (mean 58.1 vs. 53.1 nM, $p<0.05$). GFJ increased urinary (aTHF+THF):THE-ratio (mean 0.81 vs. 0.91, $p<0.05$). The serum profile showed significantly increased F $C_{t=160}$ (mean 202 vs. 306 nM, $p<0.01$), E $C_{t=160}$ (mean 43.6 vs. 51.7 nM, $p<0.05$), and F AUC (mean 53226 vs. 63746, $p<0.05$). The saliva profiles of F and E showed similar patterns to those in serum.
Conclusions
The results show that the widely available nutrition compounds liquorice and GFJ interact significantly with glucocorticoid replacement therapy; both increase bioavailable cortisol. Most likely, liquorice derivatives act mainly by reducing renal 11ß-HSD type 2 inactivation of cortisol. Possible explanations for the effects of GFJ are increased absorption, increased regeneration of cortisone by 11ß-HSD type 1, or inhibition of cortisol degradation by CYP3A4. Both patients and physicians should be aware of this altered cortisol metabolism, which may also be exploited therapeutically as GFJ and liquorice counteract the nadir of cortisol after tablet intake.

Nothing to Disclose: PM, KL, EAL, ESH

P2-670

Determination of Aldosterone-to-Renin Ratio in Normotensive Pediatric Population To Be Used as Screening of Primary Hiperaldosteronism.

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Primary aldosteronism (PA) is the most frequent cause of secondary hypertension in adults, prevalence between 5 to 12%, being higher in patients with severe or resistant hypertension. The PA is suspected when an aldosterone-renin ratio (ARR) is higher than 25. In paediatric population, the prevalence of PA and the normal ARR are unknown. **Aim:** To determine the normal ARR in a paediatric healthy normotensive population. **Patients and method**: A prospective clinical study in 211 healthy normotensive children from the local community with age from 4 to 15 years old (mean 10.81 ± 2.76 years), of whom 98 (46.7%) were men. All children were subjected to a complete physical evaluation which included at least 3 measurements of arterial blood pressure (BP). The BP of children and their parents were classified according the Fourth Report of Task Force and JNC 7 guidelines. Children undergoing chronic disease, severe obesity (z score >2.5) and who were taking drugs that could affect the renin-angiotensin system were excluded. After 10 minutes resting in sitting position: Plasma renin activity (PRA) and serum aldosterone (SA) were measured and ARR was calculated. Values were expressed as mean ± SD. According the BP, we divided the population into 2 groups: one constituted by normotensive children with hypertensive parents (NH), and the second one with normotensive children and parents (NN). There were no differences in body mass index, gender or age between both groups**. Results:**

Group (n)	Age (years)	SA (ng/dl)	PRA (ng/ml*h)	ARR
Total (211)	10.8 +/- 2.8	7.47 +/- 5.9	2.7 +/- 1.8	3.83 +/- 6.23
NH (113)	10.9 +/- 2.8	7.7 +/- 7.0	2.4 +/- 1.4	4.25 +/- 7.96
NN (98)	10.6+/-2.8	7.2 +/- 4.4	2.9 +/- 2.1	3.35 +/- 3.23

Only 1/211 subjects showed an ARR >25, who belonged to NH group and the genetic analysis confirmed the chimeric gene CYP11B1/CYP11B2. In the NN group, the ARR is 9.2 for percentile 95 value. **Conclusions:** This study demonstrated that the normal ARR in paediatric healthy normotensive population without hypertensive parents is lower than those communicated for adult population. Therefore, the ARR cut off used for adults seem to be too high and we propose a value of 10 as a new threshold for screening the PA diagnosis in paediatric population

Sources of Research Support: Chilean Grant Fondecyt 1100356 and 1070876.

Nothing to Disclose: RB, MA, AM-A, CC, LB, CA, CL, CC, HG, CF

P2-671

3Tesla-MRI of the Brain in Patients with Cushing´s Syndrome: Correlation between Hippocampal Volume and Memory Performance.

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Introduction: Patients with Cushing´s syndrome (CS) often present neuropsychological disturbances.
Aims: To study memory performance in patients with CS with the Rey Auditory Verbal Learning Test (*RAVLT*) memory test, and to correlate memory scores with hippocampal volume evaluated by a 3T MRI.
Material and methods: 29 right-handed patients with CS patients (10 with active disease) were evaluated. *RAVLT* provides measures of immediate and delay *memory* and verbal learning. It was administered as a part of a larger neuropsychological battery in a standard manner consisting of five learning trials, presentation of a distractor list, recall after the distractor list and recognition after 20-min, to evaluate learning capacity, immediate memory, delayed memory, and recognition.
A 3Tesla-MRI (3D-FFE) of the brain permitted automatic segmentation of subcortical structures using Freesurfer in a GRID computing facility so that volume of the hippocampi was automatically quantified with ITK-SNAP software.
Results: No differences in hippocampus volumes or *RAVLT were seen between Active and Cured CS.* The left-hippocampus volume (normalised by intracranial volume) was positively correlated with scores of memory performance measured with RAVLT ($p<0.02$). Retention index of RAVLT score (a measure of delayed memory) was most closely correlated to hippocampus volume ($p<0.001$). A linear regression to find variables predictive of delayed memory showed that last trial of RAVLT (which evaluates immediate memory, necessary to establish delayed memory) and normalized left-hippocampus were the best predictors ($R^2=0.79$, $p<0.001$).
Conclusions: We present an association between the dominant left hippocampus volume and memory performance in patients with CS. Left-hippocampus volume and immediate memory predict the delayed *memory measured with RAVLT in CS patients. Moreover, poorer immediate memory functioning correlates with smaller volumes of hippocampus, which in turn predicts the capacity to recall information (delayed memory), a common complaint of CS patients. These findings establish a relation between structural and functional characteristics of memory and opens up new approaches to relate memory functioning and cortisol levels.*

Sources of Research Support: FIS080302 and ERCUSYN PHP800200.

Disclosures: SW: Speaker, Novartis Pharmaceuticals; Study Investigator, Novartis Pharmaceuticals.
Nothing to Disclose: ER, AS, MJP, YV, PP, JY, EG, VP, BGA

P2-672

Accuracy of Several Parameters of Hypothalamic-Pituitary-Adrenal Axis Activity in Predicting the Improvement of the Metabolic Consequences after Recovery from Adrenal Subclinical Hypercortisolism.

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Background: Previous data suggest that in patients with adrenal incidentalomas (AI) and subclinical hypercortisolism (SH) the surgical treatment leads to the improvement of the metabolic syndrome. It is not known if the parameters of hypothalamic-pituitary-adrenal axis activity can predict these beneficial effects.
Patients and Methods: We retrospectively evaluated data from 55 AI patients (44 females, 11 males patients, age 29.6±11.2 years, BMI 28.1±5.6 k/m^2) who underwent monolateral adrenalectomy (size 3.4±1.2 cm). Before surgery, in all patients urinary free cortisol (UFC), cortisol after 1 mg-overnight dexamethasone suppression test (DST), ACTH, and midnight serum cortisol (MSC) were measured. During the follow-up (18-54 months), the improvement/worsening of body weight (BW), blood pressure (BP), glucose (GL) and cholesterol (CHOL) levels was defined in the presence of a >5% weight decrease/increase and following the European Society of Cardiology or the ATPIII criteria, respectively. The accuracy of UFC, DST, ACTH and MSC with different cut-offs, taken as single parameter or in combination, in predicting the improvement of at least 2 among BW, BP, GL and CHOL after surgery, was measured.
Results: The criteria characterised by the presence of at least 2 out of UFC>70 mg/24hours, ACTH<10 pg/mL, DST>3.0 mg/dL (UFC-ACTH-DST combination criteria) showed the best accuracy (sensitivity 65.2%, specificity 68.8%, accuracy 67.3%); the best sensitivity (91.3%) was reached by DST<1.8 mg/dL and the best specificity (96.9%) by DST>5.0 mg/dL (but at the expense of a <50% specificity and sensitivity, respectively). The UFC-ACTH-DST combination criteria was predictive of improvement even after adjustment for age and presence at baseline of obesity, type 2 diabetes, hypertension and dyslipidemia (OR 3.9, 95%CI 1.1-14.5, p=0.040). Furthermore, this criterion was tested in a group of 53 patients with AI followed-up for an 18-54 months period with a conservative approach. The UFC-ACTH-DST combination criteria showed the best accuracy in predicting the worsening of at least 2 among BW, BP, GL and CHOL (sensitivity 55.6%, specificity 82.9%, accuracy 73.6%) even after adjustment for age and presence at baseline of obesity, type-2 diabetes, hypertension and dyslipidemia (OR 5.3, 95%CI 1.2-234.5, p=0.028).
Conclusion: The UFC-ACTH-DST combination criterion is the most reliable in predicting the improvement of the metabolic consequences in surgically treated AI patients.

Nothing to Disclose: CEV, VM, ASS, MT, CB, FC, LI, AC, AA, LV, PB-P, MA, BA, AS, IC

P2-673

Modulation of Basal and Pulsatile Modes of ACTH and Cortisol Release after Epidural Glucocorticoid Administration.

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[1]VA Med Ctr Salem, VA and [2]Mayo Clin Rochester, MN.

While inhibition of corticotropic function is reported to occur with epidural glucocorticoid injection, the underlying mechanism(s), and time to recovery are not well defined. In the present study, pituitary-adrenal function was studied in 8 men after epidural administration of 80 mg triamcinolone. The research consisted of 4 separate sessions (baseline, 1, 4, and 12 weeks). During each visit, blood was collected in a fasting state at 10-min intervals for a period of 4 hours, with ovine CRH (1µg/Kg) injected after the 6th blood draw (min 60). ACTH and cortisol concentrations were measured in each blood sample, and their respective secretory and disappearance properties were assessed by deconvolution analysis. As a group, pre-CRH mean (± SEM) circulating concentrations of ACTH (5.7 ± 0.3 vs 25.4 ± 1.7: P <0.01) and cortisol (1.5 ± 0.03 vs 13.2 ± 0.3 ug/dL: P < 0.01) were significantly decreased at week 1. Although full recovery of ACTH occurred at week 4 (27.2 ± 2.7 vs 25.4 ± 1.7 pg/mL: P = NS), respective serum cortisol concentrations of 10.5 ± 0.3 and 11.6 ± 0.1 ug/dL at weeks 4 and 12, continued to be lower than the pre-treatment values (P<0.01). Similarly, corticotropic response to CRH stimulation was blunted at week 1 with significant decreases in the mean (± SEM) ACTH (12.8 ± 0.2 vs 41.9 ± 3.6: P <0.01) and cortisol (2.9 ± 0.2 vs 20.4 ± 0.9: P < 0.01) concentrations. Normalization of ACTH response to CRH stimulation at week 4 (39.8 ± 2.3 vs 41.9 ± 3.6: P = NS) preceded that of cortisol response, observed at week 12 (20.1 ± 0.9 vs 20.4 ± 0.9: P = NS). Deconvolution analysis of post-CRH time series did not reveal any change in ACTH and cortisol disappearance rates, but was significant for: (1) blunted pulsatile ACTH and cortisol release and decreased mass of hormone secreted per burst at week 1, while compared to the other study sessions; and (2) suppression of basal ACTH and cortisol secretion (non-pulsatile) maximally at week 1, and normalizing at week 12 for ACTH and week 4 for cortisol. In summary, epidural glucocorticoid administration markedly represses HPA output via suppression of both basal and pulsatile modes of ACTH and cortisol release. The average recovery time appears to be 4 weeks for ACTH, and potentially 12 weeks or more for cortisol. These inferences warrant future confirmation in a larger patient population.

Sources of Research Support: Salem V.A. Medical Center Research Institute.

Nothing to Disclose: AI, DG, BD, JDV

P2-674

Amplified Mass of Hormone Released Per Burst as the Mechanism for Augmented Corticotropic Activity after Oral Dextrose Intake.

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While corticotropic activation is uniformly observed with fasting, reports of nutrient modulation of the HPA axis are sporadic and inconclusive. In the present study, the corticotropic response to oral dextrose administration was assesses in 32 healthy men in the age range of 19-78 yrs and BMI 21-38 Kg/m^2. Each subject was studied after an overnight fast on 2 separate occasions 1-4 weeks apart. During each session, blood was drawn at 10-min intervals starting at 0800-0900 hr for a total period of 6.5 hrs, with either 75 grams of dextrose or identical volume of water ingested orally after the 4th blood draw at min 30. Circulating concentrations of ACTH (pg/mL) and cortisol (µg/dL) were measured in each blood sample, and their respective secretory and disappearance properties were assessed by deconvolution analysis. Nonparametric statistics were used for comparison of the data. As a group, oral intake of dextrose was associated with significant increases in the mean (±SE) 6.5 hr sum mass of ACTH (886 ± 78 vs 737 ± 52: P=0.006) and cortisol (490 ± 25 vs 422 ± 19: P=0.004). Deconvolution analysis of the 6.5 hr time series was significant for post-dextrose increases in pulsatile release (ng/L/6.5 h) of ACTH (128 ± 23 vs 88 ± 16: P=0.03) and (µg/dL /6.5 h) cortisol (52 ± 4.3 vs 39 ± 2.6: P=0.006) associated with corresponding increases in the masses of ACTH (38 ± 7.8 vs 20 ± 3.9 ng/L: P=0.004) and cortisol (14 ± 1.3 vs 11 ± 0.9 µg/dL: P=0.02) secreted per burst. While basal secretion was not affected for either ACTH or cortisol, only ACTH pulse frequency (3.9 ± 0.25 vs 4.5 ± 0.22: P=0.04) was negatively affected by dextrose administration. Nutrient-induced changes in ACTH and cortisol were independent of age and BMI per regression statistics. In summary, the results of this study demonstrate corticotropic stimulatory effect of oral glucose, characterized by augmented pulsatile secretory mode with increased mass of ACTH and cortisol secretion per burst. Generalization of these findings to other nutrients/mixed meals, and potential inter-individual variability warrant future investigation

Sources of Research Support: Salem V.A. Medical Center Research Institute.

Nothing to Disclose: DL, BD, JDV, AI

P2-675

Hair Cortisol Levels in Patients with Adrenal Insufficiency on Glucocorticoid Replacement Therapy.

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INTRODUCTION: Patients with adrenal insufficiency require life-long treatment with exogenous glucocorticoids, such as hydrocortisone and prednisone, to replace this deficiency. Several studies have shown impaired subjective health status in these patients, which may be caused by glucocorticoid over-replacement. To improve assessment of long-term effects of glucocorticoid replacement on health, availability of a method that would measure long-term systemic glucocorticoid exposure would be useful. Our laboratory has developed a technique to measure cortisol in hair to obtain a historical record of systemic cortisol exposure. The objective was to compare hair cortisol content in patients receiving glucocorticoid (specifically hydrocortisone) replacement therapy with levels in control subjects. **METHODS**: Using a mail-out study approach, hair samples, participant demographics, medical history and perceived stress questionnaires were collected from 57 patients across North America, diagnosed with primary or secondary adrenal insufficiency. The patient data was compared to a matched control group (N=57) of subjects residing in the same household. Cortisol was measured from the most proximal 2cm of hair, representing the most recent 2 months of exposure. A modified cortisol enzyme immunoassay was used for analysis. **RESULTS:** Hair cortisol concentrations, reported as median (range), were 222.0 (22.7-1438.0) ng/g and 182.0 (57.7-1479.0) ng/g in the patient and control groups, respectively ($P>0.05$). Although these findings were not significant, the patient and control groups differed in their perceived stress scale scores of 16.2 ± 7.2 and 12.7 ± 6.2, respectively (mean ± SD, N=56, $P<0.005$). **CONCLUSIONS**: We found that hair cortisol concentrations of patients with adrenal insufficiency were not significantly different than their control group. However, the patients' perceived stress scores were significantly greater than their matched controls, a finding which is consistent with previous studies. As most data is based on prescribed cortisol dosing or short-term cortisol levels, this study will provide clinicians with further information regarding long-term exposure to glucocorticoids. The trend toward higher cortisol levels in the patient group may suggest some are over-treated and, hence, may be at risk for the adverse effects of cortisol. Further research with a greater sample size may be needed to detect this important difference.

Nothing to Disclose: RSG, MJR, GK, SVU

P2-676

Stimulated Free Salivary Cortisol Correlates with Body Composition Markers of Fat Mass in Healthy Non-Obese Men and Women.

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Objective: The metabolic syndrome has several similarities with Cushing's syndrome suggesting a link between the hypothalamic-pituitary-adrenal (HPA) axis and the metabolic syndrome in pathological conditions. We hypothesized that high peak total and free salivary cortisol during physiological stress (i.e. hypoglycaemia), would be associated with higher total and regional fat mass even in healthy non-obese men and women.

Subjects and Measurements: Thirty healthy subjects (15 men) with a median BMI of 24 kg/m^2 (range 19 – 30) underwent an insulin tolerance test (ITT), with plasma and salivary sampling at 0, 15, 30, 45, 60, 75 and 90 min. Body composition was assessed by: BMI, waist-to-height (WHt) ratio, total and abdominal fat mass (FM), total fat % and total lean mass (TLM) as measured by dual energy X-ray absorptiometry.
Results Baseline and peak salivary cortisol were positively correlated with baseline ($r=0.75$; $p<0.01$) and peak ($r=0.50$; $p=0.01$) plasma cortisol. No gender difference was observed ($p>0.2$), and no association was observed as regard Se-estradiol, -testosterone, and -thyroid hormone levels (all $p>0.1$). Further, no correlation was observed between baseline salivary, and baseline /peak plasma cortisol and body composition markers. In contrast, mean peak salivary cortisol was associated positively with BMI ($r=0.45$; $p=0.03$), WHt ($r=0.43$) and body composition markers of fat mass (totalFM: $r=0.51$; TF%: $r=0.43$; abdFM: $r=0.45$; all $p<0.04$), whereas not to TLM ($r=-0.06$; $p=0.8$).
Conclusion Our data indicate that peak free salivary cortisol is associated with body composition markers of fat mass. These results may indicate a physiological relationship between the HPA axis regulation and lipid metabolism even in healthy non-obese individuals, but finding the mechanism requires further studies.

Nothing to Disclose: MCK, LH, UF-R

P2-677

Dose Individualization of Cortisol Replacement Therapy in Adrenal Insufficiency with a Dual-Release Hydrocortisone Formulation Using Body Weight and Pharmacokinetic Nomograms Derived from Population Pharmacokinetic Modelling.

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[1]Uppsala Univ Uppsala, Sweden and [2]Sahlgrenska Academy, Univ of Gothenburg Gothenburg, Sweden.

Patients with adrenal insufficiency receiving glucocorticoid replacement therapy have sub-optimal outcomes with increased cardiovascular mortality and reduced quality of life. Dose monitoring is currently based only on clinical judgment since a biomarker of glucocorticoids is lacking. We have developed a dual release hydrocortisone (HC) tablet for oral replacement therapy and dose nomograms to facilitate individualized dosing.
Methods: Cortisol serum concentration time profiles were performed in 62 patients after administraton of oral once daily dual release HC tablets (20-60 mg) on 116 occasions, and in healthy volunteers (after betametasone suppression) after single dose administration of 5 (n=14) and 20 mg (n=16). All data was simultaneously modeled using population pharmacokinetic (PK) modeling approach in NONMEM. The model predicted HC exposures in a typical patient after different doses, which were compared to normal endogenous exposure in order to define a clinical exposure target. Based on this clinical exposure target and the model, a weight and a PK nomogram were developed.
Results: Oral clearance was estimated to increase linearly with increasing weight thus forming the basis and rationale for the weight nomogram. The PK profile simulated using the weight nomogram and the actual exposure profile from the subjects studied were on average identical (2% difference). The unexplained between-patient variability in clearance was 26%. The weight nomogram reduced the risk of patients receiving too high HC doses. The difference in serum cortisol concentration before and 6 hours post-dose was found to be the best input into the PK nomogram. The PK nomogram is a more precise dosing tool compared to the weight nomogram as it takes into account endogenous cortisol levels and unexplained between-patient variability in cortisol clearance.
Discussion: Population PK modeling and simulation is a powerful tool for guidance in glucocorticoid dosing. The derived nomograms provide an opportunity for informed, objective dose individualization. The weight nomogram provides an accurate dosing recommendation whereas the nomogram based on serum cortisol sampling is a more accurate tool adjusting for endogenous serum cortisol concentrations and interindividual variations in cortisol clearance. Nomograms for HC replacement can optimize individual replacement doses and thus improve long-term outcome of patients with adrenal insufficiency.

Sources of Research Support: DuoCort Pharma AB.

Disclosures: USHS: Coinvestigator, DuoCort Pharma. SS: Founder, DuoCort Pharma. HL: Founder, DuoCort Pharma. GJ: Founder, DuoCort Pharma.

P2-678

Night Shift Work Affects Circadian Rhythm of Cortisol, Cortisone, and DHEA.

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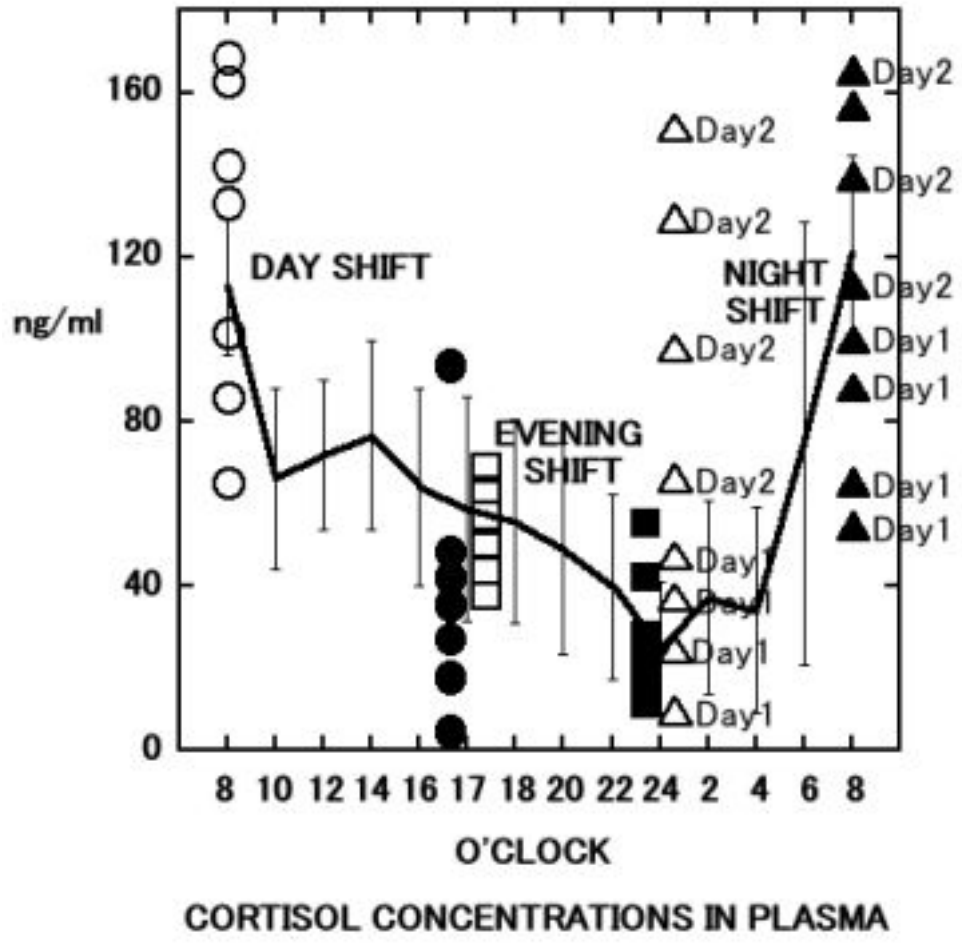
Introduction
Working at night is associated with increased risk of cardiovascular disease, peptic ulcer disease and breast cancer. To examine the influence of shift work, concentrations of cortisol, cortisone, and DHEA in plasma and saliva were compared based on working conditions.
Material and Methods
Twenty-one nurses who enrolled voluntarily in this study were healthy and had regular menstrual cycles. The nurses worked three different shifts from 8:00 to 17:00 (day shift, N=7), from 17:00 to 24:00 (evening shift, N=6), and from 24:00 to 8:00 (night shift, N=8). Blood and saliva were obtained from each nurse at the start and end of of their shift. The effects of shift work were evaluated by comparing hormone concentrations of plasma and saliva obtained every two hours from 8:00 for 24hours and at 17:00 from six off-duty nurses. The data from the off-duty nurses were served as a control group to see the circadian rhythms. Concentrations of cortisol, cortisone and DHEA were determined by LC-MS/MS.
Results
Circadian rhythms of cortisol, cortisone and DHEA were observed in plasma and in saliva simultaneously. The nadir of these hormone concentrations was observed at 0:00 A.M. and the peak was at 8:00 A.M. These concentrations in saliva were well correlated with those in plasma, respectively. All hormone concentrations at the end of the day shift did not differ from those of the control group. All hormone concentrations of those in the evening shift did not differ from those of the control group. Despite finding no differences in any of the hormones at the end of the first day of the night shift, all hormone concentrations at the beginning of the second day of the night shift were significantly elevated. Concentrations of cortisone and DHEA were also elevated at the end of the second day of the night shift.



Conclusions
Circadian rhythms of cortisol, cortisone, and DHEA were maintained during the day shift and evening shift, but were affected by night shift work. Working at night for 8 hours might affect the circadian rhythms of these hormones and this disorder might persist for at least 16 hours.

Nothing to Disclose: FM

P2-679

Quantitative Analysis of Late-Night Salivary Cortisol and Urinary Free Cortisol in Diagnosis of Cushing's Syndrome (CS).

BFC Barone[1], PCL Elias MD, PhD[1], M Castro M.D, PhD[1] and AC Moreira MD, PhD[1].

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Diagnosis of clinical hypercortisolism is dependent on biochemical tests. Because of the variability of hypercortisolism in CS, the recent guideline recommends at least two measurements of late-night salivary (SF) or urinary (UFC) cortisol, with the cut-offs of late-night SF>150ng/dl and UFC>43 ug/24h (upper limit of normal range - ULNR) being suggestive of CS (1). The present study compared the variability of SF and UFC in order to increase the confidence of these tests. We evaluated 38 patients with confirmed hypercortisolism (33F and 5M, mean age 32 yrs; range: 9-69 yrs). Late-night SF and UFC samples were concomitantly obtained up to three consecutive days in all subjects, with a total of 114 SF and UFC samples. SF measurements were performed by RIA with assay sensitivity of 60ng/dl. UFC was measured by HPLC, with a normal range of 0.3 to 43.0ug/24h. Using ACTH levels, high dose DST, oCRH test, bilateral simultaneous inferior petrosal sinus catheterization, when appropriated, and imaging, 25 patients had diagnosis of Cushing's Disease (CD), confirmed by transsphenoidal surgery; whereas 13 patients had ACTH independent adrenal Cushing´s syndrome (adrenal CS): 8 adenomas; 2 carcinomas, 2 macronodular adrenal hyperplasia, and one Carney Complex, all submitted to adrenalectomy. Mean late-night SF were 3003±2695 ng/dl (P5-P95: 709-8432) on CD and 1678±1419 ng/dl (P5-P95: 164-4385) on adrenal CS (P<0.0005). UFC were respectively 522±1303 ug/24h (P5-P95: 18.5–2467) and 216±257 ug/24h (P5-P95: 16.7–801) (P<0.02). There was a positive correlation between late night SF and UFC (r=0.59; P<0.0001). All patients with CD and all but one with adrenal CS had all samples of SF>150 ng/dl. However, 22 in 25 CD patients and 8 in 13 adrenal CS patients had UFC samples >43 ug/24h. Moreover, we also used a more stringent cut-off criteria: SF>350 ng/dl and UFC>129 ug/24h (3xULNR) (2). All CD patients and 7/13 adrenal CS patients presented all SF samples >350ng/dl, and 16/25 CD and 4/13 adrenal CS patients had UFC >129ug/24h. The mean relative increase of SF was 16.9x and UFC 9.2x based on the ULNR (P<0.0001). SF and UFC coefficients of variation (CV) were 36.5% and 31% (NS). Our data indicate that although the variability of SF and UFC are similar, the increment of SF is higher than UFC in CS. These quantitative analyses on SF and UFC support the best performance of SF compared to UFC in the initial evaluation of Cushing's syndrome.

(1) Nieman LK et al, JCE&M 2008; 93:1526
(2) Castro M & Moreira AC, Arq Bras Endocrinol Metab 2007; 51:1191

Sources of Research Support: FAPESP and CNPq.

Nothing to Disclose: BFCB, PCLE, MC, ACM

P2-680

Usefulness of the 10:00-11:00 PM Urinary Cortisol/Creatinine Ratio *Versus* Late-Night Salivary Cortisol To Diagnosing Cushing's Syndrome.

OD Bruno MD[1], MA Rossi[1], MC Albiero MD[1] and L Juarez-Allen MD[1].

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Measurement of late-night saliva cortisol (LNSC) is one of the best tests for detection of Cushing's syndrome (CS) because of its simplicity for collection and its high levels of sensitivity and specificity. However, this test is not widely available in routine laboratories, at least in Argentina. We have communicated our results by measuring the urinary cortisol/creatinine ratio (UCCR) in spot 10:00-11:00 PM samples compared with 11:00 PM saliva cortisol in different groups of normal and obese people and Cushing's patients[1]. In this communication, we compared UCCR and LNSC obtained simultaneously in controls, obese and Cushing's patients. Twenty three patients with CS (15 females, 8 males; 24-65 year-old) (9 pituitary-dependent, 3 adrenal, 11 undetermined), 27 normal volunteers aged 23-64 years (18 females, 9 males) with normal body mass index and 28 obese patients aged 23-75 years (25 females, 3 males) were investigated. They were told to have dinner at 08:00 PM, drink water normally, not to brush their teeth and void urine at 10:00 PM. Then, they collected saliva samples at 10.30 PM into a plastic tube and urine at 11:00 PM into a recipient containing 50 mg sodium borate. Both, saliva and urine samples were kept at 4º C until delivered to the laboratory. Saliva cortisol was measured by RIA (DPC) using a standard curve adjusted to read values between 0.69 and 138 nmol/L. Urinary cortisol was measured by RIA after extraction with dichloromethane and creatinine measured by the method of Jaffe; results were expressed in ng cortisol/mg creatinine. Saliva cortisol values were 1.8 $\pm$1.2 (mean $\pm$ SD) nmol/L in normals, 2.4 $\pm$1.5 in obese and 24.8$\pm$16.8 in CS patients ($p< 0.001$vs. normals and obese). Urinary cortisol was 12.2 $\pm$ 8.5 ng cortisol/mg creatinine in normals, 10.9 $\pm$ 8.1 in obese and 400.8 $\pm$ 381.8 in CS ($p< 0.001$ vs. normals and obese). All data obtained correlated with an r value of 0.85, $p< 0.001$ ($n= 78$). Sensitivity and specificity for diagnosis of CS were comparable for saliva and urine cortisol (91/96% and 100/96%, respectively). In conclusion, simultaneous measurement of LNSC and the CCR showed comparable high sensitivity and specificity figures which fulfill the necessary requirements to diagnose CS. The measurement of spot 10:00-11:00 PM urine cortisol/creatinine ratio is easy to perform, more readily available in routine laboratories and constitutes a valuable alternative to late night salivary cortisol in the study of Cushing's syndrome.

(1) Rossi MA, Albiero C, Juarez-Allen L, Bruno OD. Evening 10-11 PM urinary cortisol/creatinine ratio: an alternative to midnight salivary cortisol in the diagnosis of Cushing's syndrome. Workshop: Novel insights in the management of Cushing's syndrome.European Neuroendocrine Association (ENEA, Napoli, Italy, December 4-6, 2009.

Nothing to Disclose: ODB, MAR, MCA, LJ-A

P2-681

Assessment of the Circadian Rhythm of the HPA Axis in End-Stage Renal Disease (ESRD) Using Salivary and Serum Cortisol.

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Assessment of late-night salivary cortisol is now standard in the diagnosis of endogenous Cushing's syndrome along with measurement of 24-hour urine free cortisol and low-dose dexamethasone-suppressed serum cortisol. The latter two tests are problematic in patients with renal failure because of anuria and changes in dexamethasone metabolism, respectively. We hypothesized that measurement of late-night salivary cortisol would be a valid approach to evaluate ESRD patients for Cushing's syndrome. However, assessment of late-night salivary cortisol has not been adequately validated for use in screening patients with ESRD for endogenous Cushing's syndrome. We evaluated the circadian rhythm of cortisol in 7 patients (6 male/1 female; aged 45-62 years) with ESRD on chronic hemodialysis and with no known history of adrenal disease. Patients were admitted to the Translational Research Unit the night before sampling was started; an IV catheter for blood sampling was inserted at 0700 the next morning. Then, on this non-dialysis day, blood was sampled every two hours from 0800 hr to 0600 hr the next morning. Saliva was sampled every two hours from 0800 to 2200 hr (bedtime) and then on awakening at 0600 hr. Indices of HPA circadian activity were plasma cortisol at 2400 hr, salivary cortisol at 2200 hr, and the ratio of 0800 hr to 2200 hr plasma or saliva cortisol. Salivary cortisol (y-axis) correlated well with plasma cortisol (x-axis); slope = 0.032±0.004; y-intercept not different from 0 nmol/L; N=63. All four indices of HPA axis rhythm were abnormal in 3 of 7 ESRD subjects indicating a failure to achieve a normal late night nadir. In these 3 patients, plasma cortisol at 2400 hr was 257-325 nmol/L (normal <210 nmol/L), salivary cortisol at 2200 hr was 10.8-15.1 nmol/L (normal <6.0 nmol/L), and the 0800 to 2200 hr ratios were 0.7-1.2 for plasma cortisol and 0.4-0.6 for salivary cortisol (normal >2). All four indices of HPA axis rhythm were normal in the other 4 patients with ESRD. We conclude that salivary cortisol is an excellent surrogate for plasma cortisol in ESRD patients. A significant number of ESRD patients appear to have abnormal HPA axis activity such that an elevated late-night plasma or salivary cortisol level must be interpreted with great caution.

Sources of Research Support: Lemann Endowment Grant.

Nothing to Disclose: HR, HT

P2-682

Growth Pattern in the First Three Years of Life in Children with Classical Congenital Adrenal Hyperplasia (CAH) Diagnosed by Newborn Screening and Treated with Low Doses of Hydrocortisone (HC).

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Context: Linear growth is the best clinical parameter for monitoring metabolic control in classical CAH. Since newborn screening is available nowadays, diagnosis of CAH is no longer delayed and since it is clear that children with CAH are relatively androgen insensitive during the first two years of life, children with CAH are treated with lower doses of HC today.
Objective: To analyze growth pattern in children with CAH diagnosed by newborn screening and treated with relatively low doses of HC during the first three years of life.
Patients: 51 patients (27F, 24M) were diagnosed with classical CAH by newborn screening. Diagnosis was confirmed by mutation analysis of the *CYP21A2* gene in all patients. Patients were treated with relatively low doses of HC (9-15 mg/m² BSA). 47 patients were treated with fludrocortisone additionally.
Results: At birth height SDS (H-SDS) was 1.1±1 in girls, and 0.9±1.5 in boys, respectively. After three months H-SDS decreased to 0.4±0.9 SDS in girls and to 0.1±1.3 SDS in boys. Over the three year period H-SDS further decreased to -0.4±1.8 SDS in girls and to -0.8±1 SDS in boys and approached the genetic height potential (target H-SDS of girls -0.5±0.3 and target H-SDS of boys -0.9±0.7). At the age of 9 months growth velocity was slightly decreased in girls (18.2±1.9 cm) and in boys (17.3±1.6 cm) when compared to a healthy reference population (girls 19.0±3.9 cm and boys 18.7±4.7 cm, Prader et al). During the second and third year of life growth velocity (GV) was in the normal range (GV second year: CAH girls 11.9±1.4 cm, CAH boys 12±1.7 cm versus reference girls 11.1±2 cm and reference boys 11.0±3.5 cm, GV third year: CAH girls 8.6±1.4 cm, CAH boys 8.1±2 cm, versus reference girls 8.5±1.5 cm and reference boys 8.3±1.3 cm).
With “low-dose” HC treatment mean plasma 17-hydroxy-progesterone levels were still elevated at 3 months and subsequently decreased to the treatment goal of below 600 ng/dl. At the age of 3 years bone age was appropriate for chronological age in both girls (BA 2.7±0.5 years) and boys (BA 2.9±0.5 years). BMI-SDS was average over the study period (BMI-SDS at 3 years: 0±1 SDS in girls versus -0.1±1.1 SDS in boys).
Conclusion: Birth length is above average in children with classical CAH, which might be the result of untreated hyperandrogenism in utero. With relatively low doses of HC treatment growth velocity decreases slightly during the first nine months and H-SDS then approaches the genetic height potential.

Nothing to Disclose: WB, HS, HPS

P2-683

Females with Congenital Adrenal Hyperplasia (CAH) and Hyperandrogenism Have Similar Measures of Insulin Sensitivity Compared to Females with CAH and Normal Androgen Levels.

MK Crocker MD, MBA[1], SP Mehta BS[2], CA Van Ryzin NP, CPNP[1,2] and DP Merke MD, MS[1,2].

[1]The Eunice Kennedy Shriver Natl Inst of Child Hlth and Human Development, Natl Inst of Hlth Bethesda, MD and [2]Natl Inst of Hlth Bethesda, MD.

Context: Patients with congenital adrenal hyperplasia (CAH) have been reported to have lower insulin sensitivity than healthy controls.(1) The role of androgens in insulin resistance is unknown; however, an interrelationship between hyperandrogenism and hyperinsulinism has been described in patients with polycystic ovary syndrome.
Objective: The aim of the study was to evaluate the insulin-androgen relationship in CAH by comparing measures of insulin resistance between hyperandrogenemic and normoandrogenemic female patients.
Design: Females over the age of 12 with genetically confirmed CAH who were seen at the National Institutes of Health Clinical Center were retrospectively identified as hyperandrogenemic if their testosterone concentrations were greater than the normal range for age and pubertal stage. Hyperandrogenemic patients were age- and phenotype- (salt-wasting, simple virilizing, or non-classic) matched with normo-androgenemic females with CAH. Women on antiandrogen or diabetic therapy were excluded.
Outcome Measures: Clinical and hormonal values were compared between groups including fasting insulin and glucose, homeostasis model assessment of insulin resistance (HOMA-IR), and body mass index (BMI).
Results: Nine women with high testosterone concentrations and twenty matched controls were identified. Average age was similar between the two groups (hyperandrogenemic *vs.* normoandrogenemic: 22.2 ± 4.2 *vs.* 22.2 ± 6.7 years, p=0.99). The difference in total testosterone concentration was significant (122.8 ± 60.1 *vs.* 27.1 ± 19.6 ng/dL, p=0.001), as was the difference in free testosterone concentration (3.1 ± 1.4 *vs.* 0.9 ± 1.7 ng/dL, p=0.004). Both groups were on similar doses of glucocorticoids for body surface area (12.1 ± 9.1 *vs.* 17.1 ± 9.4 mg/m2/day hydrocortisone equivalents, p=0.19), and a similar number were receiving oral contraceptive pills (22% *vs.* 25%, p=1.0). Average fasting insulin concentrations (11.1 ± 2.9 *vs.* 10.8 ± 5.7 microunits/mL, p=0.67), fasting glucose concentrations (85.8 ± 5.5 *vs.* 85.5 ± 8.8 mg/dL, p=0.92), HOMA-IR (2.6 ± 1.8 *vs.* 2.4 ± 1.3, p=0.83), and BMI (29.8 ± 6.8 *vs.* 26.4 ± 6.3 kg/m2, p=0.2) were not statistically different between the two groups.
Conclusion: Measures of insulin resistance did not differ between hyperandrogenemic and normo-androgenemic women with CAH, suggesting that hyperandrogenism does not play a major role in the insulin resistance observed in some patients with CAH.

(1) Kim M, Merke DP, Semin Reprod Med 2009; 27: 316

Sources of Research Support: The Intramural Research Programs of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), and the National Institutes of Health Clinical Center; The Congenital Adrenal Hyperplasia Research, Education and Support (CARES) Foundation; DPM is a Commissioned Officer in the United States Public Health Service.

Nothing to Disclose: MKC, SPM, CAVR, DPM

P2-684
LH and Adrenal Function in Postmenopausal Women.

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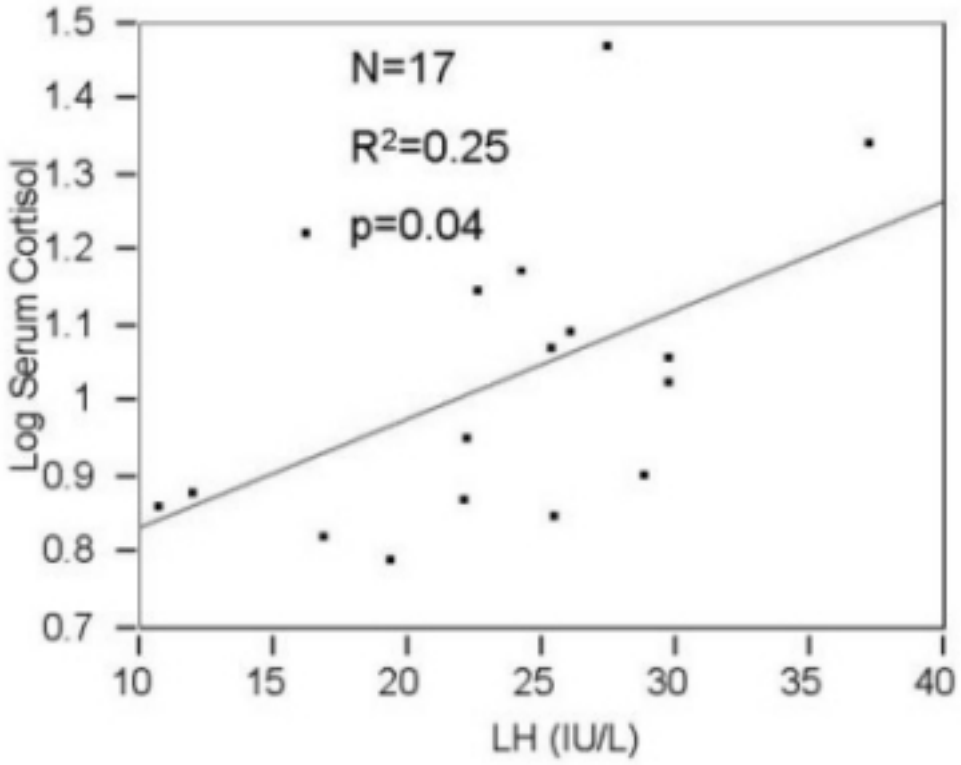
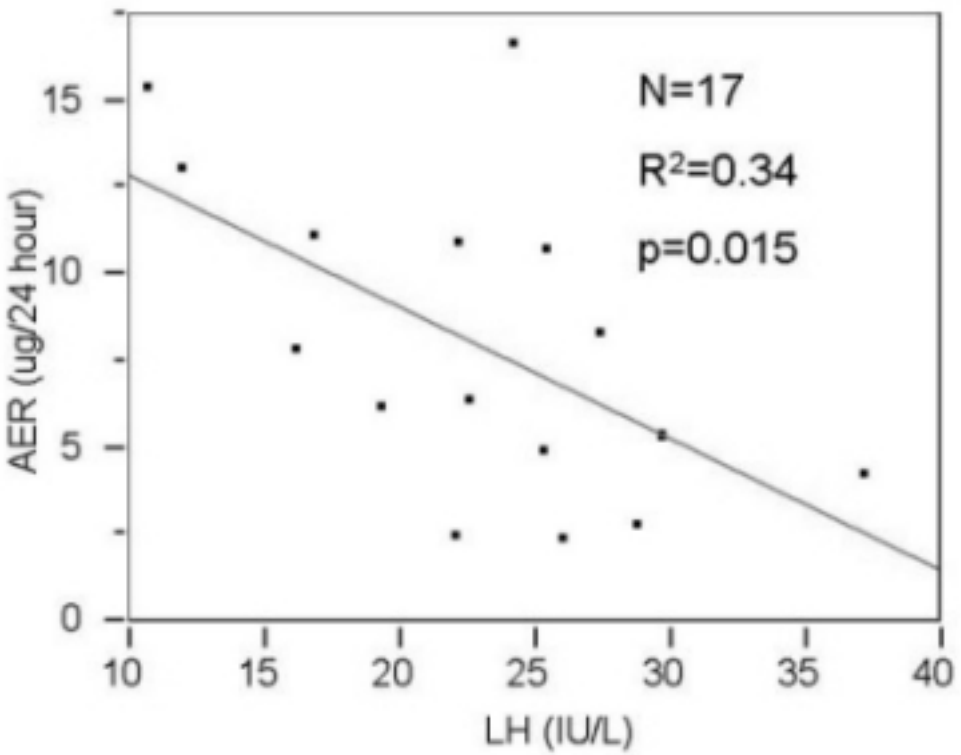
[1]Brigham and Women's Hosp Boston, MA.

In postmenopausal women, a relationship between luteinizing hormone (LH) and cortisol levels has been suggested. LH receptors on the adrenal gland have been shown to mediate adrenocorticotropin hormone (ACTH) -independent Cushing's Syndrome. In contrast, FSH receptors have not been found on the adrenal gland.
We tested the hypothesis that in the postmenopausal state, where LH levels are persistently elevated, secretion of cortisol and aldosterone is also increased.
We studied 17 hypertensive postmenopausal women in high sodium balance to control for confounding effects of the endogenous renin-angiotensin system. Twenty-four hour urine samples were collected to confirm high sodium balance. Serum cortisol, aldosterone, LH and FSH levels were measured. Twenty-four hour urinary free cortisol (UFC) and aldosterone excretion rates (AER) were also measured. Comparisons of LH and FSH levels to levels of serum cortisol, serum aldosterone, UFC and AER were performed by calculation of Pearson's correlation coefficient.

In these women serum LH correlated significantly with log-transformed serum cortisol (r^2 = 0.25, p=0.04) in high sodium balance (mean Urinary sodium 235 mmol/24 hour). In addition, serum LH had a significant inverse correlation with measured AER (r^2 = 0.34, p=0.015). We found no correlation of LH with serum aldosterone or with 24-hour UFC. In addition, we did not find any correlation between FSH levels and serum cortisol, serum aldosterone, UFC or AER.





In hypertensive, postmenopausal women, serum LH levels correlate significantly with serum cortisol levels (positively) and urinary AER (negatively). LH stimulation of the adrenal gland may shift adrenal activity toward cortisol secretion. Elevations in LH may play a role in the development of intermediate phenotypes of hypertension and cardiovascular disease in older women.

Nothing to Disclose: ARS, EWS

P2-685

The Dynamics of Post-Operative Serum ACTH Values Following Transsphenoidal Surgery for Cushing's Disease.

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Introduction: Rapid assessment of adrenal function is critical following transsphenoidal surgery (TSS) for Cushing's disease (CD) in order to determine surgical efficacy. Because serum ACTH declines after successful surgery, we hypothesize that there may be a role for ACTH measurement as a rapid indicator of adrenal function in the postoperative assessment for CD.
Objective: To assess ACTH dynamics in conjunction with serum cortisol measurements following TSS in patients with CD.
Methods: Following surgery, glucocorticoids were withheld and paired serum ACTH and cortisol levels were measured every 6 hrs. Post-operative hypocortisolemia was defined as serum cortisol <5 µg/dl. Multiple linear regression analyses were used to determine if post-operative decreases in ACTH level at different time point were associated with remission.
Results: We studied 12 subjects, all female, mean age 44.6 years (range 25 – 55), including 13 operations. 9 subjects attained hypocortisolism. Serum ACTH levels decreased more in subjects with hypocortisolemia (0.9 pg/mL per hour, $p = 0.0028$) vs. those with persistent disease (0 .2 pg/mL per hour, $p = 0.26$) within the first 48 hours after surgery. In all patients with hypocortisolemia, a sustained serum ACTH value < 20 pg/mL was noted by 19 hours postoperatively (range 1 – 19 hours). Four of the 9 patients with hypocortisolemia achieved serum ACTH values < 20 pg/mL by 13 hours and the remaining 5 patients achieved this value by 19 hours. The sole subject who underwent a total hypophysectomy had ACTH < 20 pg/mL at 1 hour after TSS. Hypocortisolemia occurred between 3-36 hr following achievement of a serum ACTH < 20 pg/ml. None of the patients with persistent disease had a sustained ACTH value < 20 pg/mL.
Conclusions: A reduction in postoperative serum ACTH levels differentiates subjects with surgical remission from CD versus patients with persistent disease. In addition, an absolute serum ACTH value < 20 pg/mL precedes attainment of hypocortisolemia and may be of prognostic importance, supporting use of ACTH measurements in the postoperative management of CD.

Nothing to Disclose: LS, MM, KK, ERL, RLD, OC, LK

P2-686

Time and Factors Associated with Recovery of the Hypothalamic-Pituitary-Adrenal Axis after Withdrawal of Exogenous Steroids.

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[1]Tan Tock Seng Hosp Singapore, Singapore.

Aims: Prolonged use of exogenous steroids either in the form of medical formulations or traditional Chinese medications leads to a severe and sustained suppression of hypothalamic- pituitary –adrenal (HPA) axis with manifestation of Cushing's syndrome. Data on the factors associated with the recovery of the HPA axis is limited. The aim of the study is to illustrate the response of HPA axis to a tapering dose of corticosteroid and to identify predictable factors and the time associated with the recovery.
Methods: A retrospective study of the entire patient cohort with exogenous Cushing's syndrome from June 1999 to December 2008 was done with acquisition of all relevant data.
Results: Using the criteria of a suppressed 8 am cortisol of less than 260 mmol/L, 63 patients were identified. The median age was 65 (57-74.5), numbers of male patients 30 (47.6%). The reasons cited for the use of exogenous steroids included use of traditional Chinese medications either for general well being or osteoarthritis of the knee-37 (57.8%), iatrogenic steroids for skin problems such as eczema or psoriasis -16 (25.4%), and connective tissue disorders 5 (7.9%). Full blown features of Cushings syndrome was present in 45 (71.4%) of patients. Out of these 32 patients also had a low initial ACTH <4.5pmol/L level. Only 27 (42.9%) patients had a full recovery of HPA axis (defined as normal ACTH 0 pmol/L to 10.2 pmol/L, cortisol >260mmol/L and a normal short synacthen test 60minutes cortisol >550mmol/L). Thirteen of the patients who had a full recovery had an initial rise of ACTH (6.4pmol/L to 36.9pmol/L) during replacement. The median duration to complete recovery was 22 months (16-38). The median duration ACTH rise was 10 months (7-13) and the median duration to complete recovery after ACTH rise was 9 months (5-14).
Conclusion: The above suggests the HPA axis recover with an initial surge of ACTH after a period of suppression. Hence ideally these patients should be given a morning physiological dose of hydrocortisone and followed up 3 monthly with ACTH and cortisol levels. Once the ACTH level rises, recovery is certain and further trailing down may be done until full recovery of the axis

Nothing to Disclose: HTK, RD

P2-687

Post-Operative Day 3 Cortisol as a Predictor of HPA Axis Integrity after Transsphenoidal Surgery.

Maryam I Khan MD[1,2], Ian E McCutcheon MD[1], Graciela M Nogueras-Gonzalez MD[1], Jessica K Devin MD[1], Camilo Jimenez MD[1], Mouhammed A Habra MD[1], Nicholas B Levine MD[1] and Steven G Waguespack MD[1].

[1]Univ of Texas MD Anderson Cancer Ctr Houston, TX and [2]Baylor Coll of Med Houston, TX.

INTRODUCTION: Early assessment of the hypothalamic-pituitary-adrenal (HPA) axis after transsphenoidal surgery (TSS) is essential for determining the need for corticosteroid replacement in these patients at risk for adrenal insufficiency (AI). Morning serum cortisol in the immediate post-op period correlates with long-term outcomes, but the exact cortisol level that accurately predicts HPA axis integrity remains unknown.
OBJECTIVE: To determine if a post-operative day #3 (POD3) AM cortisol ≥10 mcg/dl is predictive of long-term adrenal sufficiency (AS). Secondary objectives were to explore other cortisol cut offs as better predictors of long-term AS.
METHODS: We retrospectively reviewed case records of 486 TSS patients to determine study eligibility. Serum cortisol measured at POD3 was compared with results of dynamic assessment of adrenal function 3-10 weeks after TSS and random cortisol levels measured at least 6 months after TSS. AS was defined as a peak cortisol ≥18mcg/dl during dynamic testing, typically with a 1 mcg cosyntropin stimulation test. AI was defined as a peak cortisol <18 mcg/dl.
RESULTS: Eighty-four patients were eligible for the study. POD3 cortisol was measured a mean of 21 hours (range: 17.5-31) after the last dose of hydrocortisone. Of the 47 patients who had POD3 cortisol ≥10, 36 (77%) had AS whereas 11 patients (23%) had AI at a mean of 43.0 days post TSS. Of the 37 patients with a POD3 serum cortisol <10, 19 (51%) had AS and 18 (49%) had AI at a mean of 41.3 days post TSS. The diagnostic sensitivity of a POD3 serum cortisol ≥10 mcg/dl for prediction of early AS was 65 %(95%CI: 0.51-0.78), with an odds ratio of 3.1 (95%CI 1.1-8.80). Diagnostic specificity was 62 %(95%CI: 0.42-0.79). Decreasing the POD3 cortisol cut off to ≥8.5mcg/dl resulted in a sensitivity of 84% and specificity of 52%. At a mean follow up of 987 days (range: 190-4455), only 2 patients with POD3 cortisol ≥10 required corticosteroid replacement, both of whom had multiple anterior pituitary hormone deficiencies and evidence of pituitary dysfunction in the perioperative period.
CONCLUSION: POD3 cortisol ≥10 mcg/dl appears to be a reliable predictor of HPA axis integrity, correctly identifying 45/47 (96%) patients with long-term AS. In the absence of clinical symptoms of AI or other clinical factors suggestive of pituitary injury, routine corticosteroid replacement may not be necessary in patients with POD3 serum cortisol ≥10 mcg/dl.

Nothing to Disclose: MIK, IEM, GMN-G, JKD, CJ, MAH, NBL, SGW

P2-688

Opioid Analgesics Suppress Male Gonadal Function but Opioid Use in Males and Females Does Not Correlate with Symptoms of Impaired Sexual Function.

D Wong BSc[1], D Gray MD[1], M Simmonds MD[1], S Rashiq MD[1], I Sobolev MD[1] and D Morrish MD[1].

[1]Univ of Alberta Edmonton, Canada.

Impaired gonadal function in men and women using opioid analgesia has been described but the frequency of abnormalities and correlation with symptoms of hypogonadism is not well established. We conducted a prospective study of patients attending a Multidisciplinary Pain Clinic. 65 women (47 opioid users, 18 non-opioid analgesic controls) and 32 men (26 opioid users, 6 control) were enrolled. History of sexual dysfunction (men: reduced libido, erectile dysfunction; women: decreased libido, oligo/amenorrhea), and hormonal testing (men: total testosterone [TT], free testosterone [FT], prolactin, LH; women: free testosterone, total testosterone, prolactin, DHEAS, SHBG, progesterone, LH, FSH, estradiol) were obtained. In men, low FT (20/26) was commoner in opioid users (p=0.04) but other hormones were not different. In the group of males with abnormal hormone levels, there was no difference in the frequency of sexual dysfunction compared to normal hormone values, and no difference in the frequency of opioid versus non-opioid use. In women, opioid users had lower FT (p=0.02). Low DHEAS was more frequent in women on opioids (p=0.03). Menopausal women taking opioids more frequently had a low DHEAS (p=0.046) and premenopausal women taking opioids more frequently had a low TT (p=0.03). Frequency of sexual dysfunction (decreased desire) was the same in opioid users (32/47) and controls (13/18; p=0.75) and also did not relate to any hormone abnormality. Menstrual abnormalities also did not relate to opioid use.
We conclude that opioids frequently cause low FT in men, but there is no relationship between abnormal hormone levels and symptoms of sexual dysfunction. Since symptoms are not useful in determining the occurrence of hypogonadism, we thus suggest all men should be screened for low FT. Women had lower FT on opioids but this did not correlate with sexual dysfunction symptoms. Therefore, measurement of FT or other hormones was not considered useful in women.

Nothing to Disclose: DW, DG, MS, SR, IS, DM

P2-689

Relative Adrenomedullary Failure May Exist in the Critically Ill Despite Elevated Catecholamine Levels.

MS Almehthel MD[1], D Li MD[1], M Pudek MD[1], J Ronco MD[1], BW Paty MD[1], G Francis MD[1], E Ur MD[1], GC Wong MD[1], G Redekop MD[1], J Prior MD[1] and SM Sirrs MD[1].

[1]Univ of British Columbia Vancouver, Canada.

There are insufficient data for the range for urinary catecholamines (UCAT) response in the critically ill to help interpret UCAT levels in this population particularly in the setting of labile blood pressure or adrenal incidentaloma. Relative adrenocortical insufficiency has been described in the critically ill but relative adrenomedullary failure has not been previously explored.
Patients admitted to the critical care areas (CCA) of our institution were eligible for inclusion unless they were suspected clinically of pheochromocytoma (PHEO) or required drugs affecting UCAT. 24-hour urine collections were obtained on admission to the CCA and repeated prior to hospital discharge. Urine epinephrine (EP), norepinephrine (NEP), metanephrine (MET) and normetanephrine (NMET) were measured using high-performance liquid chromatography (HPLC) with electrochemical detection. Data were analyzed using non-parametric methods to establish the physiologic response range for UCAT levels in critical illness [(1,2)]. UCAT levels were correlated with the APACHE IV scores using Spearman's rho test.
43 subjects (30 males), ages 18 to 88 (mean age 56), were included from ICU (46%), CCU (26%), Neuro-ICU (16%) and Trauma ICU (12%). UCAT levels in the critically ill showed marked variability with the responses ranging from within normal limits to 3-6 fold above the upper limit of normal. Levels of EP and MET showed a strong negative correlation with the APACHE IV score suggesting that relative adrenal medullary failure may be a component of critical illness. The lack of correlation of NEP and NMET and the severity of critical illness was expected as the major secretory output of the adrenal medulla is EP.

	Critically-ill patients	Normal population (1,2)	Patients with essential hypertension (1,2)	Correlation with APACHE IV score
Epinephrines (nmol/day)	0 – 507	0 – 160	0 – 160	r -0.60, p 0.006
Norepinephrines (nmol/day)	49 – 2723	89 – 470	Up to 900	r -0.37, p 0.11
Metanephrines (µmol/day)	0.32 – 5.88	0.26 – 1.73	0.26 – 1.73	r -0.72, p <0.001
Normatanephrines (µmol/day)	1.12 – 13.03	0.48 – 2.42	Male: up to 4.4	r -0.34, p 0.145

Conclusions: We have described a response range for UCAT levels in the critically ill which overlaps with the levels in patients with PHEO and postulate that relative adrenomedullary failure may be a component of critical illness.

1. Standard operating procedure for free Catecholamines in urine by HLPC with electrochemical detection, Vancouver General hospital laboratory manual.
2. Standard operating procedure for metanephrines in urine by HPLC with electrochemical detection, Vancouver General Hospital laboratory manual.

Sources of Research Support: Vancouver Coastal Health Research Institute.

Nothing to Disclose: MSA, DL, MP, JR, BWP, GF, EU, GCW, GR, JP, SMS

P2-690

Establishment of Reference Value of Insulin Tolerance Test, Low-, and High-Dose ACTH Stimulation Test for Assessment of the Hypothalamo-Pituitary-Adrenal Axis in Normal Korean Subjects.

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Backgrounds: The insulin tolerance test (ITT) is widely regarded as the gold standard test when evaluating hypothalamic-pituitary-adrenal (HPA) axis. Although a peak cortisol value > 18 μg/dl is usually interpreted as a sufficient response to the ITT, the cut-off value was measured by a fluorimetric assay. Fluorimetric assay for cortisol have a 20-30% positive bias compared to the current, more specific RIAs. Moreover, cortisol RIAs are not standardized. The aim of this study was to investigate the validity of cortisol RIAs during ITT and ACTH stimulation tests (high-dose [HDT, 250 μg] and low-dose [LDT, 1 μg]) in normal Korean subjects, and to establish the reference value of each test.
Methods: We measured cortisol using two different RIA kits (group A = CIS bio international; group B= Diasorin) from thirty healthy volunteers (10 males, aged 24-55) during ITT, LDT and HDT. As well, we analyzed ITT data from normal subjects (N = 204; 70 males, aged 15-88) of another University Hospital (group C) using Diasorin RIA kit. All subjects achieved adequate hypoglycemia (< 40 mg/dl) during ITT.
Results: Measured cortisol levels by two different kits were significantly correlated with each other (Pearson's correlation = 0.976, P < .0001) and Bland-Altman plots (agreement analysis) showed the mean difference of peak cortisol levels between two kits was 0.95 μg/dl (95% CI, 0.83-1.10). The cortisol levels were not significantly different between the two kits. The 95th percentile of peak cortisol levels during ITT were 14.1 μg/dl for group A, 14.4 μg/dl for group B and 14.7 μg/dl for group C. The pooled analysis of group B and C (N = 234) showed that the mean peak cortisol level was 23.1 ± 1.3 μg/dl (11.8-49.6) and the 95th percentile of peak cortisol level was 14.7 μg/dl. The mean cortisol levels during LDT for group A and B were 24.7 ± 3.2 μg/dl (19.6-32.9) and 24.7 ± 3.6 μg/dl (19.9-32.3), respectively. The mean cortisol levels during HDT for group A and B were 28.9 ± 4.2 μg/dl (22.4-36.5) and 30.7 ± 4.9 μg/dl (23.3-41.2), respectively. The 95th percentile of peak cortisol level of LDT and HDT were 20 and 23 μg/dl, respectively.
Conclusion: Using an RIA method, valid results could be obtained during ITT, LDT and HDT. The cut-off levels for peak cortisol response during ITT, LDT and HDT in healthy Korean volunteers were 15 μg/dl, 20 μg/dl and 23 μg/dl, respectively.

Nothing to Disclose: JHA, HYC, HJC, MJK, KWK, SWK, CSS, SWK, SYK

P2-691

In Vivo Dose-Response Reconstruction from Paired Hormone Concentration-Secretion Time Series Reveals Rapid Intrapulse Autoregulation.

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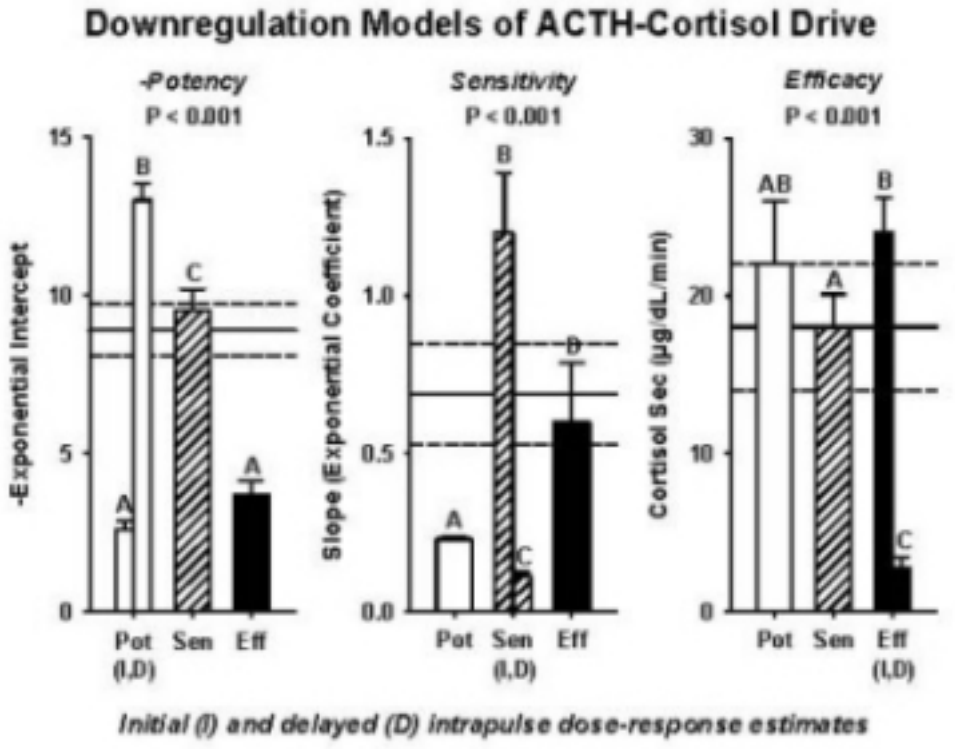
Pituitary adrenocorticotropin (ACTH) drives adrenal glucocorticoid (cortisol) pulses via a time-delayed asymptotic dose-response process. We test the postulate that dose-response dynamics change within single pulses: ACTH stimulates cortisol secretion unequally during the initiation and termination of a cortisol secretory burst. The dose-response function would then be (allowably) up- or downregulated in potency, sensitivity or efficacy within each successive ACTH-cortisol pulse pair. In 28 healthy adults, ACTH and cortisol were measured every 10 min for 24 hr in the unstressed state (8,120 measurements). A dual-waveform deconvolution model was used to construct cortisol secretion rates and reconstruct ACTH concentration profiles. One four-parameter (basal, potency, sensitivity and efficacy) ACTH concentration-cortisol secretion dose-response function was estimated in each subject without allowance for hysteresis (base model), and also three other functions variously allowing for delayed hystereses in (i) ACTH potency (ii) adrenal sensitivity and (iii) ACTH efficacy. Model residual error was 40% lower in the potency and sensitivity models and 20% lower in the efficacy than the base model ($P < 0.001$). The time shifts for hysteretic inflection were model-independent (*viz.*, 23 ± 1.1 min). Half-maximally effective ACTH concentrations (EC_{50}) differed markedly by model before and after permissible hysteretic inflection: (a) 11 ± 1.1 and 58 ± 3.8 ng/L ACTH in the potency model; (b) 10 ± 0.93 and 304 ± 87 ng/L ACTH in the sensitivity model; and (c) 23 ± 3.3 for no hysteresis and 14 ± 1.5 for the efficacy model [$P < 0.001$]. Efficacy hysteresis and no hysteresis yielded the lowest random effects on ACTH efficacy (combined mean 6.5 ± 2.0 *vs* 18 ± 2.8 for the potency/sensitivity models, $P < 0.001$). In the efficacy model, estimated maximal ACTH drive varied 9-fold (from 24 ± 2.2 before hysteresis to 2.7 ± 0.073 after hysteresis ug/dL/min cortisol secretion $P < 0.001$). The collective results introduce the basis for modeling physiological downregulation within the span of hormone interpulse intervals *in vivo*.



Sources of Research Support: AG029362, AG019695 and DK73148 from the National Institutes of Health (Bethesda, MD).

Nothing to Disclose: DMK, FR, JDV

P2-692

Bilateral Pheochromocytomas in a Child Who Had Hemihypertrophy and Alteration in VHL Gene.

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Background
Pheochromocytomas are rare neoplasm in children. Isolated hemihypertrophy and hemihypertrophy linked to other genetic disorders have rarely been associated with development of pheochromocytomas. Only two cases have been described to date. We report a 5 year old male with bilateral adrenal pheochromocytomas and right lower extremity hypertrophy.
Clinical Case
A 5 year old boy presented with complaints of excessive sweating and poor weight gain for 6 months. On examination his blood pressure was elevated (180-205/41-136), weight was 17 kg (50%ile) and height was 107cm (50-75%ile). Lower extremity length and width discrepancies were noted with the right lower extremity measuring 2.2 cm shorter than the left and 2-4 % girth discrepancy with the left lower extremity hemihypertrophy. Renal US showed a solid mass which was adjacent to the superior pole of the right kidney measuring 4.5x3.5x5.3cm. CT of abdomen revealed bilateral adrenal masses (4.7x3.6x3.8cm) on the right and (4x1.9x1.9cm) on the left suggesting the diagnosis of pheochromocytoma. Urinary norepinephrine (18070ug/24hr), epinephrine (12ug/24hr) and dopamine (311ug/24h) were elevated. Plasma metanephrine (0.39 nmol/L) and normetanephrine (62.6 nmol/L) were also elevated. I-123 MIBG scan revealed bilateral adrenal masses evident on CT examination with abnormally increased uptake. No other tumors were identified. Patient underwent bilaterally total adrenalectomies.
DNA test disclosed the presence of heterozygous variant in VHL gene (c.245G>T). Parents testing were negative; therefore this was a de novo event.
Conclusion
Hemihypertrophy can be an isolated finding, or it can be associated with certain malformation syndromes, namely Beckwith-Wiedemann syndrome, Neurofibromatosis type 1, and Proteus syndrome. Hemihypertrophy has not been described with VHL, and the association between pheochromocytoma and hemihypertrophy has only been previously described twice in patients who did not have an alteration in the VHL gene.
Pheochromocytoma is not a common tumor in children, but the incidence rate is increased with conditions like von Hippel-Lindau, multiple endocrine neoplasia type A and B and neurofibromatosis type 1.
We conclude that in patients who present with hemihypertrophy, the physician should be aware of the symptoms of pheochromocytoma. Besides screening for abdominal tumors, urinary and/or plasma catecholamines and/or their metabolites may need to be checked.

Nothing to Disclose: ZA, DBV, AL, RCA

P2-693

Constitutional Mosaic Genome-Wide Uniparental Disomy in a Female Patient with Beckwith-Wiedemann Syndrome and an Ectopic Virilizing Adrenocortical Tumor Diagnosed in the Second Decade of Life.

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Introduction: Beckwith-Wiedemann syndrome (BWS) is the most common overgrowth syndrome and is caused by defects in imprinted gene expression at 11p15. The overall tumor risk is 5%-10% but it increases almost four-fold in patients with hemihyperplasia (HP) and decreases in the second decade of life. Wilms' tumors (WT) are among the most frequent, usually discovered before the forth year of age. Adrenocortical tumors account only for 7% of all cases. Very few pediatric patients with Genome-Wide Paternal Uniparental Disomy (GWPUPD) have been reported recently. Some of their clinical features are consistent with known imprinting disorders associated with paternal UPD of specific chromosomes. **Clinical Case**: A 21-year-old woman with HP consulted for hirsutism and amenorrhea. Her past medical history included a right nephrectomy and adrenalectomy followed by adjuvant chemotherapy for a WT at the age of 3 years, and a 80% pancreatectomy at the age of 9 for a nesidioblastosis previously controlled with diazoxide for 7 years. A 7 cm retroperitoneal mass, anterior to the left kidney and independent from the adrenal gland was found at the CT scan. Histopathology after surgical removal described an encapsulated 7 cm tumor with adrenal cortex differentiation and uncertain malignant potential. Nowadays she is 26 and remains asymptomatic with normal adrenal and ovarian functions. Genetic study revealed not only a paternal 11p15.5 UPD, a defect found in 10-20% of BWS patients, but also an almost complete massive loss of heterozygosity (LOH) in all chromosomes due to high-degree (85%) GWPUPD, that was demonstrated through high-density genomic SNP-arrays, microsatellite analyses, MS-MLPA, high resolution melting analysis, and pyrosequencing of several imprinted genes. MS-MLPA also showed abnormal methylation patterns at Prader-Willi/Angelman syndrome and pseudohypoparathyroidism loci. **Conclusions**: As far as we know, this is the first reported case of an adult patient with GWPUPD and also of an ectopic virilizing tumor in a BWS. Our case highlights the importance of maintaining a close follow-up in patients with BWS even after the fourth year of age, especially in those with HP or unusual clinical findings, and incorporating appropriate genomic technologies to the genetic study of suspected imprinting/UPD disorders presenting with dysmorphic features and/or cancer, in order to make an early diagnosis of these patients and improve their prognosis.

Nothing to Disclose: BL, CA, LFP, AF, GPdN, MN, EC, PL

P2-694

Adrenal Failure Caused by a Retroperitoneal Malignant Mesothelioma.

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Background: There is substantial interest in malignant mesothelioma because its incidence has increased over the past 20 years and is expected to continue to increase over the next few decades in response to the widespread use of asbestos in the past (1). Malignant mesothelioma is aggressive tumor and shows resistance or little response to chemotherapy in many cases. It can arise from serous surfaces anywhere in the body including the pleura and peritoneum. In addition, the diagnosis of mesothelioma is often difficult because precise diagnosis based on imaging findings alone is not possible.
Clinical case: A 62-year-old man presented with clinical signs of acute abdominal pain and adrenal insufficiency. Computerized tomographic scans revealed bilateral adrenal tumors and clinical examination revealed adrenal insufficiency. Despite the intensive examinations including whole-body CT, FDG-PET and gastrointestinal endoscopy, the bilateral adrenal tumors were the only tumors to be detected. In order to obtain an appropriate diagnosis, a laparoscopic tumor extirpation was performed on his left adrenal tumor. Examination of the excised specimen by immunohistochemistry revealed that the tumor cells were positive for epithelial membrane antigen (EMA), cytokeratin (CK) 5/6, thrombomodulin, D2-40, cytokeratin AE1/AE2 and low-molecular-weight CK (CAM5.2). Based on these immunohistochemical findings and the clinical manifestation of bilateral adrenal tumors, retroperitoneal malignant mesothelioma was diagnosed. After laparoscopic removal of left adrenal tumor, a rapid growth and hepatic infiltration of the right adrenal tumor was observed. Therefore, systemic chemotherapy was commenced with a combined regimen including cisplatin (CDDP) and 5-fluorouracil (5-FU). It is noted that a systemic chemotherapy with CDDP and 5-FU was dramatically effective to the retroperitoneal malignant mesothelioma and lead to a complete remission of the disease.
Conclusion: We reported the first case of retroperitoneal malignant mesothelioma presenting as bilateral metastatic adrenal tumor with the clinical symptom of adrenal insufficiency. The definitive diagnosis of mesothelioma could be achieved by immunohistochemical study of the tumor specimens using a panel of markers. Malignant mesotheliomas might be considered when we come across retroperitoneal tumor such as adrenal tumor.

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Nothing to Disclose: IA, MN, HS, TY, RT

P2-695

Estrogen and Androgen Co-Secreting Adrenalcortical Carcinoma in a Postmenopausal Woman.

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Adrenalcortical carcinoma (ACC) is a rare entity, with an incidence of 1 to 2 cases per million people per year. Only a small proportion of them (<5%) secretes estrogens and leads to feminizing syndrome in males. Even rarer are estrogen-secreting tumors in postmenopausal women and only have a few cases been described. Moreover, the direct production of estradiol by ACC and the mechanisms involved in the process have been recently described. We report a case of an estrogen and androgen-secreting ACC in a postmenopausal woman.
A 65-year-old woman was referred to us for evaluation of an adrenal mass. She had undergone an anexohysterectomy due to abnormal vaginal bleeding, objectifying endometrial hyperplasia without atypia. At that time, CT scan showed a 38 mm right adrenal mass whose relationship with her clinical condition had not been properly evaluated. A year later the tumor size was 51 mm. In the initial consultation, she complained of right flank pain but the physical examination was unremarkable.
Complete blood tests and urinalysis were all within normal ranges and endocrine studies revealed high levels of estradiol (E_2), total testosterone (To), androstenedione (A), 17-hydroxiprogesterone (17OHP) and sex hormone binding globuline (SHBG) but FSH and LH levels were undetectable.

Table 1

	E2 <28pg/mL	To <0.6ng/mL	SHBG <110nmol/L	LH >14UI/L	FSH >30UI/L	A <1.0ng/mL	17OHP <0.6ng/mL
Before Surgery	413	2.91	167	<0.1	<0.1	>10.0	6.8
After Surgery	<28	0.49	65	19.6	32.7	1.5	2.2

A complete right adrenalectomy, with an uneventful postoperative course, was performed and hystopathological studies showed a high-grade ACC that met at least seven of the Weiss's criteria. After tumor removal, plasma E_2 concentration fell to undetectable level while FSH and LH levels increased and total testosterone and SHBG concentration decreased, reaching normal values. She started adjunctive chemotherapy with mitotane, in increasing doses up to 3 grams per day. A and 17OHP levels also fell but persisted above the normal upper limits (table1). Four months after adrenalectomy, a CT scan showed no evidence of recurrence.
Co-secreting ACC in postmenopausal women is a rare condition. Estrogen-secreting tumors should be suspected in the presence of abnormal vaginal bleeding once endometrial carcinoma has been ruled out and blood tests show increased levels of estrogens with suppressed gonadotrophins concentrations

Nothing to Disclose: RMG, EA, LJA, DS

P2-696

Adrenocortical Carcinosarcoma, a Very Rare and Extremely Lethal Histologic Variant of Adrenocortical Carcinoma (ACC).

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Background: ACC is a rare endocrine malignancy with poor survival even when detected early (35% surgical cure rate). Functioning cancers (60% of ACC) may declare themselves before spread with clinical syndromes of hormonal excess, but non-functioning tumors are often not diagnosed until local or distant spread occurs, resulting in poor prognosis. We present a case of a rare variant with a course so aggressive as to match the most lethal cancers.
Clinical Case: A 45 year old previously healthy male suddenly developed intractable abdominal pain, nausea, and vomiting. CT scan revealed a 20x14 cm adrenal mass with a large internal hematoma, multiple hepatic metastases, and retroperitoneal lymphadenopathy. Hormonal studies showed elevated cortisol and catecholamines, which were attributed to pain from adrenal apoplexy, (plasma cortisol 30ug/dL, normal range [NR] 7-25, ACTH 59pg/mL, NR 9-46, 24h UFC 372ug, NR 20-90, total plasma metanephrines 363pg/mL, NR <205; total 24h urinary metanephrines 1976ug, NR 90-690) with normal androgens. The patient underwent en bloc resection of the adrenal mass, left kidney, spleen, pancreas, left colon, rib and left lobe of the liver. Pathology showed a biphasic tumor composed of high-grade carcinoma (70%), and rhabdomyosarcoma (30%), consistent with a diagnosis of adrenocortical carcinosarcoma, with extensive hemorrhagic necrosis, and widespread metastases.
After surgery, the patient's condition deteriorated relentlessly, with the onset of respiratory and renal failure. Serial CT scans showed recurrent tumor that grew in just 2 months to occupy the entire left half of the abdomen, from the left dome of the diaphragm to the pelvis. The remarkably aggressive tumor re-growth and the patient's poor functional status ruled out the use of mitotane, and he was discharged to hospice care. He died shortly thereafter.

Conclusion: There are only 7 reports of adrenocortical carcinosarcoma in the literature[1]. The extraordinary rarity of this histologic pattern of ACC, and its equally extraordinary lethality, make it a very difficult cancer to study. This leaves the interesting question of its origin unanswered. Specifically, it is not known if it arises from dedifferentiation of a single clone into two different cell lines, or from a parallel process affecting two different clones. Evidence from anaplastic endocrine cancers that have a biphasic histologic picture (e.g. anaplastic thyroid cancer) supports a single clonal origin[2] .

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Nothing to Disclose: MED, KS, RS, RHR

P2-697

Malignant Pheochromocytoma with Severe Intestinal Paralysis Based on a Significant Hypercatecholamine State: The Effectiveness of α-Metyrosine in Dysmotility of Digestive Tract.

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[Background] Peristalsis of digestive tracts is controlled by sympathetic nerve, and the hyperstimulation of α receptor by catecholamine in pheochromocytoma often provokes severe dysmotility resulting in paralytic ileus. [Clinical case] A 77-year woman relapsed pheochromocytoma with metastasizes in liver, lung and retroperitoneum space, after laparoscopic adrenalectomy 5 years ago. Hormonal data on admission were consisted with the recurrence of pheochromocytoma, elevated plasma catecholamines; Dopamine (DA): 137 pg/ml (normal <20), Norepinephrine (NE): 20941 pg/ml (normal<450) and elevated urinary excretion of both catecholamines and metabolites of them; U-DA: 1025.8 µg/24hr (normal<960), U-NE: 5805.9 µg/24hr (normal<170) and U-normetanephrine (NM): 12.60 mg/24hr (normal<0.33). During the course of 3 cycles of the chemotherapy with CVD (cyclophosphamide, vincristine and dacarbazine), she had been suffered from severe constipation, and she developed into the intestinal paralysis by progress of 4 months. Various medications, which contribute to bowel motility, and decompression by trans-anal tube were ineffective, normalization of NE level by administration of α-metyrosine immediately improved the condition. [Clinical Lessons] In great majority of pheochromocytoma cases complicated with paralytic ileus reported in Japan in the past 20 years, plasma NE was elevated at the level more than 10000 pg/ml. The result was not concerned with either malignant/benign or male/female. Of 17 pheochromocytoma cases diagnosed at our hospital in the past 10 years, 4 cases with significant elevation of plasma NE (over 12000 pg/ml) demonstrated severe constipation, including 2 paralytic ileuses. The analysis with the 17 cases indicated that, utilizing the cut-off value for the risk of dysmotility, the plasma NE level (>5330 pg/ml) had adequate sensitivity (80%) and specificity (90.9%), and the U-NE excretion (>1660 µg/24hr) had sensitivity (80%) and specificity (92.3%), respectively. [Conclusion] We have found that the higher frequency of digestive tract paralysis is present, in phechromocytoma patients with higher levels of peripheral plasma NE. Patients in the hypercathecholamine state, in particular with NE level more than 10000 pg/ml, are in high risk of serious dysmotility and paralytic ileus, and it is desirable to start α-metyrosine at an early stage of their treatment.

Nothing to Disclose: KI, KY, MY, MM, YO, HN

P2-698

Pheochromocytoma in a Normotensive 89-Year-Old Man: An Atypical Presentation.

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Background: Although hypertension is the most common clinical manifestation of pheochromocytoma, approximately 5-15% of patients present with normal blood pressure. The frequency of normal blood pressure is even higher in patients with adrenal incidentalomas (1, 2).
Case: An 89-year-old male with a history of 3rd-degree heart block with pacemaker and remote TB presented with shortness of breath. Chest CT showed bilateral pulmonary nodules and a 3-cm left adrenal mass. The patient had a core biopsy of the adrenal mass given the patient's age and risk of pulmonary biopsy. The pathology was consistent with pheochromocytoma, immunostains strongly positive for synaptophysin and chromogranin. The endocrine service was called for consultation.
Further history revealed that the patient had episodes of shortness of breath, falls, weight loss, and intermittent lower limb swelling over the last year. The patient denied any history of hypertension, but beta-blockers had been started prior to pacer placement, more than one year before presentation. Physical exam noted a comfortable patient, BP 118/59, not orthostatic, BMI 28. Exam was otherwise nonrevealing. Tests to determine the functionality of the adrenal gland revealed increased plasma free metanephrines of 267 pg/mL (0-62), plasma free normetanephrines of 298 pg/mL (0-145), and increased 24-hour urine metanephrines of 1262 ug/24 hrs (35-460); normal potassium and ACTH stimulation testing. Additional work-up included CT head (normal), thoracentesis (nonspecific), ECHO (normal) and PET scan. The PET revealed the 3.2 x 2.7 cm left adrenal mass with very mild uptake and no evidence of malignancy. The pulmonary lesions were thought to be reactive changes with plan for serial CT. Given the patient's age and overall clinical status, the decision was made to manage the patient conservatively.
Conclusions: 1-Normal blood pressure is seen in a subset of patients with pheochromocytoma. As more adrenal lesions are noted incidentally with the increased frequently of CT scanning, a more patients will be assessed for pheochromocytoma. 2-Starting beta-blockers in patients with pheochromocytoma may precipitate cardiopulmonary dysfunction (hypotension, pulmonary edema, pleural effusion, lower extremity edema) as a result of cardiomyopathy or pulmonary venoconstriction and altered pulmonary capillary permeability (3). 3-Clinicians should consider pheochromocytoma with a history of "spells", particularly in the geriatric population.

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Nothing to Disclose: NS, AMT

P2-699

Bilateral Adrenal Vein Sampling (BAVS) with Glucagon Stimulation and 18-Fluorodeoxyglucose Positron Emission Tomography Scanning (^{18}FDG PET) in a Filipino Patient Suspected with Bilateral Pheochromocytoma.

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BACKGROUND
Diagnosis of pheochromocytoma depends on biochemical evidence of excess catecholamines and adrenal mass on imaging (CT, MRI, 123I-MIBG, PET) if lesion is >1cm. BAVS can be helpful in localizing unilateral tumor <1cm or lateralizing the more dominantly secreting adrenal gland in bilateral pheochromocytoma.
CASE
A 39 year-old Filipino female presented with 5 months history of paroxysmal attacks of palpitations, anxiety, headaches, dizziness and diaphoresis. Ambulatory BP monitoring recorded a highest BP of 213/157mmHg. Baseline plasma metanephrine was elevated at 1552 (NV 12-61pg/mL). CT and MRI showed an equivocal 1.5x0.9cm left adrenal mass which was confirmed with 123I-MIBG and 18FDG PET. Glucagon-stimulated BAVS, however, revealed elevated baseline and stimulated metanephrines on both adrenals with dominant secretion on the right. Right vs left metanephrine (pg/mL) results were 3289 vs. 1498 at baseline and 5080 vs. 1473 after stimulation. The stimulated epinephrine and norepinephrine gradients on both adrenals were similar (1.4 vs. 1.2), suggestive of a bilateral lesion.
DISCUSSION/CONCLUSION
This is the first report utilizing BAVS and 18FDG PET in suspected pheochromocytoma. BAVS showed predominant catecholamine secretion on the right adrenal. The non-detection of the right lesion by PET could be explained by a probable presence of an occult tumor (<1cm) with lower metabolic activity albeit greater secretory property. However, the presence of other endocrine disease (carcinoid tumor) should be ruled out.

Nothing to Disclose: SWL-U, AMG, LAEM, AGM, MLP, KP, LBM-A

P2-700

Bilateral Pheochromocytoma in a Filipina Who Underwent Glucagon-Stimulated Bilateral Adrenal Venous Sampling and Genetic Testing: A Case Report.

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BACKGROUND
In occult pheochromocytomas, catecholamine-induced stimulation by glucagon with adrenal venous sampling (AVS) may be necassary. We present the first Filipino pheochromocytoma patient who underwent genetic testing at the National Institutes of Health, Bethesda, USA.
CASE
A 28 year old female had 12 years history of hypertension, palpitation, flushing, headache, anxiety and sweating. Highest BP 240/110, usual BP 130/90, while on five antihypertensive agents. Thyroid function was normal with secondary hyperaldosteronism (PRA 9.93ng/mL/hr; aldosterone 45.28ng/dL). Twenty-four hour urine tests showed vanillylmandelic acid 28.1umol/24hrs (<49.5) and metanephrines 0.74mg/24hrs (<1). Adrenal CT scan was normal. Glucagon-stimulation of catecholamine excess together with bilateral AVS showed predominance of catecholamine output on the right (R vs L, pg/mL: epinephrine, E, 154 vs 86, norepinephrine, NE, 31 vs 26, metanephrine, 4,975 vs 3,421, and normetanephrine, 935 vs 649), with E:NE gradient 4.9 and 3.3 respectively. Genetic mutation testing at the NIH did not show any succinate dehydrogenase gene mutation or deletion. She underwent laparoscopic right adrenalectomy. Histopathology showed adrenal medullary hyperplasia positive for chromogranin immunostaining. BP was maintained at 120-130/80 on terazosin and bisoprolol.
CONCLUSION
Glucagon stimulation test may be important in the diagnosis of adrenal medullary hyperplasia or very small pheochromocytomas that often do not present with any positive biochemistry.

Nothing to Disclose: AMG, SWL-U, LAEM, AGM, MLP, KP, LBM-A

P2-701

An Unusual Case of Cushing's Syndrome Caused by Ectopic Adrenocortical Carcinoma.

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Background: Adrenocortical carcinoma (ACC) is an extremely rare disease. Co-secretion of androgens and cortisol is highly suggestive of a malignant adrenocortical tumor. In addition, ACCs arising from ectopic adrenal glands associated with hypercortisolism are very rare. We report here a case of a woman with Cushing's syndrome (CS) secondary to ectopic ACC. Clinical Case: A 31 year old woman was admitted to our hospital for evaluation of edema of the lower extremities. Physical examination revealed acne on her face and back, hirsutism and weakness of the lower extremities. Results of hormonal data were consistent with those of ACTH-independent CS, i.e., ACTH level was less than 2 pg/ml while the serum cortisol level was 30 µg/dl with no circadian rhythm. In addition, DHEA-S level was 1060 µg/dl. Urinary 17-KS was 27.8 mg/day and urinary 17-OHCS was 18.5 mg/day. There was no suppression of cortisol following 1mg and 8mg dexamethasone challenges. CT scan and MRI revealed a lymph node measuring 3 cm in the left supraclavicular fossa and a multinodular mass in the abdominal para-aorta, although neither adrenal gland showed abnormalities. I-131 adosterol scintigraphy demonstrated no accumulation in the masses or in the adrenal glands. Histological examination of the left supraclavicular mass revealed diffuse atypical cells with clear cytoplasms, frequent mitosis, and vascular and sinusoid invasion. Further immunohistochemical evaluation revealed that SF-1, DHEA-ST, 3βHSD were diffusely positive while chromogranin A was negative in these tumor cells. Therefore, the patient was subsequently diagnosed as CS secondary to ectopic adrenocortical carcinoma. Six courses of EDP therapy (etoposide, doxorubicin and cisplatin) with mitotane were given. While tumor size was reduced and steroid hormone secretion was initially well-controlled, a rapid progression of multiple metastases was eventually detected following the six courses of chemotherapy. Patient rapidly developed multi-organ insufficiency and died one month after the completion of the chemotherapy. An autopsy also revealed an enormous mass occupying the retroperitoneum, atrophic but otherwise normal adrenal glands, and ovaries with metastatic lesions were detected but the presence of non-neoplastic ectopic adrenal gland was not identified. Conclusion: We report here a rare case of CS secondary to ectopic adrenocortical carcinoma which originated in the retroperitoneum.

Nothing to Disclose: AY, NW, NH, HU, NS, YI, YA, KS, HS, GY

P2-702

Should the Growth Hormone (GH) Dose in the Treatment of GH Deficient Children Be Calculated Per Body Weight or Per Body Surface Area?.

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CONTEXT: In the treatment of children with growth hormone deficiency (GHD) there is no consensus on whether the recombinant growth hormone (GH) dose should be calculated per body weight (BW) or per body surface area (BSA). Despite the lack of hard evidence to support either approach, the per BW approach is used predominantly in clinical practice. OBJECTIVES: To assess the influence of the GH dose, calculated per BW and per BSA, on height velocity (HV). PATIENTS AND METHODS: Data was retrospectively collected from a cohort of 62 prepubertal Brazilian children with GHD during their first year of treatment. GH dose had originally been calculated per BW, and its equivalent dose per BSA was calculated for this study. First, simple linear regression was used to correlate HV to each GH dose approach. Then, a subgroup of patients (n=35) with fixed GH dose per BW (0.6-0.7U/kg.wk) were divided into terciles according to per BSA GH dose; HV, BW, height, and age of the lower vs. higher terciles were compared separately by t test. RESULTS: The cohort consisted of 40 boys and 22 girls, mean±SD, age 9.8±4.1 yr, height 106.4±20.3 cm, height SDS -4.6±1.6, weight 18.7±6.5 kg, GH dose 0.58±0.19 U/kg.wk and GH dose 14.4±5.2 U/m^2.wk, and HV 10.4±3.5 cm/yr. Simple linear regression demonstrated a significant correlation between HV and both per BW and per BSA GH doses: per BW GH dose explained 32.5% ($p<0.001$) of HV, whereas per BSA GH dose explained 22.7% ($p<0.001$) of HV. In the subgroup with fixed per BW GH dose, the patients receiving the highest tercile of per BSA GH doses (18.65±0.65 U/m^2.wk) had similar HV (11.5±4.2 vs 12.9±4.6 cm/yr, $p=0.24$), but significantly ($p<0.001$) higher age (12.3±4.4 vs 6.0±3.0 yr), height (120.4±35.1 vs 90.0±26.9 cm) and weight (25.1±7.9 vs 12.1±4.1 kg) than those of the lowest tercile of per BSA GH doses (15.19±0.75 U/m^2.wk). Data had insufficient power for multiple linear regression. DISCUSSION: One of the arguments for calculating GH dose per BSA instead of per BW is that the total GH dose gets increasingly higher in the latter, when compared to the former, as the patient grows (1). A higher total dose implies in increased cost. CONCLUSION: In this study, GH dose calculated per BW had a slightly stronger correlation with and better power of predicting HV than dose per BSA. However, comparison and analysis of larger cohorts, pharmacodynamic and pharmacokinetic studies are needed to resolve this issue which has also an economic impact.

(1) Wit JM, Horm Res 2007; 68:244–247

Sources of Research Support: FAPESP 03/13506-8.

Nothing to Disclose: MGMR, VNB, LC, AJ, BBM, IJPA

P2-703

84-Month Results of a Phase III Study about Treatment of Growth Hormone Deficient Children (GHD) with the Recombinant hGH Omnitrope®.

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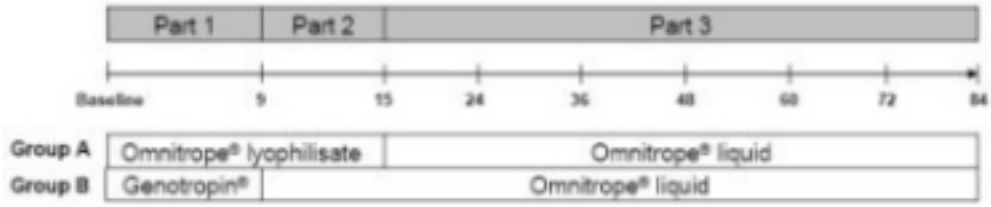
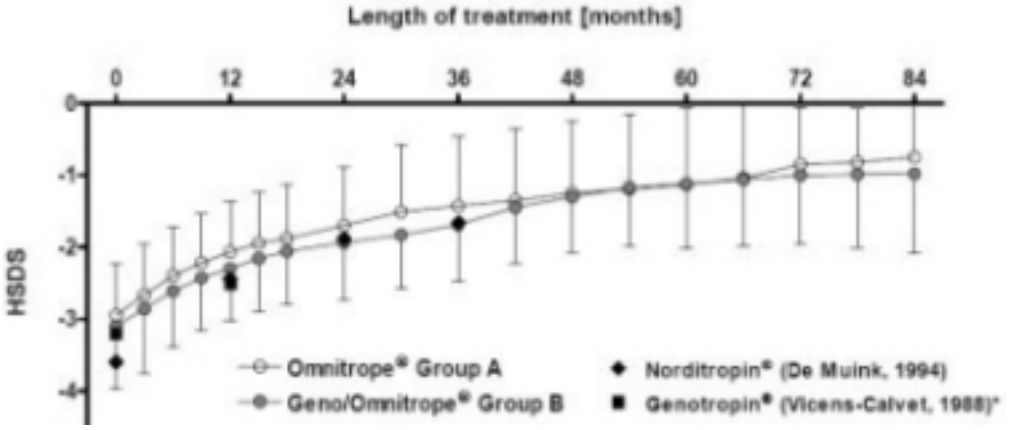
AIM:
Growth-retardated GHD children treated with Omnitrope and Genotropin were documented in a controlled phase III study. Aim was to compare efficacy and safety of Omnitrope® versus Genotropin® and to assess safety and efficacy of Omnitrope® over a period of 84-month of treatment.



METHOD:
89 treatment-naïve, prepubertal children (HSDS -3.0 ± 0.8, height velocity (HV)SDS -2.3 ± 1.1) with GHD (peak < 10 ng/ml, in 2 tests) were randomised (Part 1) to Omnitrope® lyophilisate (Group A, n=44) or Genotropin® (Group B, n=45) for 9 months and received subcutaneous dose of 0.03 mg/kg/day. In Part 2, patients receiving Omnitrope® lyophilisate continued the same treatment for a further 6 months, while patients on Genotropin® were switched to Omnitrope® liquid for the subsequent 6 months. In Part 3, patients in both groups received Omnitrope® liquid for a period up to 69 months.
RESULTS:
The development of height, HSDS, HV, HVSDS and IGF-1, IGFBP-3 levels were comparable between both groups of patients and confirmed the well-known growth response of GHD children to rhGH treatment. IGF-1 levels increased in both treatment groups to mean levels in the upper normal range. Imunogenisity (anti-GH antibodies) during treatment with Omnitrope® liquid was acceptably low and of no clinical significance. Over 84 months of treatment Omnitrope® was well tolerated and safe.



CONCLUSION:
The clinical comparability between Omnitrope® and Genotropin® was demonstrated. Safety and efficacy of treatment with Omnitrope® were proven in a phase III study over a period of 84 months[1].

(1) Romer T et al., Horm Res 2009; 72:359-369

Disclosures: AB: Researcher, Sandoz. MZ: Clinical Researcher, Sandoz. PHS: Medical Advisory Board Member, Sandoz.
Nothing to Disclose: TR, FP, BK, RW, MW, EK, JS

P2-704

Description of Etiological and Epidemiological Features of 1018 Patients Evaluated for Short Stature in Our Service, Showing the Delayed Arrival of the Patient to an Endocrinologist.

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Background: Short stature is defined as height more than two standard deviations below the mean for a particular sex and age based on a population reference. For some patients with growth deficiency, it's important to diagnose earlier their etiological cause in order to optimize their final height. We evaluated 1018 children followed in the growth evaluation outpatient service of Clinical Hospital of Porto Alegre – RS, Brazil .They were allocated in two groups: the first group, called constitutional short stature (CSS), was formed by those patients diagnosed with constitutional short stature, familial short stature and both at the same time; the second one, organic short stature (OSS), was consist of primary hypopituitarism(H), chronic diseases, genetic diseases, growth hormone deficiency (GHD) and pan-hypopituitarism(PH) as diagnoses. A third group was formed during analyzes, with those children witch were sent to specialty investigation for short stature but didn't carry growth disease. There were more boys than girls in our cohort (621:61%), as expected in our culture that emphasizes male's height. Only 9,25% (n=63, from 681 diagnoses) of children were diagnosed as not having short stature disease, while 46,69% (n=318) constitutes the CSS group, and 34,65% (n=236) the OSS group. The mean age of first medical appointment of those without short stature was 8,98 years old, compared to the 10,45 years old in the CSS, and to the 9,22 years old in the OSS (p=ns). It's important to notice that within the OSS group, the average ages weren't lower: Turner's syndrome, 10.9 years; GHD, 9.7 years; H, 10.29 years; PH, 9.91 years. There were 298 children born with less than 2.5 kg (Short for gestational age), 152 of them belonged to CSS group, suggesting that the “constitutional” diagnose of short stature inside this group could be justified by the insufficient "growth catch up" during the first childhood. Conclusion: Unfortunately, children with short stature due to diseases which can be earlier managed for optimizing their final height, as GHD, pan-hypopituitarism and Turner's disease, are arriving late to medical care, and this will certainly affect their full growth potential, besides enhancing the costs of the public health budget. Beyond that, low birth weight should be remembered in patients with CSS.

Nothing to Disclose: FC, PBDL, VB, MAC

P2-705

Increlex® Therapy in Children Who Were Treatment-Naïve or Previously Treated with Growth-Promoting Therapy: First-Year Results from the Increlex Growth Forum Database (IGFD) Registry.

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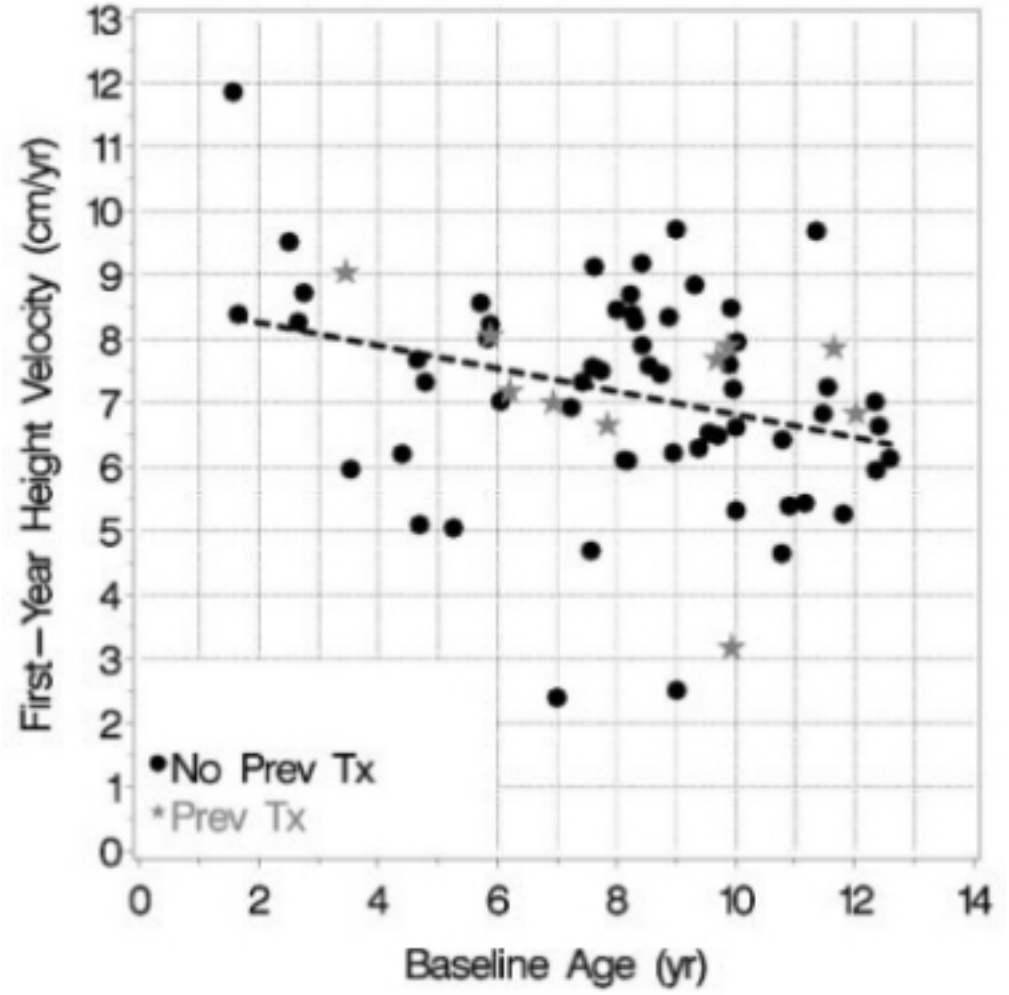
Introduction: The safety and efficacy of Increlex® (mecasermin [rDNA origin] injection) therapy was evaluated in children enrolled in the IGFD Registry.
Methods: This is a multi-center, open-label, observational study monitoring the safety and efficacy of Increlex in children who received Increlex from a qualified practitioner and whose parents (or legally authorized representatives) gave informed consent. Targeted adverse events (AEs) were AEs observed in previous growth-promoting therapy studies.
Results: As of October 2009, there were 685 US patients (safety analysis patients) with at least one follow-up visit (822 patient-years; 5th and 95th percentile age range, 4.0-15.6 years). Hypoglycemia (7.2% of patients) and headache (5.3%) were the most frequently reported targeted AEs; both AEs were more likely to occur during the first year of therapy. Four serious adverse drug reactions were reported: headache (n=2), intracranial hypertension (n=1) and slipped capital femoral epiphysis/avascular necrosis of the hip (n=1); 4 other serious AEs were not considered drug-related. Sixty-nine children remained prepubertal and had height and dose information available at one year (efficacy analysis patients). Ten of these children were previously treated with growth-promoting therapy and 59 were treatment-naïve. These 10 children had higher baseline BMIs (median BMI SDS, 0.2 vs -0.4) and were older (median age, 8.8 vs 8.4 years) and shorter (median height SDS, -3.1 vs -2.4) than the treatment-naïve patients. Using multiple regression analysis, first-year height velocity (HV) was related to both age (regression coefficient b=-0.180, p=0.0087, Figure) and dose (b=0.0184, p=0.033). Previous therapy was not a significant predictor of first-year HV. First-year HV was also not predicted by baseline height SDS, IGF-1 SDS, BMI SDS or maximum stimulated GH.
Figure. HV vs age (n=69)



Conclusions: Increlex was generally well tolerated in these children for the doses studied (up to 120 µg/kg twice-daily). In prepubertal children, first-year HV was related to both age and dose, and prior use of growth-promoting therapy was not a significant predictor of first-year HV.

Sources of Research Support: Ipsen, US.

Disclosures: BR: Speaker, Ipsen; Study Investigator, Ipsen; Medical Advisory Board Member, Ipsen. DB: Speaker, Ipsen; Study Investigator, Ipsen. JK: Employee, Ipsen. JH: Employee, Ipsen. JWF: Consultant, Ipsen, Genentech, Inc. SB: Employee, Ipsen; Consultant, Ipsen.

P2-706

Metabolic Outcome of GH Treatment in Prepubertal Children with Idiopathic Short Stature Compared with GH-Deficient Children.

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Background: Few studies have evaluated the metabolic outcomes of growth hormone (GH) treatment in idiopathic growth stature (ISS). Moreover, children with ISS appear to need higher GH doses than children with GH deficiency to achieve the same amount of growth, and are therefore at increased risk of adverse events during treatment. This finding requires investigation.
Objective: To compare metabolic outcome and height gain during GH treatment, as well as the relationship of these variables to GH dose, in children with ISS and GH-deficient.
Hypothesis: Metabolic outcome is similar in children with ISS and GH-deficient.
Study design: Short prepubertal children with either isolated GH-deficient (n=89) or ISS (n=39) participated in a 2-year randomized trial of either individualized GH treatment with six different GH doses (range, 17–100 µg/kg/day) or a standard dose (43 µg/kg/day).
Results: Similar metabolic changes are seen in ISS and GHD. Height changes (Δ) correlated with Δinsulin-like growth factor I (IGF-I), Δleptin and Δbody composition. Moreover, principle component analysis identified an anabolic and a lipolytic component. Anabolic variables [change in lean body mass (LBM) SDS and IFG-I SDS] clustered together and correlated strongly with height response and GH dose, whereas lipolytic variables [change in fat mass SDS and leptin] appeared to be separate from the anabolic cluster. Regression analysis showed GH dose-dependency in ISS, and to a lesser degree in GH-deficient, for change in LBM SDS and change in height SDS, but not for changes in fat mass.
Conclusions: Children with ISS showed similar metabolic outcomes to GH treatment as GH-deficient children.

Sources of Research Support: This investigator initiated study TRN 98-0198-003 was funded by Pharmacia/Pfizer. Financial support was also obtained from Swedish Research Council no 7509 and no 7238, University Hospital (ALF) and West Sweden Region (VGR) grants.

Disclosures: KA-W: Principal Investigator, Pfizer, Inc. BK: Coinvestigator, Pfizer, Inc.
Nothing to Disclose: JD, RD, JG, ZH

P2-707

Presenting Features and Long-Term Effects of Growth Hormone (GH) Treatment of Children with Septo-Optic Dysplasia (SOD).

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Background. SOD is a cause of congenital GH deficiency (CGHD) with associated hypothalamic manifestations. No studies of a large SOD cohort, describing presenting features and long-term outcomes of GH, with comparisons to non-SOD CGHD, have been published.
Methods. Data from KIGS, Pfizer International Growth Database, were compared for 395 SOD subjects and 158 CGHD without non-pituitary midline pathology. Enrollees were prepubertal with ≥1 y of growth data (median; 10, 90 %ile). Wilcoxon rank-sum test was used to detect differences between groups.
Results. Compared to non-SOD, SOD had higher birth length (BL), birth wt (BW), and mid-parental ht (MPHt) SDS and similar peak GH. At GH start (GHs), age and BA were similar, while ht, wt, and BMI SDS were higher in SOD. After 1 y GH, both groups had similar delta ht SDS, but wt and BMI SDS were still higher in SOD. At near-adult ht (NAH) (>10 y GH), SOD (n=59) and non-SOD (n=23) had similar ht, wt, and BMI SDS.

Results

	SOD/ONH Median	SOD/ONH 10, 90	Non-SOD/ONH Median	Non-SOD/ONH 10, 90	P-value
BL SDS	0.27	-1.3, 2.0	-0.62	-2.5, 1.2	<0.001
BW SDS	-0.31	-1.7, 1.1	-0.58	-2.5, 1.2	0.021
MPHt SDS	-0.23	-1.8, 1.3	-1.04	-3.0, 0.5	<0.001
Peak GH (ng/mL)	2.60	0.8, 7.2	3.30	0.5, 10.8	0.053
Age (y) GHs	3.99	0.9, 9.8	4.47	0.7, 10.7	0.498
BA (y) GHs	3.50	1.4, 8.7	4.00	1.2, 10.0	0.784
Ht SDS GHs	-3.00	-4.8, -0.9	-3.68	-6.7, -1.9	<0.001
Wt SDS GHs	-1.56	-4.2, 0.5	-2.56	-5.7, -0.6	<0.001
BMI SDS GHs	0.22	-1.8, 2.1	-0.18	-2.1, 1.6	0.001
Delta Ht SDS 1 y GH	1.02	0.0, 2.0	1.10	0.3, 2.7	0.066
Wt SDS 1 y GH	-0.91	-3.3, 1.3	-1.81	-3.9, -0.0	<0.001
BMI SDS 1 y GH	0.05	-1.8, 2.3	-0.43	-2.1, 1.2	<0.001
Ht SDS NAH	-0.87	-2.8, 0.6	-1.37	-2.7, 0.2	0.430
Wt SDS NAH	-0.53	-2.6, 2.7	-0.22	-2.1, 1.9	0.988
BMI SDS NAH	0.32	-1.9, 2.5	0.59	-0.9, 2.5	0.581

Conclusions: Compared to CGHD w/o midline defects, SOD, despite comparable ages and GH secretion, presented with less severe short stature. Their higher wt/BMI SDS at GH start and at 1 yr suggest initial effects from hypothalamic hyperphagia which improve after long-term GH.

Disclosures: MEG: Principal Investigator, Pfizer, Inc.; Poster Reviewer, Pfizer, Inc.; Scientific Board Member, Pfizer, Inc. HK: Employee, Pfizer, Inc.
Nothing to Disclose: AV, CF, MDB

P2-708

Long-Term Findings from a Randomized Controlled Trial: An Effective Lifestyle Intervention in Overweight Children ("Obeldicks Light").

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[1]Pediatric Nutrition Med Datteln, Germany and [2]Fac of Hlth Scis Bielefeld, Germany.

Background: Randomized controlled trials (RCT) have demonstrated the effectiveness of lifestyle interventions in obese children. However, the effectiveness of interventions for overweight, but not obese children has not been demonstrated yet by RCTs.

Methods: A total of 66 overweight (BMI >90^{th}≤97^{th} percentile) children (mean age 11.5±1.6years, 58% females, mean BMI 23.4±1.5 kg/m²) were randomized into a control group (CG) (n=32; no intervention for a duration of 6 months) or intervention group (IG) (n=34; 6 months intervention "Obeldicks light" based on physical activity, nutrition education, and behavior counseling). BMI, waist circumference, skinfold thickness, bioimpedance analyses, blood pressure, physical activity based on questionnaires, and three-day-weighed dietary records were determined at baseline (T0) and 6 months (T1) later. Additionally, BMI was determined 6 (T2) and 12 months (T3) after end of intervention. Degree of overweight was calculated as BMI-SDS. Comparisons were performed on an intention-to-treat approach.
Results: The drop-out rate was 3% in IG and 16% in CG. At T1, 94% of the children in IG decreased their BMI-SDS and 24% of them were normal weight. The changes between T0 and T1 in BMI-SDS differed significantly ($p<0.001$) between IG and CG (CG: +0.05±0.19 BMI-SDS; IG: -0.26±0.22 BMI-SDS). Similar findings were observed for blood pressure, waist circumference, skinfold thickness, and fat mass based on bioimpedance analyses. In the IG, energy, fat and sugar intake decreased significantly between T0 and T1, while no significant changes were observed in the CG. The IG and CG did not differ significantly in respect of physical exercise at T0 and T1. In the long-term follow-up, BMI-SDS did not differ significantly between T1 and T2 (mean BMI-SDS change 0.0±0.37, $p=0.887$) and between T1 and T3 (mean BMI-SDS change 0.0±0.35, $p=0.918$) in the IG.
Conclusions: The lifestyle intervention was associated with an improvement of dietary patterns and was effective in reducing degree of overweight, fat mass, waist circumference, and blood pressure. Most importantly, the reduction of BMI-SDS at end of intervention was maintained 6 and 12 months later.

Sources of Research Support: German Federal Ministry of Research (grant numbers 01EL619 and 01EL0603).

Nothing to Disclose: TRR, AS, KW, EF, PK

P2-709

Eating Behaviour in Sardinian Obese Children and Adolescents: Role of *FTO*.

Anastasia Ibba[1], Sabrina Pilia[1], Patrizia Zavattari[1], Patrizia Civolani[1], Maria Rosaria Casini[1], Alberto Loche[1], Luigi Minerba[2] and Sandro Loche[1].

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Background. Obesity is a chronic multifactorial disease. The FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase, and polimorphism within the *FTO* gene have been consistently associated with obesity in several studies across multiple European population. The *FTO* KO mouse shows postnatal growth retardation and a significant reduction in adipose tissue and lean body mass. The pathways through which *FTO* confers increased obesity risk are still unknown. Some recent studies suggest that the FTO protein may influence either energy intake and eating behaviour, or energy expenditure.
Objective. The aim of the study was to investigate the association between *FTO* polymorphisms and eating behaviour in a cohort of obese Sardinian children and adolescent. Sardinia is the second Italian island with a population of about 1.6 million people. The Sardinian population is genetically distinct and homogeneous. Evidence for a different genetic background from other Europeans, including Italians is supported by the higher frequency of some genetic diseases (i.e. Thalassemia and Wilson disease).
Subjects and Methods. Two hundred and forty-nine subjects (127 F e 122 M; aged 3-19 years, BMI-SDS 2.66±0.38) participated in the study. Obesity was defined as BMI >95° centile according to the Italian 2006 growth reference charts. All children were genotyped for the intronic *FTO* SNP (rs9939609). Seventy-eight children had the AA genotype (mutant type), 55 the TT (wild type) and 116 were heterozygous. All children or their parents filled the Child Eating Behaviour Questionnaire (CEBQ). Eating behaviour was measured using two scales: satiety responsiveness and enjoyment of food.
Results. Results of the association analysis are shown in the figure. There was no difference in eating behaviour scores between the three different genotypes.

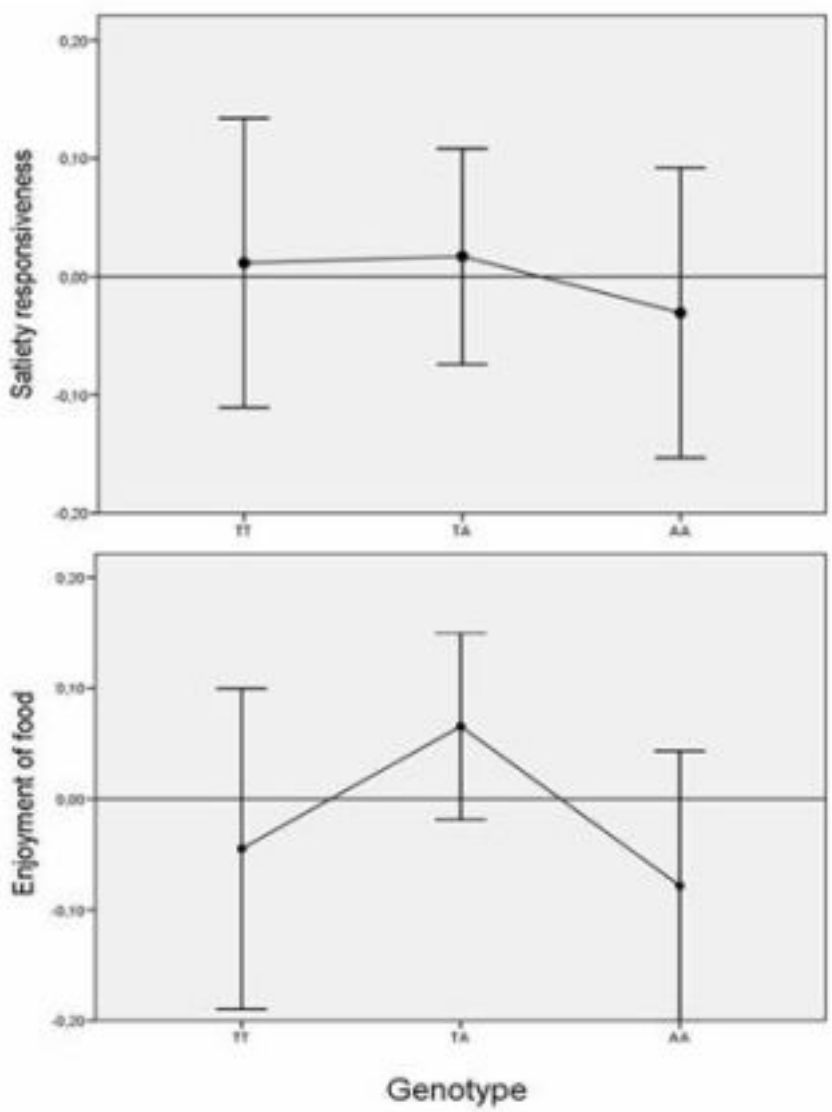

Figure
Result of FTO association analisys (mean±SD). Error bars represent ±SE).

Conclusions. Results of the CEBQ were not different between the three different genotypes in a selected cohort of obese children from a genetic isolate. Although more sophisticated tecqniques may eventually unravel subtle differences, our results do not support a major role of *FTO* in eating behavior.

(1) Wardle J et al., J Clin Endocrinol Metab 2008; 93:3640
(2) Cecil JE et al., N Engl J Med 2008; 359:2558

Sources of Research Support: Grant from Assessorato Igiene, Sanità e Assistenza Sociale, RAS.

Nothing to Disclose: AI, SP, PZ, PC, MRC, AL, LM, SL

P2-710

Urinary 6-Sulfatoxymelatonin Levels in Girls and the Relationship with Obesity.

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Aim; Short sleep duration is associated with obesity. 6-sulfatoxymelatonin (6-SM) in urine is the principal metabolite of melatonin which is closely related with sleep. The aim of this study was to evaluate the difference of urinary 6-SM levels between obese girls and normal weight girls, and the relationship of urinary 6-SM with other hormones that regulate body weight and metabolism.
Methods ; Total 76 girls (age 6.3-12.4y) were included in this study. They were 31 obese girls, 15 overweight girls, and 30 normal weight girls. We examined pubertal status and bone age. We measured fasting serum levels of total ghrelin(RIA), leptin(EIA), insulin(RIA), and 6-SM(ELISA) and creatinine concentrations in the first morning urine. HOMA-IR was calculated with fasting insulin and glucose levels.
Results ; There was no significant difference in creatinine adjusted 6-SM levels between obese girls and controls. Serum total ghrelin levels in obese girls were lower than in overweight and normal weight girls($P<0.05$). Serum leptin levels in obese girls were higher than in overweight and normal weight girls($P<0.05$). Serum insulin and HOMA-IR were not different between three groups. Urinary 6-SM levels showed no correlation with BMI, ghrelin, leptin, insulin levels, and HOMA-IR. Negative correlations were found between urinary 6-SM levels and chronological age($r=-0.37$, $p<0.05$) and bone age($r=-0.35$, $P<0.05$).
Conclusion; These results suggest that melatonin is not associated with obesity and the appetite regulating hormones. Melatonin levels decrease progressively during childhood and adolescence. Further studies for the interaction between sleep and control systems of fuel metabolism in obese children and adults are necessary.

Nothing to Disclose: SYL, CHS, SWY

P2-711

Serum Sphingolipids and Serum Inflammatory Mediators in Adolescents at Risk for Metabolic Syndrome.

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Background: Obesity is associated with metabolic syndrome and inflammation. Increased levels of tumor necrosis factor-alpha (TNF-α), free fatty acids (FFAs), and interleukin-6 (IL-6), and decreased serum adiponectin (AN) mediate this low grade inflammatory state. TNF-α increases sphingomyelinase activity *in vitro*. Serum ceramide, sphingosine (Sph) and sphingosine 1-phosphate (S1P), byproducts of sphingomyelin metabolism, are elevated in genetically obese (*ob/ob*) mice.
Objective: 1) Compare serum levels of S1P, Sph, and ceramide in overweight vs. lean adolescents. 2) Correlate serum sphingolipid levels with anthropometric parameters (body mass index (BMI) and waist circumference (WC)), measures of insulin resistance (calculated homeostasis model of insulin resistance (HOMA-IR)), lipid profiles, and serum adipocytokines.
Designs/Methods: Healthy overweight adolescents (age 13-18) with BMI ≥ 85% and lean (BMI 10-85%) controls were recruited. Anthropometric measurements and fasting blood samples were collected. Serum glucose, insulin, and fasting lipid profiles were measured. Serum adipocytokine levels were measured by ELISA or colorimetric assay. Serum sphingolipids were measured by HPLC mass spectroscopy.
Results: The study enrolled 22 overweight and 11 lean adolescents. The subjects were similar in age (14.5 yr ±1.3 vs.14.6 yr ±1.1*). Overweight subjects had greater weight *z*-score, BMI *z*-score, and WC compared to controls ($p < 0.001$ for all). HOMA-IR, was higher in the overweight group (3.6(2.6, 6.4) vs. 1.1(0.7, 1.7) ¥). AN was lower (7.3 µg/ml (5.5, 10.1) vs. 22.7 µg/ml (8.6, 26.9) ¥) and FFAs were higher (73.5 µM (44, 85.3) vs. 20 µM (8, 32) ¥) in the overweight group compared to controls. No differences were seen in TNF-α, IL6, or sphingolipid levels between groups. There were significant correlations between Sph and blood glucose ($r=0.360$, $p=0.047$); Sph and triglycerides ($r=0.46$, $p=0.001$); ceramide-S1P ratio and LDL levels ($r=0.55$, $p=0.003$). Data are given as mean±SD* or median (25^{th}, 75^{th} percentiles) ¥.
Conclusions: This study population of obese adolescents has significant insulin resistance and altered adipocytokine levels compared to lean controls. Serum sphingolipid levels correlate with some risk factors for the metabolic syndrome, but not with BMI or HOMA-IR. Given that the sample size is small, future studies will address the correlation between serum sphingolipids and risk factors for the development of T2DM in adolescents.

Sources of Research Support: Sklarow Research Foundation.

Nothing to Disclose: IM, LDM

P2-712

Very Young Children Referred for Tertiary Weight Management Have Adult-Level Obesity and Associated Metabolic Co-Morbidities.

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Little is known about the metabolic risk of very young children with severe obesity. We completed a retrospective database review to describe the degree of obesity and prevalence of abnormal metabolic markers in children aged 2-5.99 years referred to two geographically distant tertiary weight management clinics: Cincinnati Children's Hospital Medical Center (CCHMC: Cincinnati, OH) and Royal Children's Hospital (RCH: Melbourne, Australia). Our cohort included 130 children seen for initial assessments (CCHMC: N=88, 2006-2009; RCH: N=42, 2006-2008). This group represented 7% and 11% of the total referrals for the two institutions, respectively. The mean age at initial visit was 4.4±1.2 years. The mean BMI was 27.0±7.3 kg/m2 and mean BMI Z-score was 3.5±1.1. These children were tall for age (mean height Z-score =1.8±1.0) and had large waist circumferences (mean waist =82.0±11.3 cm). The RCH sub-cohort had significantly lower BMI and waist circumference than CCHMC (both p<0.02). The prevalence of abnormal metabolic parameters for the total cohort and two sub-cohorts are found in Table 1.

Metabolic Parameters of the Cohort

	CCHMC	RCH	Combined
	N/total # with data (%)	N/total # with data (%)	N/total # with data (%)
BMI>30 kg/m2	20/88 (23%)	7/42 (17%)	27/130 (21%)
BMI>40 kg/m2	6/88 (7%)	0/42 (0%)	6/130 (5%)
BMI>50 kg/m2	3/88 (3%)	0/42 (0%)	3/130 (2%)
Insulin≥20 µIU/mL	15/51 (29%)	1/7 (14%)	16/58 (28%)
Glucose≥100 mg/dL	0/60 (0%)	0/7 (0%)	0/67(0%)
LDL≥130 mg/dL	5/65 (8%)	3/6 (50%) *	8/71 (11%)
HDL≤40 mg/dL	25/65 (38%)	2/6 (33%)	27/71 (38%)
SBP≥90%ile	12/77 (16%)	10/21 (48%) *	22/98 (22%)
SBP≥95%ile	7/77 (9%)	8/21 (38%) *	15/98 (15%)
DBP≥90%ile	11/77 (14%)	7/21 (33%)	18/98 (18%)
DBP≥95%ile	4/77 (5%)	5/21 (24%) *	9/98 (9%)
GGT≥35 U/L	5/49 (10%)	0/13 (0%)	5/62 (8%)
ALT≥35 U/L	12/64 (19%)	5/13 (38%)	17/77 (22%)

* RCH significantly different from CCHMC (p<0.02)

Despite lower mean BMI, the RCH cohort's prevalence of high LDL cholesterol and hypertension was significantly higher than that of CCHMC (p<0.02); however, numbers of subjects were much smaller in the former. In conclusion, it is not uncommon for young children to present with adult levels of obesity. These children have a high prevalence of insulin resistance, elevated transaminases, and hypertension which foreshadows serious metabolic disease in young adulthood.

Nothing to Disclose: NAC, JGW, AW, ZM, GW, SAX, MAS

P2-713

Association of Retinol-Binding Protein 4 (RBP4) and Obesity in Children.

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Objective
Retinol-binding protein 4 (RBP4) is secreted by liver and adipose tissue and has been associated with obesity and insulin resistance in several animal and human studies. There is limited and conflicting data on this relationship in pediatric population. The objective of our study was to compare the levels of the RBP4 in obese and non-obese children and to determine the relationship between RBP4 and BMI, waist circumference, and other markers of insulin resistance in a cohort of obese children in Huntington, WV.
Materials and Methods
This was a cross sectional study. Subjects were children aged 8-18 years (n=45) recruited at a university based pediatric clinic. The obese group (n=28) had children with BMI above 95th percentile on CDC growth curve whereas non-obese group (n=17) were children with BMI of less than 95th percentile. Both groups were matched for age, gender and pubertal status. RBP4 was measured by ELISA technique (ALPCO diagnostics, Salem, NH). We studied the relationship between plasma RBP4 levels and BMI, BMI SDS, waist circumference, triglyceride level, HOMA IR (homeostasis model assessment of insulin resistance) and other markers of insulin resistance.
Summary of results
The plasma RBP4 level was significantly higher in obese group 16.3 $\pm$ 5.02 as compared to the non-obese group 12.4 $\pm$ 4.26 with a p value of 0.0048. Using Spearman's rank correlation, there was a significant correlation between RBP4 levels and BMI (rho 0.53, p <0.001), BMI SDS (rho 0.55, p <0.001) and waist circumference (rho 0.9, p <0.001).There was no significant correlation between RBP4 levels and serum triglycerides, insulin resistance, insulin sensitivity, age and gender.
Conclusion
Plasma RBP4 levels were higher in obese children when compared with non-obese children of the same age, gender and pubertal status in our study cohort.
There was a significant correlation between RBP4 levels and BMI, BMI SDS and waist circumference. Plasma RBP-4 level did not correlate however with triglyceride levels, HOMA IR, insulin sensitivity, age and gender.

Nothing to Disclose: MMH, AY, JW, RS, TG, YE

P2-714

Associations between Circulating Serum S100b Protein and Symptoms of Anxiety and Depression in Obese Children.

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Background: S100b is a glia-specific neurotrophic protein that has been used as a biomarker for neuroplasticity and neuropathology, as well as an index of blood brain barrier integrity. A limited number of studies have shown higher serum S100b levels in adults with major depression than in control individuals. Furthermore, S100b was higher than normal in morbidly obese adults with obstructive sleep apnea and in children with migraine headaches. Since obesity, depression and migraines are co-morbid disorders, we investigated relations between this protein and anxiety and depression symptoms in obese children.
Methods: Eighty-three children and adolescents recruited from the Childhood Obesity Clinic of our Department were examined. Symptoms of anxiety were assessed using the SCARED (Screen for Child Anxiety Related Emotional Disorders) questionnaire, while depressive symptomatology was assessed using the CDI (The Children's Depression Inventory) questionnaire. Serum S-100b levels were measured with an immunoluminometric assay.
Results: There was a positive correlation between body mass index (BMI) in SD units (z-score) and anxiety and depressive symptomatology scores. S100b was not related to age, gender or BMI z-score. However, positive correlations were found between anxiety symptoms and S100b concentrations in obese children ($r=0.313$, $p=0.008$). Furthermore, positive associations were noted between depressive symptoms and s100b ($r=0.217$, $p=0.04$), as well as a significant difference ($p=0.04$) in S100b levels between children with high or low depressive symptoms based on the Greek cut-off point of the CDI questionnaire (0.37 ± 0.12 and 0.29 ± 0.12 microg/L, respectively).
Conclusions: High S100b concentrations may represent an early marker of internalizing disorders, such as anxiety and depression, in obese children, possibly reflecting a disturbance of the blood brain barrier.

Nothing to Disclose: PP, DB, CK, CK-G, KP, IP, GPC

P2-715

Independent Relationships of Obesity and Insulin Resistance with Serum Proinsulin Level in Prepubertal Children with Normal Glucose Tolerance.

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OBJECTIVE — We compared the fasting serum proinsulin levels in lean, overweight, and obese prepubertal children with normal glucose tolerance (NGT). We also evaluated the relationship between fasting proinsulin level and indices of insulin resistance (IR).
RESEARCH DESIGN AND METHODS — One hundred nine prepubertal children (mean age, 8.6 years) with NGT were included. The indices of IR included the homeostasis model assessment of IR (HOMA-IR) and adiponectin level. We recorded the presence of 1 or more of the following metabolic derangements: triglycerides ≥ 150 mg/dl, HDL cholesterol < 35 mg/dl, and hypertension.
RESULTS — Fasting proinsulin levels significantly increased with BMI category from lean (n = 52, 7.22 ± 3.01 pmol/l) to overweight (n = 14, 12.31 ± 2.91 pmol/l) to obese (n = 43, 16.51 ± 7.27 pmol/l) (P = 0.001), after controlling for age, sex, family history of type 2 diabetes and HOMA-IR. The ratio of the fasting levels of proinsulin to insulin did not differ significantly between the 3 groups. The fasting proinsulin level correlated positively with HOMA-IR (r = 0.57, P < 0.0001) regardless of BMI z-score and family history of type 2 diabetes and correlated negatively with adiponectin level. Children with NGT who had at least 1 metabolic derangement had higher proinsulin levels than those without metabolic derangement.
CONCLUSIONS — Obesity itself or obesity-related IR may independently impose b-cell overload on prepubertal children with NGT, leading to hyperproinsulinemia without causing failure to convert proinsulin to insulin when some degree of IR and metabolic derangement appears.

Nothing to Disclose: YAL, JHY, JHK, SHL, JHK, MJK, HHL, CHS, SWY

P2-716

Identification of Childhood Obesity in Inpatient Setting: A Missed Opportunity.

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The alarming rise in childhood obesity (1) has grave implications for the physical and mental health of children. This raises an urgent need for strategies to identify obesity and provide optimal intervention to these children and their families.
This study examines the identification of childhood obesity and interventions made during inpatient admission. We conducted a retrospective chart review of admission history, physical exam and management plan for all children admitted to the General Pediatric service of an urban tertiary pediatric center for a 10 week period. Eligibility criteria included ages 2 to 18 years, documentation of measured height, weight, and blood pressure on admission. Children were excluded if admitted directly to intensive care, measurements were estimated or incomplete, or if chronic disease affecting growth or development was present. Body mass index (BMI) was calculated and BMI percentiles were derived from current CDC graphs (2) for males (M) and females (F) for age. Obesity was defined as a BMI greater than the 95th percentile for age. Overweight constituted a BMI between the 85th and 95th percentiles (3). 80 children (42 M, 38 F) met age and other inclusion criteria and were used in this analysis.
Of the 80 children, 37.5% (n=30, 14M, 16 F) were obese, 11.25% (n=9, 4M, 5F) were overweight, 8.75% (n=7, 2M, 5F) were underweight (BMI less than the 3rd percentile for age and sex). 42.5% (n=34, 22M, 12F) had BMI in a healthy weight range.
Of the 30 obese children, only 8% were documented as such by a resident or attending physician in the admission assessment.
In only 1 instance was obesity included in a problem list and addressed in the assessment and plan of the patient, and a referral to an outpatient obesity clinic was made.
None of the 30 children were seen by or referred to a nutritionist during hospitalization.
AAP recommended cholesterol and diabetes screening was not performed for any of the 30 children (4,5).
This preliminary data highlights underidentification of childhood obesity in inpatient setting and lack of a coordinated approach to screen and counsel those identified. Electronic charting of BMI percentiles and flagging abnormal values followed by a flowchart to assess for comorbidities may assist in rapid identification of childhood obesity and comorbidities. Also, efforts need to be directed at nutritional screening and counseling during hospital admission when entire family's attention might be easily captured.

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Nothing to Disclose: KAS, AG

P2-717

Plasma Advanced Glycation End Products (AGEs) and Their Correlation with Inflammatory Markers in Middle School Age Children.

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CONTEXT: Plasma advanced glycation end products (AGEs) are the result of non-enzymatic glycation and oxidation of proteins and lipids. AGEs have been implicated in impaired insulin action and diabetes co-morbidities due to enhanced oxidative stress and microinflammation.
OBJECTIVE: This study investigated the correlation between the levels of the AGE N-carboxymethyllysine(CML) and multiple adiposity-related co-morbidity risk factors in healthy adolescents.
SETTING, DESIGN, AND PARTICIPANTS: We analyzed BMI, % fat, waist circumference, TNF-α, IL-6, CRP, glucose, adiponectin and CML in 40 school age children 11-15 yrs of age [mean(sem) 13.0± 0.1 yrs; female/male ratio 1/1] who participated in the ROAD consortium. Insulin secretory capacity was measured as acute insulin response (AIR, mean rise in insulin 3 and 5 minutes after 0.5gm/kg of I.V. dextrose) and glucose disposal index [GDI, $\log_{10}$(AIR x [fasting glucose] /[fasting insulin])]. CML data were log transformed to insure normality of distribution.

RESULTS: The children had a Mean (SD) BMI of 22.6 (4.8) kg/m^2. Serum CML was significantly inversely correlated to BMI, waist circumference, % fat, fat mass, waist circumference and LDL-cholesterol and directly correlated to HDL-cholesterol, adiponectin, insulin and QUICKI (all $p<0.05$). Correlations with HDL and adiponectin remained significant when corrected for age, sex, and % body fat.

Table 1

	Log CML			
			Semi Partial r adjusted for age, sex, a	
	r	p	r	p
BMI	-0.31	0.05		
% Fat	-0.33	0.02		
Fat Mass	-0.37	0.04		
Waist	-0.34	0.04		
HDL	0.35	0.03	0.34	0.03
LDL	-0.35	0.03		
Adiponectin	0.33	0.03	0.37	0.02
Insulin	0.33	0.03		
QUICKY	0.35	0.03		

CONCLUSION: In this group of middle school students log CML was inversely correlated with BMI, % fat, fat mass, waist circumference and LDL and directly correlated with HDL, adiponectin and QUICKI. The inverse correlation of AGEs with multiple adiposity-risk factors in adolescents is contrary to the direct correlation of AGEs with these same risk factors reported in adults. Further studies, including examination of pro-inflammatory AGE receptors (RAGE) and anti-oxidant receptors (AGER1) are necessary to fully evaluate these findings.

Sources of Research Support: AMDeC.

Nothing to Disclose: LM, WR, MR, RMC, IF, AMJ, DD, KP, ES, SC, LI, RR, DEC, CB-B, PWS , FJJ, RG, SS, ST, SA

P2-718

Degree of Fatty Liver in Obese, Hispanic Adolescents with Vitamin D Deficiency.

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of liver disease in children and it is described as a chronic inflammatory process that can eventually cause progressive liver dysfunction. Low Vitamin D levels are associated with NAFLD in adults and are proposed to contribute to the underlying insulin resistance and inflammation which leads to the development of NAFLD. In recent studies, 25 OHD has been shown to be inversely correlated with BMI, plasma glucose, and insulin resistance (1). We are conducting a pilot trial to determine the degree of NAFLD by magnetic resonance imaging and spectroscopy (MRS) in Vitamin D deficient, obese, Hispanic adolescents.
Materials and methods: Subjects were obese (body mass index over 85%), adolescents (ages 10-17 years) who presented with with elevated ALT (greater than 1.5 x normal) and Vitamin D deficiency (<30 ng/mL) without evidence for other causes of fatty liver disease (Hepatitis B and C profile, ANA, anti-LKM antibody, anti-smooth muscle antibody, alpha-1 antitrypsin level, ceruloplasmin, iron, and TIBC). We evaluated each subject's fasting glucose, insulin, lipid panel, and liver fat by MRS. Subjects were excluded if they had type 2 diabetes, positive fatty liver screening exams, or were taking medications known to affect the liver.
Results: Subjects (ages 10-15 years) had an average 25 OHD level of 19 ± 2 ng/mL and elevated ALT (73 ± 17 U/L) at the time of the MRS.

TABLE 1: Patient Baseline Characteristics

	Baseline (n=6)	Range
Age	12	10-15
Gender (M:F)	3:3	
BMI (kg/m2)	38 ± 2	30-45
25 OHD (ng/mL)	19 ± 2	9-23
Fasting glucose (mg/dL)	91 ± 3	81-100
Fasting Insulin (uIU/mL)	76 ± 34	20-223
Total Cholesterol (mg/dL)	158 ± 13	106-199
LDL (mg/dL)	85 ± 6	64-102
HDL (mg/dL)	35 ± 4	22-50
Triglycerides (mg/dL)	192 ± 41	43-324
ALT (U/L)	73 ± 17	31-147
AST (U/L)	37 ± 7	21-70

The average liver fat (normal levels <5%) assessed by MRS was 30 ± 4 %.

TABLE 2: MRS Results

Subject	% (Normal < 5%)
1	40
2	27
3	22
4	42
5	17
6	30
Average	30 ± 4

Conclusion: Obese, Hispanic adolescents with Vitamin D deficiency are at risk for significant NAFLD. We currently have recruited 6 subjects with a goal of 20 adolescents and we recommend further clinical trials to further evaluate the association between the degree of liver fat and Vitamin D levels and whether treatment with Vitamin D improves the degree of NAFLD.

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Sources of Research Support: Lester and Liesel Baker Foundation Award; Institute for Integration of Medicine and Science Clinical and Translational Science Award at the UTHSCSA.

Nothing to Disclose: COO, JLL, DEH

P2-719

Pilot Study of BMI Changes among Teens in a Multidisciplinary Weight Management Program: Should the Goal Be Weight Loss or Slowing the Rate of Weight Gain?.

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Background: Comprehensive weight management programs have been developed to address the obesity epidemic. While most programs look only at weight loss, we evaluated the effect of a weight management program on the rate of weight gain.
Objective: To explore the effect of a multidisciplinary weight management program in lowering BMI and also in decreasing the rate of weight gain.
Design/Methods: A retrospective analysis of teens enrolled in a 10 week weight management program. Patients were referred by their primary care providers and then met with the team for an intake before entering the SHAPEDOWN program. Changes in BMI were calculated from the time of referral to entering the program and at the initial and final program visit.
Results: 7 teens completed the SHAPEDOWN program. 5 females and 2 males ages 14-17years. Mean initial BMI was 39.6 kg/m2, mean BMI at the start of the program was 40kg/m2, and mean BMI at the end of the program was 39.5kg/m2. The mean change in BMI from the start of SHAPEDOWN to the final visit was -0.5mg/m2 (see table 1). The teens were referred to the program at different times so weight gain/month prior to entering the program was calculated. From the time of evaluation until they entered the program, the teens gained a mean of 0.48kg/month and lost a mean of 0.69kg/month during the program (see table 2).

BMI Change During SHAPEDOWN

BMI Before SHAPEDOWN	BMI at Start of SHAPEDOWN	BMI at END of SHAPEDOWN	Mean Change From Start to End of SHAPEDOWN (Range)
39.7	40	39.5	-0.5 (-1.5 to 0.9)

p=0.89

Rate of Weight Change (kg/month) Before Starting SHAPEDOWN vs. Weight Change at the End of SHAPEDOWN

Weight Change Before SHAPEDOWN kg/month	Range	Weight Change During SHAPEDOWN kg/month	Range
0.48	(-3.2 to 2.4)	-0.69	(-1.45 to 1.1)

p=0.22

Conclusion: Although the most of the teens achieved modest weight loss during the program (6/7), it is more significant when you consider that prior to entering the program, they were gaining weight at a rate of 0.48kg/month. Achieving statistical significance was difficult due to the small number of teens in our pilot program but we suspect that further studies will show the value of weight management programs in slowing down the rate of weight gain in teens.

Nothing to Disclose: BRM, PN, SW

P2-720

Differences in Metabolic Parameters in Patients with Bardet-Biedl Syndrome Compared with BMI-Z Matched Controls.

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Background: Bardet-Beidl syndrome (BBS) is a genetically heterogeneous disorder within a group of ciliopathies associated with obesity (1). In BBS mouse models, ciliary dysfunction leads to impaired leptin signaling (2), and hyperleptinemia precedes onset of obesity, suggesting a causal role for leptin resistance (3).
Objective: To compare metabolic parameters of patients with BBS and BMI-Z matched controls.
Methods: 50 patients with BBS were matched 2:1 by age, sex, race, and BMI-Z with 100 healthy controls. Groups were compared for differences in body composition (DEXA, abdominal MRI), blood pressure (BP-Z, standardized for age, sex & height), and fasting concentrations of leptin, lipids, insulin, and glucose. To account for potential confounders due to residual differences despite matching, data were analyzed using ANCOVAs.
Results: Subjects with BBS and controls were well-matched for age, sex, race, and BMI (Table). Body fat % was similar, but visceral adiposity was greater in patients with BBS. Patients with BBS had higher DBP-Z, leptin, and triglycerides, but no significant differences in the remaining variables. Analyses adjusting for covariates (age, sex, race, body fat %) yielded comparable results. *BBS1* (24%) and *BBS10* (32%) mutations were most frequent. In subgroup analyses, patients with *BBS1* mutations were similar to BMI-matched controls for all variables, whereas the differences in visceral fat, DBP-Z, leptin, and triglycerides between patients with *BBS10* mutations and controls persisted.

	BBS (n=50)	Control (n=100)	P-value
Age (y)	14.8 ± 11.1	15.3 ± 11.0	0.68
Sex (% female)	50	50	1.00
Race/Ethnicity (% Caucasian)	98	98	1.00
BMI (kg/m^2)	31.7 ± 9.7	31.5 ± 8.4	0.96
BMI Z-score	2.17 ± 0.89	2.10 ± 0.84	0.60
Height Z-score	-0.03 ± 1.38	0.83 ± 1.30	**<0.001**
Total Body Fat (% of total weight)	42.1 ± 8.5	39.4 ± 10.5	0.19
Visceral Fat (% of total fat at L2/3+L4/5)	26.9 ± 10.4	19.7 ± 6.1	**0.001**
Systolic BP (mmHg)	118 ± 15	117 ± 13	0.58
Systolic BP Z-score	1.17 ± 1.45	0.82 ± 1.23	0.12
Diastolic BP (mmHg)	70 ± 8	65 ± 8	**<0.001**
Diastolic BP Z-score	0.72 ± 0.80	0.14 ± 0.73	**<0.001**
Leptin (ng/mL)	42.1 ± 28.5	23.7 ± 16.3	**<0.001**
Cholesterol (mg/dL)	160 ± 30	167 ± 35	0.28
Triglycerides (mg/dL)	182 ± 133	112 ± 88	**<0.001**
HDL Cholesterol (mg/dL)	40 ± 12	43 ± 12	0.09
LDL Cholesterol (mg/dL)	101 ± 28	111 ± 29	0.06
Fasting Glucose (mg/dL)	86 ± 9	89 ± 16	0.11
Fasting Insulin (mU/L)	21.8 ± 22.6	15.8 ± 10.9	0.11
HOMA-IR	4.76 ± 5.34	3.65 ± 2.85	0.21

Discussion: Patients with BBS have higher leptin than expected for their degree of adiposity, consistent with leptin signaling dysfunction. Preferential deposition of fat intra-abdominally may predispose to metabolic complications. Lack of difference between patients with *BBS1* mutations vs. controls suggests variability in phenotype by genotype. In particular, the BP profile of these patients is consistent with *Bbs1* mutant mice, which are obese but normotensive (4). These results show that the obesity of BBS is distinct from nonsyndromic obesity and that there is heterogeneity in both genotype and clinical phenotype among the causative BBS loci.

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(2) Seo S et al., Hum Mol Genet 2009; 18:1323
(3) Rahmouni K et al., J Clin Invest 2008; 118:1458
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Sources of Research Support: NIH Intramural Research Programs of NICHD and NHGRI. JAY and JCH are commissioned officers of the US Public Health Service.

Nothing to Disclose: JCH, PPF, DN, JCS, YCZ, JAY, LGB

P2-721

Circulating Lipoprotein-Associated Phospholipase A2 Levels Are Elevated in Obese Children.

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Introduction: Obesity and cardiovascular disease are often co-morbid, but the pathophysiologic mechanisms that link the two are not fully understood. Lipoprotein-associated phospholipase A2 (Lp-PLA2) is involved in the modification of lipids within atheromas. Recently, circulating Lp-PLA2 was found to be predictive of thromboembolic episodes in adults, independently of a variety of other potential risk factors, including markers of inflammation, renal function, and hemodynamic stress. However, the function of this lipase and its importance as a biomarker in children, especially obese ones, has not been studied as yet.
Aim of the study: To study Lp-PLA2, a nontraditional risk factor of cardiovascular disease (CVD), in obese children.
Patients and methods: 67 lean (39 boys- 28 girls, mean BMI z-score: -0.23 ±0.78) and 66 obese (32 boys-34 girls, mean BMI z-score: 4.39 ±1.18) age-matched (p=0.08) children, aged 6-12 years, were studied. All children had a physical examination and morning blood drawn after 12h of fasting. Glucose, insulin, lipid profile and Lp-PLA2 were determined. Plasma concentrations of Lp-PLA2 were determined by a commercially available Lp-PLA2 enzyme-linked immunosorbent assay (ELISA) kit (PLAC Test). BMI z-score was calculated based on the Greek BMI growth curves and children were categorized as obese according to the Cole criteria.
Results: Plasma Lp-PLA2 levels were significantly higher in obese children (322.5 ± 77.8 ng/ml) than those of the normal weight group (278.0 ± 64.4ng/ml), ($p<0.00001$). Lp-PLA2 concentrations were significantly correlated with the BMI z-score (p=0.006). **Discussion:** We found significantly higher Lp-PLA2 levels in obese children than lean controls. Interestingly, they all had levels >200 ng/ml, which are thought to correlate with atherosclerosis and a high thromboembolic risk in adults. The positive correlation of Lp-PLA2 with BMI reveals an effect of obesity on this marker, which suggests vascular involvement caused by the increase of weight, even at a very young age. Measurement of Lp-PLA2 in plasma may be a valuable addition to the panel of tests used to identify individuals at high CVD risk.

Nothing to Disclose: SDS, PP, NL, CK, CK-G, GPC, IP

P2-722

Autonomic Dysfunction in Obese Adolescents: Another Route to Cardiovascular Morbidity?.

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Introduction: Adverse consequences of obesity include hypertension(HTN) and increased morbidity & mortality. The mechanisms underlying these consequences are not fully understood. The goal of our study was to examine overweight adolescents for autonomic dysfunction(AD). Autonomic function is a balance between the sympathetic (SNS) and parasympathetic (PNS) systems. The PNS is referred to the “rest and digest” system while the SNS is referred to the “fight or flight” system. Continuous EKG monitoring of heart rate variability (HRV) can be analyzed to quantify SNS and PNS tone. We obtained measurements at baseline and with challenges: deep breathing(DB), known to activate the PNS; valsalva(V), which activates the SNS; and standing(S), which activates the SNS initially, then the PNS. Frequency domain measures are obtained with the Fourier transformation providing power calculations at frequencies correlating with HF, solely PNS activity and LF which has PNS and SNS components. LF is further divided into sympathetic(LFA) tone and parasympathetic tone(RFA) using HRV software.
Methods: We enrolled 33 pubertal subjects with BMI > 85% for age and sex. Patients underwent HRV testing as above, and a 2 hour GTT. Fasting blood was analyzed for glucose, insulin, liver enzymes, lipid panel, fT4, TSH, free/total testosterone, adiponectin, ghrelin, & leptin. Statistical analysis was performed using Spearman correlation for HRV measures given non-Gaussian distribution. MannWhitney test was used to compare obese subjects to those with metabolic syndrome (MS).
Results: Half of subjects met criteria for MS. At baseline, subjects with higher weight & BMI had lower LFA/RFA and LF/HF ratios, suggesting higher parasympathetic tone relative to sympathetic tone. In the DB event, subjects with higher 2hr insulin & glucose had lower LF normalized units(nu) and higher HFnu. In the V event, subjects with higher BMI had higher LF. Finally, in the S event, subjects with higher weight and BMI had lower LFA/RFA and LF/HF and higher HFnu.
Conclusion: Overweight subjects had evidence of AD, which correlated with Wt, BMI, and with insulin levels in the 2 hour GTT. MS did not appear to be associated with worsened AD. AD may be a separate risk factor for the development of cardiovascular disease and morbidity in the obese patient. Identifying and addressing AD may be important in improving the long term outcome in obese individuals.

Nothing to Disclose: HKM, CO, JMB, MAP

P2-723

Distinct Serum Proteome Profile in Obese Adolescents Females with Metabolic Syndrome.

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Background: Obesity is common in adolescents and places these adolescents at a higher risk for cardiovascular disease, hypertension, diabetes mellitus, and cancer. The pathophysiology of this risk is not completely understood. The purpose of our study is to investigate the plasma protein profile of obese adolescents with or without metabolic syndrome and to identify patterns that might elucidate pathways involved in the progression of obesity related disease.
Methods: Fourteen overweight adolescent patients were selected and screened for metabolic syndrome. Blood samples were collected and anti-coagulated with sodium citrate. Low abundance signaling proteins in plasma samples were fluorescently tagged, and assayed by large scale antibody microarrays and confirmed using reverse capture protein microarrays and Western blot (1). Antibody microarray data were normalized by use of an internal standard.
Results: Female patients with the full metabolic syndrome had distinct patterns in the serum protein profiles compared to any of the other groups (male and female controls or males with metabolic syndrome) and were different from the female subjects who were obese, but without other characteristics of the metabolic syndrome. Male metabolic syndrome patients interestingly were most similar to male controls and female obese patients were similar to female controls. There were clear gender differences in the serum profiles.
Implications: These data suggest gender may be an important factor in the progression complications related to metabolic syndrome and obesity. Of interest, a recent study by Lin et al, showed individuals with metabolic syndrome had vessel wall changes and in particular, women with MS, and not men, had significantly larger common carotid artery intima-media thickness measurements than controls without MS. Further, notable changes in vessel wall thickness were present in younger women, ages 15-45, with MS(2). Thus, younger women with metabolic syndrome may have higher cardiovascular risk factors than previously thought. Our study showing distinct serum protein profiles in adolescent girls may lead to insights into the clinical findings cited in this article. Larger studies are underway to validate these findings and identify potentially involved pathways.

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Nothing to Disclose: HKM, SWR, OE, CA, HP, MAP

P2-724

Nonalcoholic Fatty Liver Disease in Children (NAFLD): Does the Severity of Disease Correlate with Mitochondrial Respiratory Chain Enzyme Activity?.

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Background and Aim: NAFLD and its severe form nonalcoholic steatohepatitis (NASH) are considered a manifestation of bioenergetics failure of hepatic cells leading to reactive oxygen and reactive lipid species production and hence cellular damage. Our aim in present study was to find any correlation between mitochondrial respiratory chain enzyme activity and the severity of NAFLD as determined by histology.
Methodology: we enrolled in a pilot study 4 children with severe obesity (BMI > 95% for age and sex) and persistent elevated liver enzyme levels, with negative markers for viral hepatitis B and C, after taking informed consent from them. Exclusion criteria were presence of diabetes mellitus, thyroid function abnormalities and any other systemic disease. Patients underwent liver ultrasound, biochemical tests and liver biopsy for diagnosing and staging their disease. We also extracted mitochondria from a portion of the biopsy and measured complex 1 and citrate synthase enzyme activity levels in these mitochondria samples.
Results: All 4 children had elevated ALT levels (ALT >40 IU/L) and fatty liver on liver ultrasound. All 4 had fasting hyperinsulinemia. 3 children had only steatosis and one had severe steatosis along with fibrosis on liver biopsy. There was no significant correlation of complex-1 and citrate synthase enzyme levels with either disease severity on liver biopsy or ALT levels or the quantitative insulin-sensitivity check index (QUICKI).

Metabolic characterization of subjects

Subjects	Age(yrs)	ALT(IU/L)	QUICKI	TG(mg/dL)	Liver Biopsy	Complex 1 activity(nmol DCIP/min/mg)	Citrate synthase activity(nmol/ DTNB/min/mg)
1	9	58	0.31	117	mild to moderate steatosis	53.1	113.3
2	11	124	0.32	139	severe steatosis, focal portal fibrosis	51.7	73.7
3	15	50	0.34	29	mild steatosis	44.9	41.1
4	20	64	0.31	162	mild steatosis	73.2	105.5

Conclusion: In previous studies, decreased mitochondrial enzyme activity has been shown to be present in patients with NASH. We could not find any correlation between disease severity and mitochondrial enzyme activity which could be explained partially by the small sample size and biopsy sampling variations.

Nothing to Disclose: RG, SJ, ST, AB, SN, AS

P2-725

Effects of a Non-Intensive Weight Management Program in Obese Children: Correlation between the Frequency of Clinic Visits and Changes of Body Mass Index and Features of the Metabolic Syndrome.

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Introduction: It is known that intensive weight management programs, based on frequent interactions with a multi-disciplinary team, lead to weight loss and improved metabolic profile of obese children. Yet, most of the beneficial effects of a short-term intervention do not often persist once the program is completed. We have previously shown that a non-intensive weight management program may have long-term beneficial effects on obese children. In this study, we evaluate the correlation between the frequency of visits and the long-term changes of BMI and of features of the metabolic syndrome.
Methods: Chart review of obese (BMI > 95th percentile), non-diabetic children followed at the Weight Management Center of the Section of Endocrinology and Diabetes at St. Christopher's Hospital for Children. During the visits, age-appropriate caloric intake and regular physical activity were recommended. Inclusion criteria were: 1) ≥2 years of follow- up; 2) fasting blood sample drawn at the first and last visits of the follow-up period.
Results/Trends: 52 children (17 males) were included in the study. At the initial visit, their age was 11.4±2.4 yrs (mean ± SD). The duration of the follow-up period was 47.3±11.1 months, while the mean number of clinic visits (CV) per year was 2.9±0.9. 41 of the 52 children underwent an OGTT at the beginning and the end of the follow-up. When children were divided in two groups according to their mean number of CV/yr (≤ 2 vs. >2; 1.6±0.4 vs. 3.2±0.7, $p<0.001$), there was no difference at baseline for any of the metabolic variables analyzed or for BMI SDS. At the end of the follow-up, children with >2 CV/yr exhibited a significant decrease in their LDL-cholesterol (114.6±36.6 vs. 98.1±22.7 mg/dL, initial vs. last visit, $p<0.05$), 2-hour glucose (114.9±24.6 vs. 92.5±17.9 mg/dL, $p<0.001$) and peak insulin (202.9±115.8 vs. 134.9±75.4 ulU/mL, $p<0.01$), while children with ≤ 2 CV/yr did not. No change in HOMA, HDL-cholesterol, or triglycerides occurred in either of the two groups. Although the mean BMI SDS at the end of the follow-up was lower than that at the beginning in both groups, the difference did not reach statistical significance.
Conclusion: Our data suggest that a non-intensive, conventional weight management program can have beneficial effects on the metabolic profile of obese children who remain in the program for several years. Yet, these beneficial effects still depend on the frequency with which children are evaluated.

Nothing to Disclose: RAK, CD, FDL

P2-726

Short Term Effects of Vitamin D Supplementation on Body Mass Index in Overweight Adolescents Receiving Second Generation Antipsychotics.

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Objective
Second generation antipsychotic (SGA) medications are increasingly used to treat psychiatric illnesses in the pediatric population. However, there is no consensus on the best approach for the treatment of the weight gain and metabolic abnormalities associated with this therapy.

Method
An 8-week open-label trial was conducted to evaluate the effectiveness of vitamin D supplementation, using ergocalciferol 2000 IU per day, to manage weight gain in 12 overweight and vitamin D deficient adolescents of average age 16.6±1.65 years who were receiving SGA. Height, weight, body mass index (BMI), as well as fasting insulin, glucose, c-peptide, lipid profile, 25-hydroxyvitamin D (25OHD), 1,25-dihydroxyvitamin D, adiponectin, and leptin levels were measured at baseline and at endpoint.

Results
At the end of the study, there was a significant increase in height, a non-significant decrease in weight and a significant decrease in BMI (p=0.018) Table 1. Serum concentrations of 25OHD rose significantly (p=0.005). There was also a significant decrease in the serum concentrations of total cholesterol (p=0.005), LDL-cholesterol (p=0.008), and HDL-cholesterol (p= 0.009). A non-significant decrease in the homeostasis model assessment of insulin resistance and adiponectin level occurred. No adverse effects were noted.

Anthropometric Characteristics of Subjects at Baseline and at Study Completion

Parameters	Baseline[a]	Final[a]	Difference ±95%CI[º]	p value (t-test)
Height (SDS)	0.32±1.29	0.62±1.40	0.30[0.10 - 0.50]	0.002∗
Weight (SDS)	2.56±1.32	2.49±1.38	-0.07[-0.20-0.06]	0.183
BMI (SDS)	2.56±0.75	2.40±0.82	-0.17 [(-0.32)-(-0.01)]	0.018∗

[a]=mean±SD; BMI=body mass index; SDS=standard deviation score; ∗=significant p value;[º]=95% Confidence Interval

Conclusions
Ergocalciferol appears to be a safe and effective intervention to decrease BMI and serum cholesterol in overweight, vitamin D-deficient adolescents who are receiving second generation antipsychotic medications. Future double-blind, placebo controlled studies are warranted.

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Nothing to Disclose: BUN, BM, LM, CC, JK, DR, LC, JAF, MML

P2-727

Predictors of Impaired Glucose Tolerance in Overweight Children and Adolescents and Comparison of Surrogate Insulin Sensitivity Indexes Derived from a Fasting Oral Glucose Tolerance Test (OGGT).

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Background: ADA recommends obtaining fasting blood glucose levels or using an oral glucose tolerance test (OGTT) to determine the glycemic status in overweight pediatric population (BMI ≥ 85%). Impaired glucose tolerance (IGT) is defined as a serum glucose of > 140 and < 200 mg/dl (7.8 to 11.1 mmol/L) measured at two hours following a 75 g oral glucose tolerance test (OGTT). The impaired fasting glucose (IFG) is a fasting serum glucose > 100 mg/dl (> 5.6 mmol/L). Objective: To determine the predictors of impaired glucose tolerance (IGT) status in a population of overweight children and adolescents.
Study design and methods: Retrospective chart review of 201 overweight pediatric patients (BMI ≥ 85%) of mixed ethnicity, who had an OGTT at our institution between the years 2007 – 2008. Multivariate logistic regression model with stepwise selection was used for data analysis. Insulin sensitivity surrogates determined by using the OGTT data include: Homeostasis model of insulin resistance (HOMA-IR), the quantitative insulin sensitivity check index (QUICKI), insulin sensitivity index-Matsuda (ISI-Mats), the area under the curve for glucose (AUCG) and area under the curve for insulin (AUCI).
Results: 87 % had normal glucose tolerance (NGT), 11 % had impaired glucose tolerance (IGT), and 2 % had silent type 2 diabetes mellitus (DM). Analysis of the following group of variables: fasting glucose (FBG), fasting insulin (FI), maximum OGGT insulin level, 2-hour insulin level, ISI-Mats, AUCI, AUCG, HOMA-IR and QUICKI revealed that an elevated FBG and 2-hour insulin level are the best predictors of IGT. A 10 mg/dl increase in FBG is associated with 90% increase in predicted odds of having IGT [odd ratio: 1.89; 95% confidence interval (CI): 1.11 – 3.24; p=0.019]. A 10 mcIU/ml increase in 2-hour insulin level is associated with 11% increase in odds of being IGT [odds ratio: 1.11; 95% CI: 1.06-1.16; p<0.001]. Except for ISI-Mats, all other parameters were found to be predictive of IGT. Among insulin sensitivity indexes, HOMA-IR was found to be the best predictor of IGT status [odd ratio: 1.21, 95% CI: 1.09-1.35; p<0.001].
Conclusion: Elevated fasting blood glucose and 2-hour insulin level on an OGTT are the best predictors of IGT status in our cohort.

Nothing to Disclose: GSB, JRB, AKS, FU

P2-728

Gut Hormones, Insulin Resistance, and Risk Factors for Cardiovascular Disease in Morbidly Obese Adolescents.

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Background: Ghrelin's orexigenic effect is mediated centrally to increase appetite through NPY and the arcuate nucleus. It has been demonstrated that both central sympathetic and peripheral effects on the endothelin-1/nitric oxide balance and endocardium may account for ghrelin's effect on reducing blood pressure, which is thought to decrease cardiovascular risk. 1,2,3 This relationship between ghrelin and blood pressure has not been explored in an adolescent population. In contrast to ghrelin, elevated CRP, as a marker of subclinical inflammation, has been associated with hypertension, hyperinsulinism and future cardiovascular risk.4,5,6,7
Objective: To determine if morbidly obese adolescents demonstrate similar correlations between gut hormones, insulin resistance and risk factors for cardiovascular disease as has been described in adults.
Methods: A subset of adolescent patients involved in a multidisciplinary program of laparoscopic adjustable gastric banding were evaluated by a meal based test to assess eating patterns in relationship to cardiovascular risk markers and the gut hormones leptin, PYY and ghrelin. Adolescents consumed a set amount of liquid meal replacement (Optifast) to evaluate fasting and post-prandial gut hormones. Oral glucose tolerance tests and anthropometric and metabolic data were taken from the nearest medical appointment. Eleven patients were evaluated who had at least two of three gut hormones measured. Multivariable analysis of metabolic risk factors for cardiovascular disease (hyperglycemia, elevated triglycerides, low HDL, elevated CRP, and hypertension) were correlated with gut hormones and insulin, using Pearson Correlation Coefficients, with a cut-off of $p < 0.1$ due to small sample size.
Results: Baseline ghrelin was negatively associated with systolic blood pressure ($p = 0.01$, $r = -0.76$) and CRP ($p=0.07$, $r=-0.59$). Fasting insulin was positively associated with CRP ($p=0.01$,$r=0.72$). PYY, PYY AUC, and fasting leptin did not have significant correlations with any risk factors for cardiovascular disease.
Conclusions: In a small cohort of morbidly obese adolescents, similar to findings in adults, ghrelin and fasting insulin were strongly associated with blood pressure and CRP, two known risk factors for future cardiovascular disease.

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Sources of Research Support: Empire Clinical Research Investigator Program.

Nothing to Disclose: SEL, RMC, DJM, JZ, SEO, IF

P2-729

Acylated, Unacylated Ghrelin and Obestatin Regulation during Oral Glucose Tolerance Test in Obese Prepubertal and Pubertal Children.

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Introduction. Three peptides, acylated ghrelin (AG), unacylated ghrelin (UAG) and obestatin are derived from a common prohormone, preproghrelin by posttranslational processing, originating from endocrine cells in the stomach. Circulating ghrelin levels are decreased in obesity and in metabolic syndrome (MS) in adulthood and increased by fasting and anorexia nervosa, but the physiological role of the three peptides is poorly understood in particular in childhood.
Aim. In order to understand the biological implications of these three peptide in obesity and MS, we measured AG, UAG, obestatin, at fasting and every 60 min during an oral glucose tolerance test (OGTT) in 60 prepubertal (PP-OB; 32) and pubertal (P-OB; 28) children. Auxological parameters, glucose and insulin during OGTT, lipid profile and blood pressure were also evaluated to divide patients according to paediatric IDF 2007 criteria for MS.
Results. In PP-OB group, 20 (62.5%) children had MS and 4 of them had glucose intolerance (impaired fasting glucose or impaired glucose tolerance). In P-OB group, 28 (100%) children had MS and 5 of them had glucose intolerance. UAG ($p<0.004$), obestatin ($p<0.05$) and nearly to significance AG ($p=0.07$) were lower in OB-P when compared to OB-PP. AG levels were higher in both prepubertal and pubertal males when compared to females ($p<0.04$). During OGTT: 1) AG levels decreased at 60 min ($p<0.04$) and returned to basal levels at 120 min in both PP-OB and P-OB; 2) UAG levels decreased for all the testing session ($p<0.0001$) in both groups and more significantly in PP-OB when compared to P-OB ($p<0.02$); 3) obestatin levels decreased at 120 min ($p<0.04$) in both groups. Fasting UAG ($p<0.01$) and during OGTT obestatin levels ($p<0.01$) were lower in children with MS when compared to those without MS. Fasting UAG ($p<0.05$) and obestatin ($p<0.02$) were also lower in PP-OB children with glucose intolerance when compared to euglycemic PP-OB.
The levels of three peptides were positively correlated each others ($p<0.004$). The AG decrease was correlated with glucose nadir during OGTT ($r: 0.357$; $p<0.02$). The UAG decrease was associated with fasting insulin, HOMA index and insulin nadir ($r: 0.372$; $p<0.02$) during OGTT. The obestatin decrease was associated with fasting HDL-cholesterol ($r: -0.497$; $p<0.002$). Conclusions. AG, UAG and obestatin levels were differently inhibited during OGTT in OB children. MS influences the glucose-induced regulation of ghrelin system also in childhood.

Nothing to Disclose: SB, FP, AP, SR, ID, GA, GB

P2-730

Waist-to-Height Ratio Is Useful as BMI To Identify High Metabolic Risk but Does Not Predict Ghrelin and Adiponectin Regulation in Paediatric Obesity.

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Introduction. Waist-to-height ratio (W/Hr) has been recently proposed to be a greater value for predicting abdominal obesity, metabolic syndrome (MS) and cardiovascular disorders in adults and children. Circulating ghrelin and adiponectin levels are decreased in obesity and MS and negatively correlate with BMI. The role of W/Hr in predicting ghrelin and adiponectin secretion in comparison to BMI has not been investigated yet.
Aim. In order to understand the usefulness of W/Hr in obesity and MS in childhood, we measured AG, UAG, obestatin, total adiponectin (Tad), high molecular weight adiponectin (HMW), glucose, insulin, lipid profile and liver enzymes at fasting in 155 prepubertal (PP; 75) and pubertal (P; 80) children. Auxological parameters, blood pressure, HOMA, QUICKI and acanthosis indexes were also evaluated. We divided patients according to paediatric IDF 2007 criteria for MS.
Results. In all the group, 74 children were obese (34 PP and 40 P) and 59 (79.7%) had MS and 8 of them had glucose intolerance (IFG or IGT). In the 81 normal weight children (41 PP and 40 P) nobody had MS or glucose intolerance. According to not normal distribution, all the parameters were log transformed.
W/Hr well correlated with anthropometric variables, components of MS, the number of IDF07 criteria other than large waist (>90th percentile), acanthosis, HOMA and QUICKI indexes (Pearson also higher than 0.300) but not with total and LDL cholesterol, and AST. The associations were maintained when weighted for pubertal status an/or sex. Log BMI presented similar correlation to W/Hr also when weighted. W/Hr correlated with UAG (r: -0.271; $p<0.02$), Tad (r: -0.412; $p<0.02$) and HMW (r: -0.403; $p<0.02$) but not with AG and obestatin. When weighted for puberty and/or sex, W/Hr maintained its correlation only with Tad and HMW. Conversely, BMI strongly correlated with UAG (r: -0.582; $p<0.0001$), AG (r: -0.356; $p<0.001$), obestatin (r: -0.153; $p<0.02$), Tad (r: -0.469; $p<0.008$) and HMW (r: -0.458; $p<0.001$) and the correlations were maintained with the same strength after corrections.
In a model composed by ghrelin and adiponectin, the best predictors were HMW (b:-0.438) for W/Hr (r2: 0.192) and much more UAG (b:-0.572) and HMW (b:-0.479) for BMI (r2:0.453).
Conclusions. W/Hr is useful as BMI in identifying MS and insulin resistance but seems to be not able to predict the alterations in ghrelin and adiponectin secretion in obesity and MS in childhood.

Nothing to Disclose: FP, SB, LT, SM, SS, GG, ED, AB, GB

P2-731

Insulinomas in Childhood: Twenty Year Experience at the Children's Hospital of Philadelphia.

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Background: Insulinomas are a rare cause of hyperinsulinemic hypoglycemia in children. Diagnosis of insulinoma in children is more challenging than in adults due to its rarity in comparison to other causes of hypoglycemia, its vagueness of symptoms, and the difficulty of confirming the lesion by imaging. Our institution's extensive experience with hyperinsulinemic hypoglycemia provides an opportunity to better define some aspects of pediatric insulinomas.
Objective: We review our institution's experience to describe the range of presentation and clinical course, to identify distinctive features, and to devise an efficient evaluation method for suspected insulinoma.
Methods: We conducted a retrospective chart review of all cases of insulinoma at the Children's Hospital of Philadelphia between 1987 and 2008. Demographics, clinical features, biochemical studies, imaging studies and pathology results were collected.
Results: Seven patients (3F:4M) were identified, between 4 and 16 years of age. All had been referred for evaluation of hypoglycemia. All had objective neuroglycopenic symptoms: syncope, seizures, confusion, or other mental status change. Whipple criteria were met in all. Median length of time between onset of symptoms and diagnosis was 6 months. Diagnostic fasts confirmed hyperinsulinism in all. Ability to fast ranged from 3-25 hrs. Insulin levels at hypoglycemia ranged from 7.3 to 292uIU/ml. Six of the 7 were confirmed to have an insulinoma by diagnostic study prior to surgery. Insulinomas were found in all regions of the pancreas. All patients were screened for MEN1 and were negative. Surgical resection was curative in all but one, whose persistent hypoglycemia required diazoxide.
Conclusion: Insulinomas are a surgically curable cause of hypoglycemia even in early childhood, and must be considered in the evaluation of hypoglycemia causing objective neuroglycopenic effects. No cases were malignant, and no cases were due to MEN1. No simple imaging method revealed all lesions, but endoscopic ultrasound may be the best current method. Biochemical studies were characteristic of hyperinsulinism, but in contrast to some forms of childhood hyperinsulinism, insulin levels were invariably measurably elevated at the time of hypoglycemia.

Nothing to Disclose: ABS, MMP, YH, CAS, DL

P2-732

Prime Time Hypoglycemia: A New Form of Factitious Hypoglycemia.

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Insulin has been misused during suicide attempts(1-3), to create purposeful hypoglycemia(1,4,5), & in Munchausen by proxy(1). For children with diabetes mellitus, hypoglycemia means sugar-containing foods, missed school, & extra attention. With a pump, insulin can be given at will. When a child on a pump has unexplained hypoglycemia, the pump can provide invaluable information.

A 13 year old male using an Medtronic Mini-Med® pump presented with hypoglycemia despite a 60% decrease in basal rates. He was healthy & taking PO. Exam was normal. BGs were controlled at home, but at school were erratic & he was often sent home. He was admitted for observation. Basal rates were returned to normal, yet overnight BGs were high. The priming history revealed multiple boluses corresponding with hypoglycemia. It was felt he manipulated the pump for personal gain. The pump was stopped, insulin injections given by an adult resumed, & hypoglycemia ceased.

A 12 year old male using an Animas One Touch ® pump presented after a hypoglycemic seizure. It was noted he often snuck food. BG was 26; despite glucagon he seized. In the hospital BG was 162 mg/dL. Basal rates were decreased 30% but BG fell to 16 mg/dL. Basal rates were reduced more & target BG raised. The pump was heard delivering insulin when a bolus wasn't given. The pump history showed multiple priming boluses correlating with low BGs. As the pump was "locked", it was assumed it malfunctioned. While awaiting a new pump, insulin injections were given with no hypoglycemia. When pump therapy resumed, inappropriate primes were again found. When confronted, the boy admitted entering these boluses.

Insulin used for priming is not included in the record of daily insulin administration, but is recorded in a separate priming history. When this history was probed, it became apparant that both boys used priming boluses to surreptitiously take insulin. We speculate they did this to sneak food. This form of insulin misuse can cause severe hypoglycemia. The priming function does not consider recent insulin delivery & the carbs eaten and insulin bolused likely do not match. The number of children misusing insulin this way is unknown. One other case of such misuse has been reported(6). This child used the prime for filling the cannula to deliver multiple, small boluses. Without specific review of the priming history, these boluses would be missed. The priming history should be routinely checked in addition to the total daily dose.

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Nothing to Disclose: JO, NS, MG, TAW

P2-733

Pediatric Diabetic Ketoacidosis Associated with Transient Hematuria and Hydronephrosis.

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Background: Hematuria and myoglobinuria associated with diabetic ketoacidosis (DKA) in type 1 diabetes (DM1) has been described in adults, often with pre-existing renal impairment, but is rare in children. Thrombus formation or rhabdomyolysis can occur from the hyperosmolar, hypercoagulable, dehydrated state seen in DKA. We report a child with DKA and transient macroscopic hematuria and hydronephrosis.
Clinical Case: A 13 year old male presented with 2 months of weight loss (9 kg) and 7 days of polyuria, polydipsia, fatigue and sore throat. Past history and family history were negative. There was moderate dehydration and Kussmaul respiration. Hemoglobin 172 g/L (N:125-170), glucose 26.1 mmol/L (N:3.6-11.1), CO2 <5 mmol/L (N:21-31), chloride 99 mmol/L (N:98-111), sodium 135 mmol/L (N:133-145), potassium 3.9 mmol/L (N:3.3-5.1), anion gap 31, serum osmolarity 300 mmol/L, creatinine 69 umol/L (N:40-85), and urea 7.1 mmol/L (N:2.0-7.0). He was diagnosed with new onset DM1 and DKA. Management included fluids and insulin infusion at 0.1 units/kg/hour. He developed gross hematuria 12 hours after treatment was started. There was no dysuria, abdominal pain, fever, urgency, frequency or obstructive symptoms. Blood and urine investigations ruled out cytomegalovirus, adenovirus and bacterial infections. Coagulation studies were normal as were complement levels; tests for group A streptococcus were negative. Kidney ultrasound (US) showed slightly echogenic cortex and bilateral hydronephrosis without ureteral dilatation or evidence of nephrolithiasis. Abdominal radiography was unremarkable. Screening for myoglobinuria was transiently positive, but serum creatine kinase was normal (135 U/L, (N:0-195)), ruling out rhabdomyolysis. The hematuria improved spontaneously by day 3. Kidney function remains normal. US performed 1 month later revealed complete resolution of the hydronephrosis.
Clinical Lessons: Renal complications, including hematuria and hydronephrosis as seen in our case, are rare in children with DKA. Our patient's bilateral hydronephrosis could have been either non-obstructive associated with polyuria which was aggravated by aggressive hydration, or obstructive secondary to renal stones or thrombosis for which there was no clinical evidence. Damage to the renal pelvis or mucosa is possible in acute hydronephrosis and can manifest as hematuria. Overall, clinicians should be aware of and monitor for the possibility of renal involvement in children with DKA.

Nothing to Disclose: TM-S, LH, SG, JH

P2-734

Attempted Filicide with Massive Insulin Overdose in an Eight-Year Old Boy: Clinical Course, Insulin Serum Profile and Cognitive Outcome.

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Introduction: Massive overdose of insulin in children and adolescents is rare. There are hardly any data on clinical course, serum profile of exogenous and endogenous insulin and cognitive outcome after long-term hypoglycaemia due to insulin overdose.
Clinical case: We report the case of an eight-year old boy without diabetes mellitus, in whom the father applied 300 I. U. insulin aspart before attempting to commit suicide. Boy and father were found unconscious about 16 hours later. The child was comatose (GCS 3) and showed tonic-clonic convulsions; leading to intubation and application of i.v. glucose at the scene, with a blood glucose conc. (BG) below the detection limit. Until arrival at the ICU, BG remained low. Only after setting up a central line and administration of glucose 20%, BG normalized. Despite normalization of BG, high doses of phenobarbitone were necessary to achieve seizure control. The patient remained ventilated for 3 days; an intermittent cerebral CT and MRI showed no signs of brain edema. On admission, c-peptide was undetectable (BG undetectable), but serum insulin conc. was 130 mU/l, despite the fact that insulin aspart has a cross-reactivity of less than 10% with our insulin assay. Endogenous insulin secretion, as mirrored by c-peptide serum levels, started to rise after 30 hours; with a more congruent insulin and c-peptide serum pattern appearing about 60 hrs after insulin overdose. Electrolyte levels remained mostly within the normal range during the inpatient period. After extubation, a complete physical and neurological recovery could be observed. Intelligence testing was performed as an outpatient, revealing an IQ above average (HAWIE; IQ 125), which was not significantly different from a result obtained 15 months earlier (performed due to concentration problems).
Conclusion: In pediatric insulin overdose, prolonged periods of hypoglycaemia can be survived without apparent sequelae. In our case, the pharmacokinetic of insulin aspart was severely altered with significantly prolonged duration of hypoglycaemic effect of the insulin analogue.

Nothing to Disclose: JW, FS, BG

P2-735

Bilateral Basal Ganglia Infarctions in a Neonate Born during Maternal Diabetic Ketoacidosis.

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Introduction:
In the ovine fetus, beta-hydroxybutyrate (β-OHB) may cross from the maternal circulation to that of the fetus via the placenta (1). In both the presence and absence of maternal hyperglycemia, increased levels of β-OHB produce decreased fetal oxygen tension, decreased glucose uptake by the fetal brain and increased fetal lactate levels (2-3). In human children with diabetic ketoacidosis (DKA), β-OHB accumulates rapidly in the basal ganglia (4), the same structures most likely to be compromised during episodes of DKA (5). Herein we describe what may be the first reported case of a neonate with bilateral basal ganglia infarctions who was born during an episode of maternal DKA.
Clinical Case:
A female infant was born at 33 weeks gestation to a mother with White's Class C diabetes mellitus. The pregnancy was complicated by sub-optimal glucose control (last HbA1c = 8.1%, n <6.4%) and polyhydramnios, the latter leading to the onset of preterm labor. Upon admission, the mother was diagnosed with DKA (glucose 575 mg/dL, n <200 mg/dL; bicarbonate 12 mM, n = 22—30 mM); anion gap 24 mM, n = 6—20 mM; and moderate ketones; all serum). Monitoring of the fetal heart tones revealed recurrent late decelerations, which persisted despite correction of the mother's metabolic state with rehydration and insulin (glucose 179 mg/dL; bicarbonate 21 mM and anion gap 12 mM; all serum, 2 hours prior to delivery). Given the persistent fetal distress, caesarian section was performed. First measured 2 hours after delivery, the maternal serum β-OHB level was elevated (1.7 mM, n <0.4 mM).
The infant was intubated upon delivery, but she was extubated within 24 hours. No significant physiologic or metabolic abnormalities were noted early on, though the infant did demonstrate rhythmic movements consistent with seizures. On day-of-life nine, MRI revealed bilateral infarction of the anterior basal ganglia; there was no evidence of diffuse white matter injury, only tiny punctate hemorrhages adjacent to the lateral ventricles.
Conclusion:
Given the severity of the intrapartum maternal DKA in this case, we suspect that β-OHB crossed from mother to fetus in an amount sufficient to cause or exacerbate injury to the basal ganglia. The radiographic evolution of these lesions and the infant's neurodevelopmental delay were substantial, leading us to conclude that normalization of maternal β-OHB levels may be a critical aspect of goal-directed therapy in cases of maternal DKA.

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Nothing to Disclose: MBS, CAC, WAC

P2-736

Acute Adrenal Crisis in an Asthmatic Child Treated with Inhaled Fluticasone Proprionate.

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Adrenal suppression is a potential complication of inhaled corticosteroid use, particularly with higher doses and prolonged use. This problem is usually limited to biochemical abnormalities, with no obvious clinical effects. Acute adrenal crisis is rare, but with increasing reports over the past decade. We report a case of a 7 yr old child who presented to the emergency department with a 1 day history of fever, cough, and vomiting, with increasing lethargy. He had a history of asthma, and was maintained on inhaled fluticasone for several years, with a dose of 220 mcg /day for the past year. Oral prednisone was not used for the past year. He had a significant history of hypovolemic shock 10 months prior, following a bout history of acute gastroenteritis, which required both fluid resuscitation and vasopressors. He recovered completely. In the present visit, his vital signs showed a BP 108/60, HR128/min, RR 24/min, temp 101.2 F. It was noted that he appeared mildly Cushingoid, and his height was stunted. Work up showed normal CBC and electrolytes, but with cortisol of <0.03 ug/dl (normal PM cortisol 2.3-11.9 ug/dl). He was given IV normal saline and hydrocortisone, which gave immediate relief. A 250mcg ACTH stimulation test showed a peak cortisol of 1.3ug/dl at 60mins. A 3 day ACTH infusion was done, with cortisol peaking to 13.3ug/dl. The initial ACTH level was 409 pg/ml (normal 6-48pg/ml). Adrenal antibodies were negative. A diagnosis of adrenal suppression secondary to inhaled steroids was deemed most likely. He was weaned off inhaled steroids, and switched to Singulair. The family was taught about stress dosing with hydrocortisone. 1 month after steroid discontinuation, AM cortisol was 4ug/dl (normal 6.2-19.4ug/ml) and ACTH level was 103pg/ml. After 9 months, his AM cortisol was 10.6ug/dl and ACTH was 67pg/ml. His asthma remained well controlled. He had catch-up growth, and his Cushingoid appearance resolved. Absorption of inhaled medications via the lungs into the bloodstream remains an important source of systemic steroids. Fluticasone has increased propensity for adrenal suppression owing to its very high lipophilicity, which causes higher binding in peripheral tissues and longer elimination half-life. This case shows that acute adrenal crisis may be a consequence even at the usual prescribed doses, stressing the importance of using the lowest dose of inhaled steroids needed to control symptoms and having an increased awareness of this complication.

Todd G, et al. Arch Dis Child 2002; 87:457-461.

Nothing to Disclose: AES, SR

P2-737

Bilateral Adrenalectomy for Control of Ketoconazole-Refractory Hypercortisolism Due to Ectopic ACTH Syndrome in a Pediatric Patient.

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Background: Ectopic ACTH syndrome is rare in pediatric patients. Medical therapy of this syndrome with ketoconazole has been reported. We present a case of ketoconazole-refractory hypercortisolism caused by ectopic ACTH secretion from a tumor of unknown primary which responded to bilateral adrenalectomy.
Clinical Case: A 15 yo boy presented with weakness. Electrolyte abnormalities included potassium 1.4 mEq/L (3.3-4.8 mEq/L), sodium 153 mEq/L (135-145 mEq/L), bicarbonate 47 mmol/L (18-27 mmol/L) and glucose 170 mg/dL. Blood pressure was 143/83. Skin pigmentation was markedly increased. The patient did not show features of Cushing syndrome. Lab results were consistent with an ACTH secreting tumor: ACTH 1042 pg/mL, (7-51 pg/mL), cortisol 146.0 mcg/dL, (<25 mcg/dL), 24h urinary free cortisol 10,016.4 mcg/24h (<56 mcg/24h), urine aldosterone <6 mcg/24h (3-17 mcg/24h), and plasma renin activity <0.2 ng/mL/h (0.7-0.3 ng/mL/h). Brain MRI showed a normal pituitary. Abdominal CT showed a 19cm x 18cm x 8cm left hepatic lobe mass with lymph node and pancreas involvement. Tumor pathology showed moderately differentiated NET with positive staining for ACTH. Stains were negative for insulin, glucagon, TTF-1 (lung marker), CDX2 (GI marker), HepPar1, PCEA, S100, and CD10 (hepatocellular markers).
The patient experienced severe symptoms of hypercortisolism including hypokalemia, weakness, fatigue, and mental status changes. Ketoconazole was started and increased to 400 mg tid (20 mg/kg/d) over 2 weeks. Cortisol normalized (6.5-18.7 mcg/dL) and ACTH remained elevated (3298 pg/mL). Symptoms improved. He developed ARDS from influenza A. Ketoconazole was held for 48h and one dose of hydrocortisone was given for hypotension. After resuming ketoconazole, the cortisol continued to increase to 184.0 mcg/dL with worsening symptoms but no adrenal crisis. Metyrapone and aminoglutethimide were unavailable. Bilateral adrenalectomy was performed. 24h after adrenalectomy, cortisol had decreased to 26.6 mcg/dL and ACTH remained elevated (2982-6678 pg/mL). Symptoms of hypercortisolism improved within a few days. Subsequently the patient underwent left hepatic lobectomy after which ACTH decreased to 157 pg/mL, however, disease has continued to progress.
Conclusion: Bilateral adrenalectomy results in definitive control of hypercortisolism in pediatric patients with ectopic ACTH syndrome that does not respond to medical therapy and should be considered early in the clinical course.

Nothing to Disclose: AHS, AEP

P2-738

Use of Intravenous Etomidate in a Child To Control Acute Psychosis Induced by the Hypercortisolemia Secondary to Severe Cushing's Disease.

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Background: Psychosis secondary to paediatric Cushing's disease (CD) is extremely rare and presents a significant management challenge. **Case history:** A 14.7 yr old child with CD presented with acute psychosis and self-inflicted injuries. She was diagnosed with CD aged 14.3 yr following a 2.8 yr history of weight gain, hirsuitism and mood swings. Transsphenoidal pituitary surgery (TSS) was performed at another center and post-operatively she had persistent hypercortisolemia (09.00 h cortisol 1280 nmol/l). Six weeks later she presented acutely to our center with self-inflicted knife wounds to her neck, chest and she had also ingested bleach. After medical and surgical stabilization, a psychiatric assessment diagnosed severe psychosis with cognitive impairment and she was commenced on risperidone. Other neurological and metabolic investigations were negative but an elevated midnight sleeping cortisol of 983 nmol/l indicated lack of cure of CD following TSS. Bilateral simultaneous inferior petrosal sinus sampling (BSIPSS) confirmed central ACTH secretion (IPS:P ACTH ratio after iv CRH, 9.0) and lack of ACTH lateralization suggested a central adenoma position. Transsphenoidal removal of the adenoma was attempted again but was unsuccessful (D7 post-op 09.00 h cortisol 719 nmol/l). Post-operative hypercortisolemia was treated with metyrapone 750 mg 8-hrly resulting in an initial clinical improvement. Subsequently her mental state deteriorated to a catatonic state and oral therapy was no longer tolerated (09.00 h cortisol 986 nmol/l). She was transferred to the intensive care unit and low-dose iv etmoidate infusion was commenced at 3.0 mg/hr with dose titration according to serum cortisol. At 48 hr the dose was increased to 3.5 mg/hr and serum cortisol decreased to 186 nmol/l at 62 hrs. Hydrocortisone replacement (0.5 mg/hr) was added and titrated to maintain serum cortisol levels between 200-300 nmol/l. After 5 days, an improvement in her mental state was noted and the etomidate was tolerated without any side-effects or sedation. The treatment was continued until oral medication could be restarted and pituitary radiotherapy as definitive treatment could be undertaken. **Conclusion:** Psychosis is an extremely rare complication of pediatric CD and may preclude the compliance with oral therapies. In such cases, the use of low-dose etomidate in an intensive care setting is safe and effective in managing such patients.

Nothing to Disclose: LFC, CRH, JAA, LM, FA, PCH, MOS, ABG, HLS

P2-739

Transient Pseudohypoaldosteronism in Association with Nephropathy and/or Uropathy in Infancy.

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Pseudohypoaldosteronism-I (PHA1) is a rare condition resulting in severe hyponatremia and hyperkalemia in infancy. While most cases are due to mutations in the mineralocorticoid receptor gene (autosomal dominant) or Epithelial Sodium channel (ENaC) gene (autosomal recessive), PHA1 may develop in association with medications or tubulointerstitial disease and is typically transient. We present two cases of transient PHA1 associated with uropathy and nephropathy.

Case 1: A 3-week-old former 25-week premature, phenotypically normal female infant, developed hypotension, hypoglycemia, and a gradual decline in renal function preceding her electrolytic derangement. At DOL #25, she developed severe hyponatremia (109 mmol/L; 135-145), hyperkalemia (9.1 mmol/L; 3.7-5.9) with decompensating renal function and sepsis (Cr 2.35 mg/dL; 0.2-0.6). Workup revealed elevated aldosterone level (320 ng/dL, 17-154) and normal adrenal steroid profile, including normal stimulated cortisol (41 mcg/dL, >18).

Case 2: A 5-month-old, phenotypically normal, term female infant, presented with hypotension, vomiting, lethargy and weight loss, associated with severe hyponatremia (105 mmol/L, 135-145) and hyperkalemia (8.6 mmol/L, 0.2-0.6). Her workup revealed a UTI, as well as an ectopic and dilated left ureter with poor left renal function (on renal DMSA scan and slightly high creatinine (0.85mg/dL, 0.3-0.6). Her aldosterone was also elevated (900 ng/dL) and adrenal steroid profile was normal, including normal stimulated cortisol (50 mcg/dL,>18).

Both infants responded, within two days, to fluid and electrolyte resuscitation, systemic antibiotics, fludrocortisone, and stress dosing of hydrocortisone. Their electrolytes remained normal after discontinuation of mineralocorticoid treatment once renal function and uropathy were corrected.

The mechanisms of transient PHA1 with underlying nephropathy and/or uropathy remain unclear. High intrarenal pressure, inflammation, and immaturity of the tubular function may contribute to tubular resistance to aldosterone action.

Transient PHA1 should be included in the differential diagnosis of severe hyponatremia and hyperkalemia in infancy in association with urinary tract pathology, particularly in females with normal genital appearance. High glucocorticoid doses may play a significant role in its treatment. Since urinary tract malformations are present in majority of transient PHA1 cases, imaging studies should also be considered in workup.

Nothing to Disclose: CC, KJL, EBF, ASC

P2-740

Total and Site-Specific Fat and Lean Mass as Predictors of Bone Density in Female Adolescent Athletes.

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Total lean mass (TLM) predicts bone mineral density (BMD) in adolescent athletes, attributable to the pull of muscle on bone being bone anabolic. Fat mass is also of importance, and data suggest that subcutaneous fat (SQF) positively and visceral fat (VF) negatively predicts BMD in obese adolescents. Amenorrheic athletes (AA) have lower total fat mass (TFM) and BMD than eumenorrheic athletes (EA). We hypothesized that (i) TFM (surrogate for SQF) and TLM positively predict BMD, whereas trunk/extremity fat ratio (TEFR) (surrogate for VF) inversely predicts BMD in adolescent endurance athletes, and (ii) lower extremity lean mass (LELM) better predicts BMD at all sites, whereas upper extremity lean mass (UELM) predicts spine and WBBMD.
We measured BMD and body composition using DXA in 18 EA and 21 AA (12-18 y). We report total hip and height adjusted BMD [lumbar bone mineral apparent density (LBMAD) and WB bone mineral content/height (WBBMC/Ht)]. All subjects reported ≥4h of weight-bearing training or >30 miles of running weekly.
AA had lower BMD than EA at all sites ($p \leq 0.02$). TLM did not differ, but UELM was lower in AA ($p=0.02$). TFM, UEFM and LEFM were lower in AA ($p \leq 0.004$). The table indicates BMD measures that were significantly associated with specific body composition measures.

Predictors	All athletes	EA	AA
TFM	LBMAD, WBBMC/Ht	LBMAD	
TLM	All sites	Hip BMD & Z, WBBMC/Ht & Z	LBMAD, Hip BMD
UELM	All sites	WBBMC/Ht & Z	
LELM	All sites	Hip BMD & Z, WBBMC/Ht & Z	
UEFM	LBMAD, WBBMC/Ht & Z		
LEFM	LBMAD, WMBMC/Ht	LBMAD	
TEFR			LBMAD, WBBMC/Ht & Z

$r \geq 0.32$ for all except TEFR ($r \leq -0.45$), $p \leq 0.05$

In a regression model (including TLM, TFM and TEFR), TLM and TFM were positive predictors, and TEFR a negative predictor of LBMAD and WBBMC/Ht ($r^2=0.54$, 0.56) for the whole group. TLM positively predicted hip BMD ($r^2=0.28$). In EA, TFM positively and TEFR negatively predicted LBMAD ($r^2=0.68$); TLM positively predicted hip BMD and WBBMC/Ht ($r^2=0.25$, 0.36). In AA, TLM positively and TEFR negatively predicted LBMAD and WBBMC/Ht ($r^2=0.41$, 0.59).TLM positively predicted hip BMD ($r^2=0.20$).
In AA, TLM is a weaker predictor of BMD than in EA, suggesting that estrogen may be permissive for effects of mechanical loading on bone. Stronger associations of LELM (vs. UELM) with BMD are consistent with most subjects being runners. Loss of associations of TFM with BMD in AA may be secondary to low TFM. Our data suggest that trunk fat is deleterious to bone in amenorrheic athletes.

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Nothing to Disclose: KEA, BD, LJ, MT, MM

P2-741

Biochemical Markers as Predicting Factor for Metabolic Bone Diseases in Very Low Birth Weight Infants.

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Purpose: The aim of present study was toassess the utility of serum biochemical markers for predicting the metabolic bone disease (MBD) in very low birth weight infants (VLBWI).
Method: Medical records of 104 VLBWI from 2003 to 2008 were reviewed in this retrospective study. Study patients were divided in MBD and control group according to the finding of wrist radiography performed at 4 weeks of life. We compared the serum biochemical markers including alkaline phosphate (ALP), calcium (Ca), phosphate (P) between two groups at birth, 1 and 4 weeks of life. The value of serum vitamin D (Vit. D) was measured at 4-5 weeks of life.

Results: The mean gestational age and birth weight of study patients were 30^{+6}±2.0 weeks and 1,308.2±136.7g. The incidence of MBD was 28.9% (31/104). At birth, higher values of serum ALP (438.1±129.1 mg/dL vs 360.5±122.8 mg/dL) were found in MBD group. At 1 week of life, higher values of serum Ca (11.0±1.7 mg/dL vs 10.3±1.7 mg/dL) and lower values of serum P (3.2±1.2 mg/dL vs 4.1±1.3 mg/dL) were found in MBD group. At 4 weeks of life, higher values of serum ALP activities (1,397.6±635.6 U/L vs 789.0±573.0 U/L), lower values of serum P (4.2±2.0 mg/dL vs 5.4±1.8 mg/dL) and Vit. D (17.7±7.2 ng/mL vs 30.0±15.5 ng/mL) were found in MBD group. Risk factors of MBD were male and Vit. D deficiency with high ALP at 4 weeks of life.
Conclusion: These results suggest that high ALP concentrations at 4 weeks of life may predict MBD with Vit. D deficiency in VLBWI.

Nothing to Disclose: JHY, MJK, MJK

P2-742

Two Novel Mutations in the TSH-β Subunit Gene Underlying Congenital Central Hypothyroidism.

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Isolated TSH deficiency caused by TSH-β subunit defects is a rare cause of non-goitrous congenital hypothyroidism. Patients are born with low thyroid hormones and TSH serum levels. We are reporting the molecular consequences of a novel splice-junction mutation and a novel missense mutation in the TSH-β subunit gene, found in two non-related patients with congenital central hypothyroidism and severe conventional-treatment-resistant anemia, not detected by routine TSH-based neonatal screening. Patient 1 was found to be homozygous for a G to A nucleotide change at the 5' donor splice site of exon/intron 2 at cDNA position 162 bp, the last base of exon 2 (c162G>A; the A of the ATG of the initiator Met codon is denoted nucleotide +1). This resulted in a silent change R34R in the mature protein. In vitro splicing assays showed that the mutant minigene dramatically affected pre-mRNA processing, causing exon 2 to be completely skipped and predicting a new out-of-frame translational start point in exon 3, and the production of a nonsense 25 amino acid peptide. Sequence analysis of TSHβ-subunit gene of patient 2 revealed a compound heterozygosis for the already reported 313delT (C105Vfs114X) mutation and for a second novel mutation in exon 3, substituting G for A at cDNA nucleotide position 323, resulting in a change of cysteine to tyrosine at codon 88 (C88Y). This codon change results in the loss of one of the 12 cysteine residues conserved among all dimeric pituitary and placental glycoprotein hormone β subunits. Data from in silico analysis confirmed that the C88Y mutation would be predicted to affect the subunit conformation, and either enhance TSH-β subunit intracellular degradation or diminish its ability to interact with the α-subunit. Indeed, two different bioinformatics approaches, PolyPhen and SIFT analysis predicted C88Y to be a damaging substitution. In conclusion, we are reporting two novel homozygous and compound heterozygous mutations of the TSH-β subunit gene as a cause of isolated congenital central hypothyroidism. Isolated TSH deficiency is not detected by routine TSH-based neonatal screening. The exact molecular diagnosis is mandatory for the delineation of prognosis, for genetic counseling, and for the avoidance of provocation tests of pituitary function in the newborn period or early in life. Finally the diagnosis of central hypothyroidism should be considered in any case with severe anemia of uncertain etiology

Nothing to Disclose: MSB, MC, ND, VH, YL, DMW, MAR, AB

P2-743

State Run Newborn Hearing Screening Program Detects Sensorineural Hearing Loss Early in Infants with Congenital Hypothyroidism.

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INTRODUCTION: Infants with congenital hypothyroidism (CH) are at increased risk for sensorineural hearing loss, but hearing evaluations are frequently not recommended until developmental milestones are already delayed. Newborn screening for CH has been ongoing for over 25 years in the State of Florida. In 2000, Florida mandated newborn hearing screening (via otoacoustic emission or auditory brainstem response). The purpose of this study was to determine if there is a greater incidence of abnormal newborn hearing screens in children with CH compared to the general population.
METHODS: Data from the Florida Newborn Screening program from January 2006 through December 2008 were gathered for hearing screen (HS) and thyroid laboratory results. Hearing screen results in infants with confirmed CH were compared to aggregate values obtained from the entire population of hearing screened infants.
RESULTS: From January 2006 to December 2008, 643,424 infants had known HS results entered into the State of Florida database. During this same time interval, 191 infants were diagnosed with CH. A significantly larger proportion of infants had an abnormal HS within the CH group, (n=22, 12.7%) compared to the general population (n=8362, 1.3%, $p<0.001$). Of the 22 hypothyroid infants with abnormal HS, 13 passed a secondary HS; 7 were lost to follow-up; and 2 infants (1%) were confirmed with hearing loss. Within the general population, 253 (0.03%) infants were confirmed to have hearing loss. Within the CH group of infants, 45% of infants with abnormal HS were premature, whereas only 17% of hypothyroid infants with normal HS results were $\leq$36 week gestational age ($p=0.006$). There was no statistical difference between initial screening TSH values in CH-normal HS infants [(mean +/- SD= 319.9 +/- 197.1) median =336, range= 6-791 µIU/mL] versus CH-abnormal HS infants [(mean +/- SD= 356.3 +/- 255.1), median =329, range= 7-2083 µIU/mL] ($p=0.0735$). There was a strong trend towards lower total T4 levels in the hypothyroid infants with abnormal HS [(mean +/- SD= 4.4 +/- 3.8) median=3.7, range= 0.8-17.8 µg/dL] as compared with those with normal HS [(mean +/- SD= 5.1 +/- 2.7) median=4.7, range= 1.1-13 µg/dL] ($p=0.053$).
CONCLUSION: Newborn hearing screening does appear to identify a larger proportion of infants with hearing loss in those infants with confirmed CH. Early identification of these infants should assist in early intervention and improved developmental outcomes.

Nothing to Disclose: AML, DIS

P2-744

Increased Adrenal Androgen Concentrations Associated with Advanced Bone Age in Prepubertal Children.

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Background: Traditionally, benign adrenarche is not associated with significant bone age advancement[i],[ii],[iii]. However, some children present with significant advanced bone age (ABA) without true central precocity. Since estradiol (E2) is essential in growth plate closure[iv],[v],[vi], adrenal androgens may provide substrate for paracrine aromatization to E2 in bone[vii],[viii]. We aimed to examine the relationship between adrenal androgen secretion and ABA in children. **Methods**: Retrospective review of patient medical records was done to identify children with an ABA without central precocious puberty and collected growth data and adrenal androgen levels. Controls (C) with normal BA were obtained for comparison. Unpaired t-tests for univariate analysis and multivariate linear regression for were used statistical analysis. **Results**: 44 patients with ABA (30 with adrenarche, 1 with thelarche, 13 prepubertal) , and 78 in C group were included. The ABA group had an older mean age (p=.02), were more likely male (p<.001), and had a higher BMI SDS (p<.0001) than C but otherwise similar. Unpaired 2 group t test analysis showed that group ABA had a higher DHEA (p<.0001), androstenedione (p<.001), 17-hydroxypregnenolone (17OHPreg) (p<.001), 17-hydroxyprogesterone (17OHProg) (p<.05), cortisol (p<.001), 17OHPreg:cortisol ratio (p<.01), and 17OHPreg:17OHProg ratio (p=.0547) compared to group C. In multivariate regression controlling for BMI, age, and sex, the ABA group had a higher DHEA (p<.0001, β=.0046), Androstenedione (p<.001, β=.019), 17OHPreg:Cort ratio (p<.001, β=.049), and 17OHPreg:17OHProg ratio (p<.01, β=.095). This relationship persists when subdividing by age or gender, except patients > 7 years or males with respect to androstenedione and 17OHPreg:17OHProg ratio. Within the ABA group, no difference in growth parameters or adrenal androgen concentrations were found between prepubertal patients and those with benign adrenarche or thelarche, except for a higher BA SDS in prepubertal subjects. (p<.01) **Conclusions**: Increased adrenal androgen secretion is associated with ABA in prepubertal children regardless of age, gender, BMI, or benign adrenarche. Increased 17,20-lyase and decreased adrenal 3βHSD II activities are key factors in adrenarche[ix], which can provide substrate for aromatization to E2 in bone and explain ABA in this cohort. Further studies of bone steroidogenesis in prepubertal children with ABA are needed to further support this hypothesis.

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Nothing to Disclose: JBG, YL, ZY

P2-745

Congenital Lipoid Adrenal Hyperplasia Due to a Heterozygous *De Novo* Mutation in *StAR* (Steroidogenic Acute Regulatory Protein) Gene Presenting with Ambiguous Genitalia in a 46,XY Patient.

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Background: Recessive loss-of-function mutations of StAR gene cause congenital lipoid adrenal hyperplasia (CLAH). Most patients reported presented primary adrenal insufficiency (PAI) and female phenotype regardless karyotype. Non-classic forms of CLAH have being described with late-onset adrenal insufficiency and male genitalia in 46, XY subjects. Recently an autosomal dominant form of CLAH with PAI and male genitalia was reported in a 46, XY subject with late-onset primary testicular failure carrying a heterozygous IVS1 -2G>A mutation in the acceptor splicing site causing the loss of exon 2 *(1).* **Results**: we present a patient with PAI and ambiguous genitalia. At 11 days of life the baby presented hyperpigmented skin and jaundice, vomiting, dehydration and hypotonia. Genital examination revealed a 0.5 x 0.5 cm long phallus, penoscrotal hypospadias, poorly developed corporal tissue, complete labial fusion and bilateral inguinal gonads. Laboratory tests were consistent with the diagnosis of impaired adrenal and testicular steroidogenesis. Female sex was assigned and the patient was started on hydrocortisone and fludrocortisone. LHRH test at 3 month of age showed gonadotropin response with undetectable levels of testosterone. Genetic analysis revealed the presence of the heterozygous de novo IVS1 -2G>A mutation in *StAR* protein gene and a previously described pG146A heterozygous polymorphism of the *SF1* gene in patients with hypospadias. The patient was gonadectomized at 3 years of age. Histopathological analysis showed bilateral immature testes. Basal and post-hCG testosterone production of testicular cells in culture was normal. RT-PCR from testicular tissue revealed a 397 bp band (-E2 mRNA) and a weak 511 bp wild type band while both were observed with equal intensity in cells in culture.**Conclusion:** We described for the first time a 46,XY patient with PAI and genital ambiguity carrying a heterozygous mutation in *StAR* gene suggesting that heterozygosis could affect testicular steroidogenesis in utero. In vitro testosterone production could be explained by culture conditions that allow normal splicing expression. We postulate that local testicular factors differentially regulate the expression of -E2 mRNA vs wild type determining the phenotypic differences between both patients described with this mutation. The presence of a potentially hypomorphic polymorphism in *SF1* gene may have contributed to the more severe clinical phenotype in our patient.

1)Katsumata OR 11-3 ENDO09

Nothing to Disclose: GG, MC, MSB, EB, MB, EV, NS, RM, MAR, AB

P2-746

Duplication of Functional CYP21A2 Gene in a Cluster I236N-V237E-M239K Allele: Implications for 21-Hydroxylase Deficiency Prenatal Diagnosis.

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CYP21A2 molecular analysis in prenatal DNA samples from chorionic villi is used to diagnose 21-hydroxylase deficiency (21OHD) affected fetuses and determines whether prenatal treatment should be discontinued in unnecessary cases. Haplotypes with Q318X mutation and duplicated CYP21A2 genes have been reported to occur in different populations to a varying extent. Although it was first described as a rare haplotype, recent studies have shown a relatively high frequency in clinically unaffected subjects. Discrimination between a normally functional (Q318X mutation in one of the duplicated CYP21A2 genes) and a 21-OHD (Q318X mutation without duplicated functional gene) allele is of importance, particularly for prenatal diagnosis and the respective genetic counseling. Allele specific-PCR and sequencing methods fail to detect gene copy number, being Southern blot and MLPA analysis the most accurate methods for that purpose. We report CYP21A2 molecular analysis of a salt wasting affected CAH patient, his parents, and their at-risk prenatal sample. The index case was found to be a compound heterozygote for two null mutations (del8bp/Q318X). His mother was a heterozygous carrier for del8bp mutation. His father was a compound heterozygote for Q318X and a novel haplotype with I236N-V237E-M239K (cluster E6) null mutation and a duplicated CYP21A2 gene. In a second pregnancy, dexamethasone treatment was started at 6th weeks of gestation. Molecular analysis in chorionic villous DNA sample revealed a 46,XX karyotype and compound heterozygosis for del8bp and the cluster E6/duplicated haplotype inherited from the father. Results were interpreted as an unaffected female, and prenatal dexamethasone treatment was discontinued. Delivered at term the female newborn showed normal external genitalia and normal 17OHP neonatal screening (11 nm/l; RV <40 nm/l). In conclusion, we have characterized a novel unaffected haplotype with cluster Ex6 mutation and a duplicated CYP21A2 gene. The frequency of this haplotype as well as the presence of other mutations in a duplicated CYP21A2 allele in the general population is unknown. This finding reinforces that whenever a severe CYP21A2 defect is found in carrier analysis, copy number assessment is mandatory to avoid misinterpretations of apparently severe mutations in alleles carrying duplicated genes, and therefore allowing for an appropriate genetic counseling and prenatal diagnosis.

Nothing to Disclose: RM, PR, JG, NPG, DMW, HA, LO, MAR, AB

P2-747

Pubertal Presentation in Congenital Adrenal Hyperplasia Due to P450 Oxidoreductase Deficiency.

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P450 oxidoreductase (POR) transfers electrons to all microsomal cytochrome P450 enzymes including key enzymes of glucocorticoid and androgen synthesis. Mutant POR results in the CAH variant POR deficiency (ORD) that manifests with glucocorticoid deficiency, skeletal malformations and disordered sex development (DSD) in both sexes. Neonatal presentation with undervirilisation in boys and virilisation in girls is well described. However, there is a paucity of data on the pubertal phenotype in ORD. Here we assessed the clinical and biochemical phenotype in ORD patients of pubertal age (n=6; 4f, 2m). Diagnosis was confirmed by urinary steroid analysis (GC/MS) and direct sequencing. Case 1 (46,XX; p.A287P hom) presented at 11.5 years with large bilateral ovarian cysts that required surgical intervention and only resolved after combined treatment with estrogen/progestin (HRT), GnRH superagonists and dexamethasone. Case 2 (46,XX; p.A287P hom) presented with absent puberty at the age of 17. At 24 years, after brief discontinuation of HRT, large bilateral ovarian cysts developed, resolving upon reintroduction of HRT and additional treatment with dexamethasone. Case 3 (46,XX; p.T142A/p.Y376LfsX74) presented with oligomenorrhoea and partially delayed puberty at 19 years; ultrasound revealed large ovarian cysts that remained stable on HRT. Case 4 (46,XX; p.A287P/p.R457H) presented at 14 years with primary amenorrhoea and large ovarian cysts resulting in left oophorectomy. HRT was initiated, but two years later rupture of a right ovarian cyst occurred. Case 5 (46,XY; p.R457H/p.Y576X) presented at birth with 46,XY DSD (cryptorchidism, micropenis) and at 12 years had evidence of hypogonadism requiring induction of puberty with testosterone. Treatment was ceased at 16 years after which he maintained normal testosterone levels; testicular volume was 20-25 ml. Case 6 (46,XY; pA287P/c.830+2dupT) had no apparent signs of pubertal development at 12 years except for a left testicular volume of 12 ml. In summary, delayed or absent puberty may be the first presenting sign in ORD, with the common finding of hypergonadotropic hypogonadism (LH/FSH>1). Affected females frequently present with large ovarian cysts, often not responding sufficiently to estrogen/progestin treatment. Of note, during the pubertal development of affected male individuals endogenous gonadal androgen synthesis may resume despite previously subnormal androgens consequent to mutant POR.

Nothing to Disclose: JI, SO, EMM, FC, MK, NR, MS-C, DM, MS, MD, CHLS, NK, WA

P2-748

Treatment of Young Rats with the Aromatase Inhibitor Exemestane Reduces Testicular Weight and Sertoli Cell Numbers.

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Aromatase inhibitors are currently being investigated with growth hormone for their ability to improve final height in children. Exemestane is an irreversible steroidal aromatase inhibitor used to treat breast cancer. To exclude the possibility of adverse effects on male sexual development, the drug was investigated for its potential to affect mature reproductive systems when administered to immature rats. Male and female rat pups were treated with exemestane (0, 30, 100, 300 mg/kg) once daily from PND 7-50 (male) or 7-41 (female). Plasma drug concentrations were measured on the first and last day of dosing. Maturation of the reproductive system was evaluated by monitoring the onset of vaginal patency or preputial separation. After maturation, treated rats were mated with untreated rats to evaluate the potential impact on reproductive function. After mating (male) or on GD14 (female) rats were euthanized and reproductive organs evaluated microscopically. Exemestane was well-tolerated at all dose levels and there were no effects on age at onset of vaginal patency or preputial separation. There was no drug-related effect on female reproductive function. The NOAEL for general toxicity in male and female juvenile rats and for female developmental and reproductive effects was 300 mg/kg/day which corresponds to a clinical margin of exposure of approximately 35-fold. Treatment of juvenile male rats increased the time prior to mating and caused a 10-15% reduction in mating rates, though pregnancy rates were unaffected. Testis and epididymal weights were reduced 10-15% and Sertoli cell numbers were reduced 20-30% in all treated groups but no histologic lesions and no change in epididymal sperm measures were seen. Thus, there was no NOAEL identified for male developmental and reproductive effects (margin of exposure <2-fold). These data show that an aromatase inhibitor can reduce Sertoli cell proliferation during maturation. Treatment beginning at a later time or for a longer duration is not expected to have any additional adverse effect (e.g., the effect is to inhibit Sertoli proliferation, not cause cell death). In humans, similar effects would be expected to occur in boys treated earlier than approximately age 12, though actual age is dependent on the completion of Sertoli cell proliferation in each individual. The younger the individual at treatment onset, the greater the potential adverse effect.

Disclosures: GDC: Employee, Pfizer Global R&D. REC: Employee, Pfizer Global R&D. LAB-N: Employee, Pfizer Global R&D.

P2-749

Molecular Detection of Hidden X or Y Fragment in Patients with Turner Syndrome.

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We evaluated the presence of hidden X or Y fragments in TS patients using molecular analysis and investigated the relationship between the presence of Y sequences and the development of virilization or gonadoblastoma.
A total of 58 patients were included; 45, X (n= 40), 45, X/46, X, +mar (n=18). The PCR using 11 primers (including SRY, TSPY, DYZ3, DYZ1) was performed to detect Y fragments. The confirmation of X fragments was analyzed by comparing between patients and their parents using 9 or 12 highly polymorphic X chromosome microsatellites.
Two (5%) of 45, X patients were found to be positive for Y sequences and their rekaryotype revealed 45,X/46,X,+mar.None had hidden X fragments. Origin of marker in 20 TS patients including two aforementioned was as follows; Y (n=9, 45%), X (n=2, 10%), unknown (n=9). Gonadectomy was undertaken in 6 of 9 patients with Y sequences and 4 of 6 gonadectomized patients had developed virilization (2 at birth, 2 in the second decade).

Characteristics of 9 Turner Syndrome patients with Y sequences

Age at diagnosis (yrs)	Karyotype	Virilization	SRY FISH	PCR (SRY, TSPY, DYZ3, DYZ1)	Age at gonadectomy (yrs)	Gonad (Rt/Lt)	Age at last FU (yrs)
1.4	45,X/46,X,+m	ambiguous genitalia	NE	+, +, +, -	1.3	testis/streak gonad	13.9
6.6	45,X/46,X,+m	clitomegaly	NE	+, +, +, -	5.4	testis/dysplastic gonad	30.6
14.0	45,X/46,X,+m	clitomegaly	+	+, +, +, -	14.2	GBL/atropic testis	20.1
9.6	45,X/46,X,+m	clitomegaly, hirsuitism	+	+, +, +, -	16.1	streak ovary/GBL	18.0
0.1	45,X/46,X,+m	no	+	+, +, -, -	19.3	ovary/fibrovascular tissue	28.5
12.8	45,X/46,X,+m	no	+	+, +, +, -	19.8	streak ovary/streak ovary	29.3
6.2	45,X/46,X,+m	no	NE	+, +, +, +	not yet	unknown	15.7
8.0	45,X => 45,X/46,X,+m (after rekarotype)	no	+	+, +, +, -	not yet	unknown	17.2
10.4	45,X => 45,X/46,X,+m (after rekarotype)	no	not yet	+, +, -, -	not yet	unknown	22.9

GBL, gonadoblastoma; NE, not evaluated

Two patients who developed clitomegaly in the second decade were diagnosed with gonadoblastoma. Three unvirilized TS patients with Y sequences are being carefully monitored.
Despite normal genitalia at birth, virilization can progress later in TS patients with Y sequences. It can be ominous sign of gonadoblastoma. To elucidate the usefulness of the molecular detection of X or Y fragments in TS patients, precise karyotyping should be preceded and careful monitoring of unvirilized patients who had not undergone gonadectomy is mandatory.

Nothing to Disclose: YAL, JHK, SHL, MJK, HHL, CHS, SWY, HRC, SYL

P2-750

Immunoexpression of OCT 3/4 in Dysgenetic Gonads of Several Etiologies and in Complete Androgen Insensitivity Syndrome (CAIS) Testes during Pre-Puberty or Early Puberty.

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OCT-3/4, a specific embryonic stem cell transcription factor, is essential in regulating pluripotency and differentiation. A specific role of OCT3/4 for survival of migratory primordial germ cells (GC) has been demonstrated, preventing them from premature apoptosis (1). In gonadal dysgenesis, it was detected in CIS cells and gonadoblastoma (2,3). We have previously shown that immunoexpression of OCT3/4 was absent in GC from prepubertal (PP) normal testes (4).
The aim of this study was to describe the immunoexpression of OCT 3/4 in human dysgenetic gonads of several etiologies and in CAIS testes during pre-puberty or early puberty.
Clinical material and methods:Ten DSD patients divided in 5 groups (Gr) were studied:
Gr1 (n=3): 46,XY testicular dysgenesis with *SF-1* (*NR5A1*) gene mutation, 1a: pubertal (PUB) heterozygous Y183X mutant patient, [chronological age, CA, 148 months (mo)], 1b: PP compound heterozygous W279X / g3314-3317delTCTC (IVS 4 + 8) mutant patient (CA,18 mo). 1c: PP heterozygous pG146A mutant associated with a heterozygous de novo IVS1-2G>A mutation in the *StAR* gene (CA, 33 mo); Gr2 (n=1): PP 46,XY testicular dysgenesis without gene mutation detection (CA,10 mo); Gr3 (n=2): PP 46,XX ovotesticular dysgenesis, 3a: (CA, 21 mo), 3b: (CA: 34 mo); Gr4 (n=2); mixed gonadal dysgenesis, 4a: PP 46,XY/45,X (CA, 4 mo), 4b: PUB 46,XY (CA: 144 mo); Gr5 (n=2), CAIS, 5a: PP (CA, 53 mo), 5b: PP (CA: 15 mo). Tissues from PP (0 to 5 years old, n=28) and PUB (n=5) control testes (GrC, n=33) were also analyzed. The number of seminiferous tubules with OCT3/4 positive GC was counted and referred as percentage of all seminiferous tubules wich presented GC. Expression of MAGE-A4 (marker of mature spermatogonia), Androgen receptor (AR), Aromatase (ARO), ERα and ERβ were also study.
Results: Similar to GrC, in DSD patients from Gr1, no OCT3/4 immunoexpression was found, while in all DSD patients from Grs 2,3,4 and 5 a strong OCT3/4 signal (range 18-67%) was observed in central and peripherical GCs. MAGE-A4 was present in all Grs, except Gr3, as well as in GrC. In addition, no correlation between OCT3/4 and AR, ARO, ERα and ERβ expression was found.
Conclusions: We propose that the presence of genomic SF1 gene mutations might protect dysgenetic gonad GC to develop CIS. However, the molecular mechanism remains to be elucidated. Finally, the expression of OCT3/4 in PP CAIS GC suggests that to perform late gonadectomy during pubertal age might be risky.

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