

OR18-1

Erythropoietin Provides Diabetes Protection through Direct Effects on Pancreatic β Cells.

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Diabetes mellitus is a chronic disorder of insulin insufficiency, resulting in poor glycemic control and vascular complications. The feature common to all forms of diabetes is the insufficient functional pancreatic β -cell mass that is required to maintain euglycemia. Emerging evidence has suggested that erythropoietin (EPO) may exert cytoprotective effects on non-erythroid cells. Interestingly, the EPO receptor (EPO-R) has been found on the pancreatic β cells; however, the biological effects of EPO on the β cells are not well understood.

We first assessed the effects of recombinant human EPO (rHuEPO) administration models of type 1 and type 2 diabetes, using Multiple Low Doses of Streptozotocin (STZ) (MLDS) and db/db mice, respectively. Mice were given i.p. injections of recombinant human EPO (rHuEPO) (50 μ g/kg) or saline three times per week for 4 weeks.

In both diabetes models, we observed that the rHuEPO-treated mice had reduced blood glucose levels compared to controls. The improved glycemic control in the rHuEPO-treated groups was not due enhanced peripheral insulin sensitivity, but rather enhanced β -cell mass, which was attributed to increased islet proliferation and decreased apoptosis. rHuEPO treatment also resulted in enhanced islet angiogenesis. Western blots of isolated islets from rHuEPO-treated C57BL/6 mice demonstrated activation of the JAK2/STAT5 pathway. Bcl-xL, c-Myc, c-kit, and vegf expression levels were upregulated in the rHuEPO-treated mice.

To test for the direct biological effects of EPO on the β cells, we generated β cell-specific EPO-R knockout mice. rHuEPO treatment failed to provide diabetes protection in these mutant mice following STZ, which supports the direct role of EPO in pancreatic β cells. To assess for essential downstream signaling, β cell-specific JAK2 knockout mice were also tested. These mice also failed to be protected from STZ-induced diabetes development following rHuEPO treatment. Furthermore, enhancement of β -cell mass and angiogenesis were also abolished in rHuEPO-treated knockout mice. These results show that rHuEPO directly inhibits apoptosis, and enhances proliferation and angiogenesis by activating EPO-R and JAK2 specifically in the β cells.

Our studies demonstrate that rHuEPO can exert beneficial effects directly on the pancreatic β cells. These results may lead to further elucidation of mechanisms of EPO biology relevant to β cells, which may result in novel therapeutic strategies for diabetes.

Nothing to Disclose: DC, SAS, LW, XW, MW

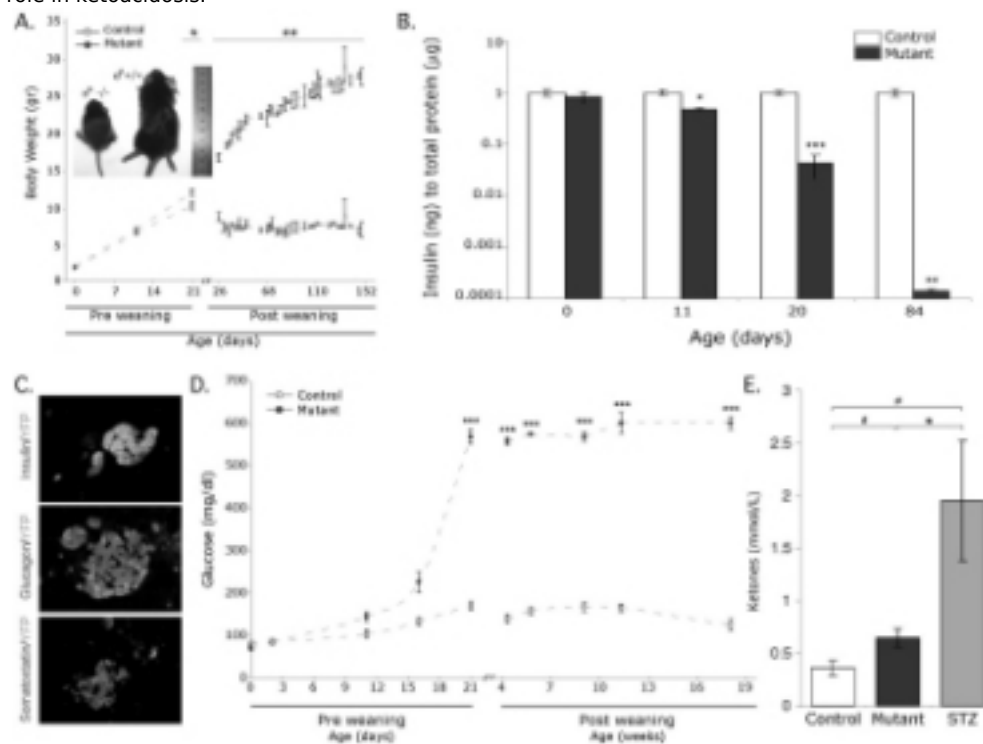
OR18-2

Dicer Is Essential for Islet Cells Survival.

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microRNAs (miRNAs) are a subset of endogenous small RNA molecules that repress gene expression post-transcriptionally. Since only little is known about their role in glucose metabolism and diabetes, we have generated a transgenic mouse model in which the gene encoding for Dicer, a key enzyme in the biogenesis of miRNA, is ablated from the developing endocrine pancreas [figure1C]. This resulted in striking metabolic abnormalities. Mutant mice exhibit severe growth retardation and by adulthood weigh up to 4-fold less than their control littermates [figure1A]. At birth, whole-pancreas insulin levels of mutant mice, did not differ from controls. However by weaning, mutant mice developed extreme hyperglycemia (>600mg/dl) [figure1D] and at the age of twelve weeks their insulin levels were reduced by four orders of magnitude [figure1B] and could not be detected by immunohistochemical examination. These results demonstrate that ablating Dicer in developmental stages results in progressive islet cell death, starting at the neonatal period. Challenging previously published reports, our findings suggest that Dicer is essential for the survival of endocrine cells in the pancreas. Surprisingly, the severe hyper-glycemic hypo-insulinemia is not accompanied by ketoacidosis [figure1E]. We suggest that mutant mice may be spared from the lethal diabetic ketoacidosis, partially because of the protective concomitant loss of glucagon expressing alpha cells, which have been shown to play a critical role in ketoacidosis.



Nothing to Disclose: IN, SH, YK, TM-Z, RO, EH, AC

OR18-3

Differential Distribution of FFA Receptors, GPR40 and GPR120, in Mouse Islet beta and alpha-Cells May Contribute to Islet Dysfunction in Obesity-Related Type 2 Diabetes.

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Pancreatic islets are the functional units controlling metabolism of nutrients, in particular circulating glucose levels. Insulin secreting beta cells and glucagon secreting alpha cells possess opposite effects on glycaemia and dysfunction of islet cells can lead to type 2 diabetes (T2D). Interestingly, hyperglucagonaemia has been observed in patients with T2D. In addition, contrary to the reduction of beta cell mass, alpha cell mass in islets of T2D patients seems to increase (1). Obesity is tightly linked to type 2 diabetes and excessive free fatty acids (FFAs) were reported to facilitate beta cell dysfunction (2). However, the effect of FFAs on alpha cells has not been studied. In order to clarify the reason for differential responses to FFAs between alpha and beta cells, receptors for long-chain FFAs were examined in both cell types. In islet, immunostaining demonstrated a peripheral distribution of alpha cells (glucagon positive) and central distribution of beta cells (insulin positive). GPR120 immunostaining showed a similar distribution to alpha cell compartments. In isolated islet cells, GPR120 was co-localized with glucagon positive cells whereas GPR40 was only on insulin positive cells. Similar trends were observed when alpha cell line (alphaTC6) and beta cell line (Min6) were stained with GPR120 and GPR40 antibodies. PCR also confirmed differential expression pattern of GPR120 and GPR40 selectively expressed in alphaTC6 and Min6 beta cell lines, respectively. Linolenic acid (C18:3) increased glucagon secretion dose-dependently in alphaTC6 cells. Long-term treatment (24-48 hours) of alphaTC6 cells by Linolenic acid induced an increase in cell proliferation and a decrease in cholesterol-induced apoptosis. Such changes are likely to be mediated by GPR120, as we observed similar increase in the cell proliferation when cells were treated with GW9508 (non-FFA agonist of GPR120). In addition, long term linolenic acid treatment also increased mRNA expression of Bcl2 in alphaTC6 cells. In conclusion, we demonstrated specific distribution of GPR120 in glucagon alpha cells in islet and long-chain FFA elevated glucagon levels through GPR120. Bearing in mind a significant reduction of beta cell function by FFA treatment, these data indicate that FFAs may regulate the islet function in a cell specific manner to influence proportional levels of insulin and glucagon through two G-protein coupled receptors, GPR40 and GPR120.

(1) K. H. Yoon et al., J Clin Endocrinol Metab, 88, 2300; 2003.

(2) Y. F. Zhao et al., J Endocrinol, 198:533; 2008.

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Nothing to Disclose: SHL, JS, YT, YM, CC

OR18-4

Mutant Preproinsulin Induced Diabetes.

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Three mutations in the Signal Peptide (SP) domain of preproinsulin (PPI): R(SP6)C, A(SP23)A, and A(SP24)D, have been recently reported to be associated with diabetes in heterozygous patients. Insulin haploinsufficiency cannot itself account for diabetes, raising questions about the molecular mechanism(s) of PPI-SP mutants in diabetes pathogenesis. We have examined the earliest events of PPI biosynthesis, including co-translational translocation, SP cleavage, and downstream proinsulin folding. Upon metabolic labeling of cells expressing recombinant PPI-SP mutants, we found that the three mutant proteins presented distinct properties. The PPI-R(SP6)C mutant loses a critical positively charged residue within the proximal portion of the SP. Here, we present evidence of incomplete delivery of the nascent PPI-R(SP6)C polypeptide across the endoplasmic reticulum (ER) membrane which was fully rescued by introducing a new positively charged residue at an adjacent position in the SP. By contrast, all of the newly synthesized PPI-A(SP24)D polypeptides were translocated across the ER membrane. However, more than 90% of these molecules exhibited failure of SP cleavage. Uncleaved PPI-A(SP24)D was retained in the ER, indicating that failure of SP cleavage interferes with further progression of the polypeptide in insulin biosynthesis. More importantly, both in β -cells and in heterologous cells, we found that PPI-A(SP24)D presented the dominant-negative behavior by specifically blocking the trafficking of co-expressed wild-type PPI. This blockade occurred even prior to cell death, suggesting that it may account for the early event of β -cell failure caused by mutant PPIs. For PPI-A(SP23)S, surprisingly, this variant (reported in a patient originally diagnosed as type 1 diabetes) showed normal ER translocation, SP cleavage, proinsulin folding and trafficking, and insulin production. We posit that PPI-A(SP23)S is not diabetogenic but rather a normal variant. In summary, distinct biological behaviors of recently described PPI mutations contribute to a broad range of clinical diabetes phenotypes. In principle, we believe that it may be feasible to design pharmacological tools to rescue the trafficking of wild-type proinsulin with normal insulin production even in the presence of PPI-SP mutants.

Nothing to Disclose: ML, LH, RL-I, PA

OR18-5

PTEN Deletion in Pancreatic Beta Cells Protects Against Type 2 Diabetes without Promoting Tumorigenesis.

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Type 2 diabetes is characterized by two main defects: pancreatic β cell dysfunction and peripheral insulin resistance. Recent genetic ablation studies have demonstrated that attenuation of phosphoinositide 3-kinase (PI3K) signaling in β cells directly leads to its dysfunction leading to type 2 diabetes. Previously, we reported that enhanced PI3K signaling through rat insulin promoter (RIP) driven deletion of PTEN (RIP cre^+ *Pten*^{fl/fl}), a potent negative regulator of this pathway, leads to increased β cell mass and function under basal condition, in addition to enhanced peripheral insulin sensitivity associated with partial PTEN deletion in insulin promoter-expressing hypothalamic neurons (1). Here, to evaluate the importance of RIP-mediated PI3K signaling in the protection against type 2 diabetes, we employed high fat diet (HFD) model and *Lep^{db/db}* mice (global leptin signaling deficiency). In both type 2 diabetes models, we observed upregulation of islet PTEN expression compared to basal states, suggestive of a causal role of PTEN in β cell dysfunction. On HFD, RIP cre^+ *Pten*^{fl/fl} mice were completely protected from glucose intolerance and hyperglycemia with enhanced glucose-stimulated insulin secretion (GSIS) and persistent increase in β cell mass. However, these mice continued to exhibit enhanced peripheral insulin sensitivity mediated by hypothalamic PTEN deficiency, which confounded the role of β cell PTEN *per se* in type 2 diabetes. To this end, we examined isolated islets from RIP cre^+ *Pten*^{fl/fl} mice, which continued to demonstrate increased GSIS in *ex vivo* conditions incubated with H₂O₂ to mimic glucotoxicity. This protective effect was further supported by *Lep^{db/db}* mice model, where RIP cre^+ *Pten*^{fl/fl} *Lep^{db/db}* mice developed severe peripheral insulin resistance to a similar degree as RIP cre^+ *Pten*^{+/+} *Lep^{db/db}* control littermates. Despite the severe insulin resistance exhibited by these RIP cre^+ *Pten*^{fl/fl} *Lep^{db/db}* mice, they were still protected against hyperglycemia with enhanced GSIS. Finally, PTEN deletion in β cells did not lead to tumorigenesis even after prolonged exposure to metabolic stressors in both models of type 2 diabetes. These findings reveal the importance of PTEN deletion in β cells in the protection against β cell defects that is observed in type 2 diabetes, thereby provide a potential therapeutic target for this devastating disease.

(1) Nguyen KT et al., Mol Cell Biol 2006; 26(12): 4511-8

Nothing to Disclose: LW, K-TN, SAS, TM, MW

OR18-6

The Extranuclear Estrogen Receptor- α Improves Pancreatic Islet Lipid Homeostasis.

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Preventing β -cell failure in Type 2 diabetes mellitus (T2DM) is a major therapeutic challenge. In islets of male Zucker Diabetic Fatty (ZDF) rats, a model of T2DM, up regulation of lipogenesis leads to triglyceride (TG) accumulation and provokes lipotoxic β -cell failure. However, female ZDF rat islets are protected from lipotoxicity. We reported that 17 β -estradiol (E2) is vital to β -cell survival via its actions through the estrogen receptor (ER)- α and the membrane G protein-coupled estrogen receptor (GPER). We find that in male ZDF rats, E2 treatment suppresses islet lipogenesis and reduces TG accumulation, thus preventing lipotoxic β -cell failure. Using a combination of ER α , ER β and GPER selective agonists in rat INS-1 β -cells, and ER α , ER β and GPER deficient mouse islets, we find that E2 reduces TG accumulation and suppresses key lipogenic genes. E2 suppresses lipogenesis via ER α , ER β and GPER with a predominant ER α effect.

Using a pancreas specific ER α deficient mouse (PER α KO^{-/-}) we find that E2 suppression of lipogenesis is mediated via ER α action in islets *in vivo*. We find that ER α suppresses lipogenesis via extranuclear signaling dependent on signal transducer and activator of transcription 3 (STAT3). In cultured WT islets, the basal phosphorylation of STAT3 (pSTAT3) is low and lipogenesis is high. Upon E2 treatment, we observe an increased pSTAT3 and a suppression of lipogenesis. Conversely, in ER α deficient islets, basal pSTAT3 is elevated, lipogenesis is low, and E2 treatment does not modify pSTAT3 and lipogenesis. These data suggest that unliganded ER α inhibits STAT3, thus releasing the inhibition of lipogenesis. Upon E2 binding to ER α , STAT3 is activated thus suppressing lipogenesis. These data provide molecular evidence for the involvement of extranuclear ER signaling in the regulation of islet lipogenesis, with implications for β -cell dysfunction in T2DM.

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Nothing to Disclose: JPT, CLM, KSK, FM-J

OR19-1

Pituitary Development Requires MicroRNAs and MicroRNA-26b Specifically Targets Lef-1, Which Modulates Pit-1 Expression.

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To understand the role of microRNAs (miRNAs) in anterior pituitary development, a group of pituitary specific miRNAs were identified and Dicer1 was then conditionally knocked out using the Pitx2-Cre mouse resulting in the loss of mature miRNAs in the anterior pituitary. The Pitx2-Cre/Dicer1 mutant mice demonstrate growth retardation compared with their littermates and the pituitaries are hypoplastic with an abnormal branching of the anterior lobe. Growth hormone (GH), Prolactin (PRL), Thyroid stimulating hormone β -subunit (TSH β) and Luteinizing hormone β -subunit (LH β) expression were decreased in the Dicer1 mutant mouse, while POMC expression was normal in the mutant pituitary. Pit-1 expression was decreased and Lef-1 expression was increased in the mutant mouse pituitary. We demonstrate that endogenous Lef-1 directly targets and represses the Pit-1 promoter. miRNA-26b (miR-26b) was identified as targeting the Lef-1 3'UTR and miR-26b represses different Lef-1 isoforms in pituitary and non-pituitary cell lines. Furthermore, miR-26b up regulates Pit-1 expression by attenuating Lef-1 expression in GH3 cells. Thus, microRNAs are critical for anterior pituitary development, and miR-26b regulates Pit-1 expression by inhibiting Lef-1 expression and may promote Pit-1 lineage differentiation during pituitary development.

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Nothing to Disclose: ZZ, SF, AG-H, BAA

OR19-2

Prolactin Gene Expression Patterns Change during Pituitary Development and Are Influenced by Tissue Architecture.

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Pituitary development, in particular the differentiation of anterior pituitary endocrine cells, remains to be fully understood, and may have implications for adult pituitary plasticity and hyperplasia. We have used prolactin-reporter transgenic rats, with the firefly luciferase reporter gene inserted into exon 1b of a 160kbp human prolactin recombinant bacterial artificial chromosome, to assess when lactotroph cells emerge in the pituitary and how transcription dynamics of the prolactin gene changes during fetal development. Bioluminescence imaging of fetal rat pituitary glands *ex vivo* detected lactotroph cells as early as ED16.5, with cells appearing initially as isolated single cells or as small isolated cell clusters. RT-PCR analyses confirmed the presence of Pit-1, endogenous rat prolactin and luciferase transcripts at this gestational age. These data indicate that transcriptionally active lactotrophs appear earlier than previously suggested and this has implications for the differentiation pathway of the lactotroph lineage.

Real-time bioluminescent imaging of ED16.5 fetal pituitaries showed that prolactin gene transcriptional activity fluctuated rapidly with time in newly emerging lactotroph cells. The transcriptional bursts in these cells appeared to be far more transient than in adult cells in primary culture with a duration ranging from 4 to 9.5 hours. In contrast, transcriptional activity in lactotroph cells in post-natal day 1.5 pituitary tissue appeared relatively stable with a minority of cells displaying a sustained increase in luciferase expression. Tissue architecture may have a role in stabilising transcription activity in PN1.5 lactotroph cells as isolated cells cultured after enzymatic tissue dispersion displayed transcriptional pulses. At ED16.5, tissue architecture had less of an influence as only closely adjacent cells (within 30µm) showed co-ordinated transcriptional activity. Overall these data suggest that transcription dynamics may be affected by the emergence of key transcriptional regulators, such as ERalpha, and/or the emergence of a putative lactotroph cell network. Furthermore, changes to chromatin structure through successive rounds of transcription may stabilise transcription dynamics. These data provide interesting insights into how transcription dynamics may be affected by regulatory factors and also how lactotroph cells function as they become specified during pituitary development.

Nothing to Disclose: KF, CVH, AM, SS, DGS, ASM, JJM, MRHW, JRED

OR19-3

Distinct Patterns of Histone Modifications Are Mediated by the Early and Late Developmental Enhancers at *Pit-1* Chromatin Locus.

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In metazoans, cis-regulatory elements for gene activation can be located hundred of kilobases from their target promoters. Long-range controls play a central and often predominant role in gene activation. Pit-1 is a pituitary-specific homeodomain transcription factor that plays a critical role in cytodifferentiation of the pituitary. Pit-1 function is essential to normal differentiation of somatotropes, lactotropes, and thyrotropes. *Pit-1* expression is under the control of multiple enhancers. Prior studies suggested that the initial activation of *Pit-1* expression in the developing pituitary is mediated by an 'early' enhancer located 5.9 kb upstream of the *Pit-1* gene and that subsequent maintenance of *Pit-1* expression is mediated by a more distal 'late' enhancer located 10.2 kb upstream of the gene. The early enhancer, on its own, is incapable of maintaining robust *Pit-1* expression in the adult pituitary and the maintenance of *Pit-1* expression by the late enhancer is via an auto-regulatory loop dependent on functional Pit-1 binding sites within the late enhancer region and at the *Pit-1* promoter. A central question in understanding *Pit-1* gene regulation is how the switching between the early and late enhancers is mediated during the development of pituitary. To address this issue we have compared the epigenetic profiles of the *Pit-1* chromatin locus in the presomatotrope and in the adult pituitary. These studies reveal that initial activation of the *Pit-1* locus is accompanied by acetylation of core histone H3 restricted to the early enhancer and the *Pit-1* promoter in the context of a broad domain of acetylated histone H4 domain encompassing the entire *Pit-1* locus. At this stage the levels of RNA polymerase II and Pit-1 recruitments are relatively low and restricted to the promoter region. In contrast, in adult pituitary broad domains of acetylated histone H3 and H4 domains are established along with the recruitment of high levels of Pit-1 to both the late enhancer and the *Pit-1* promoter. These events are paralleled by high-level RNA polymerase II recruitment at the promoter of *Pit-1* and robust *Pit-1* expression. In summary, these studies revealed that the patterns of acetylated H3 and H4 undergo dynamic changes during the switch from early to late enhancer activation. These results suggest that the mechanisms of *Pit-1* expression mediated by these two enhancers are distinct during the development of pituitary.

Sources of Research Support: University Research Foundation of University of Pennsylvania.

Nothing to Disclose: YH, SAL, NEC

OR19-4

Differential Transcriptional Regulation of CEACAM1 by Free Fatty Acids.

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High-fat feeding of C57BL/6 (BL6) mice for 30 days causes hyperinsulinemia and insulin resistance before inflammation develops. This is mediated by a reduction in hepatic CEACAM1 levels and ensuing impairment of insulin clearance. Moreover, overexpressing CEACAM1 in liver protected against insulin resistance and visceral obesity in response to high-fat diet. This implicates CEACAM1 in the early pathogenesis of diet-induced insulin resistance. Moreover, hepatic CEACAM1 levels are reduced at fasting and elevated in response to insulin surges during the first few hours of refeeding. Because both fat feeding and fasting involve PPAR α activation by free fatty acids (FFA), we investigated the role of PPAR α in the regulation of CEACAM1 expression. We showed that PPAR α activation by its specific agonist, WY-14,643, decreased Ceacam1 mRNA and protein levels in rat hepatoma cells and BL6 mice. PPAR α activation by WY-14,643 also decreased Ceacam1 promoter activity in a dose-dependent manner in human HEK 293 and H4IIE (rat hepatoma) cells. Using Chromatin Immunoprecipitation (ChIP) analysis, we demonstrated that WY-14,643 treatment enriched PPAR α binding to its response element (PPRE) located at nt -260/-247 on the mouse Ceacam1 promoter. Moreover, high-fat feeding resulted in a ~4.5-fold increase in the recruitment of PPAR α to PPRE on the Ceacam1 promoter. As FFA act as the natural ligands for PPAR α , we then tested the effect of individual fatty acids on CEACAM1 expression in primary hepatocytes. Coupled to BSA (5:1) at a concentration of 0.1-0.3mM, oleic acid (MUFA; n-9, C18:1), linoleic acid (PUFA; n-6, C18:2) and α -linolenic acid, a plant derived n-3 PUFA (C18:3) decreased CEACAM1 mRNA and protein levels. In contrast, palmitic acid (saturated FA; C16:0) did not affect CEACAM1 content even at 0.4mM. Moreover, DHA, a marine-derived n-3 PUFA, upregulated CEACAM1 protein level. Taken together, this suggests that FFA differentially regulate Ceacam1 transcription by a PPAR α -dependent mechanism. Further investigation is underway to unravel the mechanism of FFA-mediated repression or activation of Ceacam1 and to identify the co-regulatory transcription factors in this process.

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Nothing to Disclose: SKR, PRP, QYA-S, TAB, SG, YMS, ERS, SMN

OR19-5

Dietary Regulation of 11 β -HSD1 in Adipose Tissue Is Mediated by the C/EBP β Isoforms LAP and LIP.

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11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) regenerates active glucocorticoids (cortisol, corticosterone) from inert substrates (cortisone, 11-dehydrocorticosterone). 11 β -HSD1 expression is elevated selectively in adipose tissue (AT) of obese humans and in monogenic models of obesity in rodents where it plays a key role in the pathogenesis of obesity/Metabolic Syndrome. Paradoxically, in chronically high fat (HF)-fed mice 11 β -HSD1 is down-regulated in AT, probably to protect against metabolic disease. In liver, 11 β -HSD1 transcription is regulated by the CCAAT/enhancer binding proteins (C/EBP), but whether C/EBPs are involved in the striking dietary down-regulation of 11 β -HSD1 in AT has not been determined. We investigated whether alterations in the levels of C/EBPs (α , β , δ and ζ) may underlie the HF down-regulation of 11 β -HSD1 in subcutaneous AT. Mice (16/group) were fed low fat (LF, 11% calories from fat) or high fat (HF, 58%) diet for 6 weeks. C/EBP α and δ mRNA and protein levels were unchanged in subcutaneous AT, while levels of C/EBP ζ were increased with HF diet (166.8 \pm 11.8% and 140.2 \pm 4.2% for mRNA and protein, respectively; p <0.001 and p <0.05). Diet did not change AT C/EBP β mRNA levels. However, western blot analysis revealed a decrease in the Liver Activating Protein (LAP) isoform of C/EBP β (77.2 \pm 2.4%; p <0.05) and an increase in the Liver Inhibitory Protein (LIP) isoform (127 \pm 4.8%; p <0.05) following HF diet, resulting in increased LIP/LAP ratio (165.9 \pm 3.8%; p <0.001). Transfection of differentiated 3T3-L1 adipocytes with C/EBP β or ζ siRNAs showed that C/EBP β is required for the normal expression of 11 β -HSD1 in differentiated 3T3-L1 adipocytes, while C/EBP ζ is not. Chromatin immunoprecipitation assays demonstrated the presence of C/EBP β on the 11 β -HSD1 promoter in 3T3-L1 adipocytes. To test the hypothesis that the LIP/LAP ratio modulates 11 β -HSD1 expression, we generated stably transfected 3T3-L1 adipocytes conditionally overexpressing LIP using the pSLIK Tet-On system. As predicted, increased LIP expression caused a significant decrease in 11 β -HSD1 mRNA levels (p <0.05). Thus, our data suggest that the LIP/LAP ratio underlies the HF diet-mediated down-regulation of 11 β -HSD1 in adipocytes as part of a mechanism to counteract metabolic disease.

Nothing to Disclose: CLE, VK, T-MM, JRS, KEC

OR19-6

Changes in Histone Methylation during Postnatal Growth Deceleration.

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Children grow, but adults do not. The cessation of growth in multiple organs is the end result of a progressive decline in cell proliferation beginning in early life. The mechanisms responsible for somatic growth deceleration are largely unknown. We recently showed evidence that this growth deceleration results from an extensive growth-limiting genetic program that occurs simultaneously in multiple tissues. This program involves the downregulation of many growth-promoting genes, including *Igf2*, *Mdk*, *Mycn*, *Peg3*, and *Ezh2*, that are required for rapid proliferation in early life. To explore potential epigenetic mechanisms responsible for downregulation of these genes, we investigated the chromatin states of their promoter regions. Using chromatin immunoprecipitation, we found that trimethyl-H3K4, a histone modification associated with gene activation, decreased from 1wk to 4wk in multiple genes (*Igf2*, *Mdk*, *Mest*, *Plagl1*, *Peg3*, *Gpc3*) in mouse liver, kidney, and lung ($P < 0.05$ for all genes in all organs). Concomitantly, western blotting showed that the overall level of trimethyl-H3K27, a histone modification associated with gene silencing, increased from 1wk to 4 and 8wk in mouse liver, kidney, and lung, suggesting a global shift of chromatin toward a non-permissive state in these organs. Furthermore, using real-time PCR, we found that both H3K4 methyltransferase and H3K27 demethylase mRNA levels decreased with age in these organs ($P < 0.05$), implying that the observed epigenetic changes may be due to temporal changes in gene expression of these histone modifying enzymes. Taken together, our findings suggest that juvenile growth deceleration results from the downregulation of many growth-promoting genes and that this downregulation may be orchestrated by global and local histone modifications including the reciprocal decline in activation marks (trimethyl-H3K4) and increase in silencing marks (trimethyl-H3K27), which in turn may be governed by corresponding changes in histone methyltransferase and demethylase expression with age.

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Nothing to Disclose: JCKL, JB

OR20-1

The DNA Methylome of Benign and Malignant Parathyroid Tumors.

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Background: Epigenetic mechanisms participate along with genetic abnormalities to cause altered patterns of gene expression and function in tumors. The molecular pathogenesis of benign and malignant parathyroid tumors is incompletely clarified. The role of DNA methylation in parathyroid tumorigenesis has not been analyzed in an unbiased, systematic fashion.

Methods: Genomic DNA was isolated from normal parathyroid tissue (n=4), benign parathyroid adenomas (n=14), and parathyroid carcinomas (n=6). DNA was bisulphite modified using the EZ DNA Methylation kit (Zymo Research, Orange, CA) and analyzed using the the Infinium HumanMethylation27 BeadChip (Illumina, San Diego, CA). It allows interrogation of 27,578 highly informative CpG sites from more than 14,000 genes per sample at single-nucleotide resolution. The 12-sample Bead Chip features content derived from the well-annotated NCBI CCDS database and is supplemented with more than 1,000 cancer-related genes. The methylation module in the GenomeStudio (Illumina) software was used to analyze the results. Results were confirmed for selected genes in another cohort of parathyroid tumors by methylation-specific PCR.

Results: Distinct hierarchical clustering of genes with altered DNA methylation profiles in normal parathyroid tissue, parathyroid adenomas, and parathyroid carcinomas was evident. Comparing normal parathyroid tissue to parathyroid adenomas, 367 genes were significantly altered, while 175 genes differed comparing parathyroid carcinomas and normal parathyroid tissues ($p < 0.005$ for both). A comparison between parathyroid adenomas and parathyroid carcinomas identified 263 genes with distinct methylation levels ($p < 0.005$). Genes of known or putative importance in the development of parathyroid tumors showed significant and frequent hypermethylation. Such genes included those involved in regulation of the cell cycle and transcription (p15/INK4A, p16/INK4A, WT1, and RIZ1/PRDM16) and members in the Wnt/beta-catenin signaling pathway (SFRP1, SFRP2 and SFRP4)

Conclusions: The unbiased, genome-wide study of the parathyroid tumor "methylome" identified distinct hierarchical clustering of genes in parathyroid adenomas vs. carcinomas. A number of genes with altered DNA methylation patterns were identified of putative importance to benign and malignant parathyroid tumorigenesis.

Nothing to Disclose: PB, LFS, RU, GA, GW, TC

OR20-2

HRPT2 Mutations and CaSR Expression Determine Prognosis in Parathyroid Carcinoma.

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Introduction:

Parathyroid carcinoma is a rare cause of primary hyperparathyroidism, known to be associated with *HRPT2* gene mutations. Diagnosis is principally based on intra-operative evidence for local invasion, supported by pathological features of capsular invasion, vaso-invasion and increased amount of mitotic figures. It is a slow growing tumor with a median time to first recurrence of 3 years and an overall survival of 50%-86% at 5 years. The aim of our study was to evaluate the role of a genetic mutation in the *HRPT2* gene and also that of an alteration in CaSR expression as determinants of prognosis in parathyroid carcinoma.

Methods:

Clinical data were obtained from the hospital records of 25 patients with an established diagnosis of parathyroid carcinoma in whom data on somatic and/or germline mutation in the *HRPT2* gene and analysis of the CaSR expression were also available from pathological specimens obtained at surgery.

Results:

Preliminary analysis show that the presence of a *HRPT2* mutation (n=4) significantly shortens the time to development of local recurrence and/or metastases ($p=0,005$) and decreases the 5-year survival from 80% in patients without an *HRPT2* mutation to 33% in patients with an *HRPT2* mutation, albeit not significantly ($p=0,123$). The absence of CaSR expression (n=7) is also associated with significantly more rapid development of local recurrence and/or metastases ($p=0,000$) and with significant decreases in the 5-year survival to only 17% ($p=0,000$).

Conclusion:

Our data suggest that a *HRPT2* mutation and the absence of CaSR expression are strong determinants of the malignant potential and thus disease-free survival and survival in patients with parathyroid carcinoma. This advocates the need for analysis of these determinants in all patients with this malignancy. Whether a more aggressive management would alter the natural course of this malignancy remains to be established.

Nothing to Disclose: JEW, NATH, JK, JAR, J,M

OR20-3

Mouse Prkar1a Haploinsufficiency Leads to an Increase in Tumors in the Trp53^{+/-} or Rb1^{+/-} Backgrounds and Chemically-Induced Skin Papillomas by Dysregulation of the Cell Cycle and Wnt Signaling.

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Inactivation of the PRKAR1A gene leads to dysregulated cyclic adenosine mono-phosphate (cAMP) signaling and Carney complex (CNC), a syndrome that is associated with skin, endocrine and other tumors in humans. The rather specific CNC phenotype is not easily explained by the ubiquitous cAMP signaling defect; furthermore, Prkar1a^{+/-} mice developed few neoplasms at a relatively old age and did not have skin or most of the CNC tumors. To identify whether a Prkar1a defect is truly a generic but weak tumorigenic signal that depends on, or is further defined by tissue-specific or other factors, we investigated the Prkar1a^{+/-} mice when bred within the Rb1^{+/-} or Trp53^{+/-} genetic backgrounds, or treated with a 2-step skin carcinogenesis protocol. Prkar1a^{+/-}-Trp53^{+/-} mice developed more bone sarcomas than Trp53^{+/-} mice (p<0.05) and Prkar1a^{+/-}-Rb1^{+/-} mice grew more and larger pituitary and thyroid tumors than Rb1^{+/-} mice. All mice with double heterozygosity had significantly reduced life-spans compared with their single-heterozygous counterparts. Prkar1a^{+/-} mice also developed more papillomas than wild-type animals when their skin was treated with chemical. A whole-genome transcriptome profiling of tumors produced by all three models identified Wnt signaling as the main pathway activated by abnormal cAMP signaling in these tissues, along with (expected) cell cycle gene abnormalities, all confirmed by qRT-PCR array and immunohistochemical analyses. siRNA down-regulation of Ctnnb1, E2f1 or Cdk4 inhibited proliferation of human adrenal cells bearing a PRKAR1A-inactivating mutation and Prkar1a^{+/-} mouse embryonic fibroblasts and led to arrest of both cell lines at the G0/G1 phase of the cell cycle. We conclude that Prkar1a haploinsufficiency and the consequent abnormal cAMP signaling are relatively “weak” tumorigenic signals that can act synergistically with classic tumor suppressor gene defects or chemical factors to induce tumor formation, mostly through activation of Wnt-signaling and dysregulation of the cell cycle, consistent with studies in human tumors that carry PRKAR1A defects.

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Nothing to Disclose: MQA, MM, SB, AJB, KJG, KMT, CC, TW, FW, MFS, IB, MN, CAS

OR20-4

Regulation of IGF - mTOR Signalling by miRNA in Childhood Adrenocortical Tumors.

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Introduction: MicroRNAs (miRNAs) control gene expression at the post-transcriptional level and are involved in virtually every biological process, including oncogenesis. In our study, we aimed to investigate miRNA expression profiles in childhood adrenocortical tumors (ACT) and to identify the signalling pathways regulated by miRNAs. miRNA expression profiles were studied in a series of 25 childhood ACT and 5 age-matched normal adrenals by Agilent human V2 microarrays. Results were validated by Taqman qPCR. **Results:** We identified a set of miRNAs that are differentially regulated in childhood ACT, including miR-99a and miR-100. Functional analysis of these miRNAs in adrenocortical tumor cell lines showed that they coordinately regulate expression of key components of the IGF - mTOR signalling pathway (IGF-1R, mTOR and raptor) through binding sites in their 3' UTRs. In adrenocortical tumor cells, the active, Ser2448-phosphorylated form of mTOR is strikingly present only in mitotic cells in association with the mitotic spindle and with the midbody in late phases. Pharmacological inhibition of mTOR signalling by RAD001 (everolimus) greatly reduces adrenocortical tumor cell growth in vitro and in vivo. **Conclusion:** Our data reveal a new mechanism of regulation of IGF - mTOR signalling by miRNAs and lay the groundwork for clinical experimentation of drugs targeting mTOR in the therapy of adrenocortical cancer.

Nothing to Disclose: MD, AEW, BC, ET, JW, WZ, MHCP-DV, BCF, GPZ, EL

OR20-5

R337H P53 Mutation and the Expression of microRNAs in Adrenocortical Tumor.

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Introduction: In southern Brazilian children population, adrenocortical tumor (ACT) has been described to be 15 times more frequent and associated with high prevalence of the R337H germline mutation in the p53 gene(1). There are few data on microRNAs (miR) in the molecular pathogenesis of ACT. **Objective:** To compare a panel of miR expression in ACT and normal adrenal tissue and whether the different miR expression is associated with R337H p53 mutation, type of hormone excess, the age of patients, tumor size, tumor stage or mortality. **Material and Methods:** We studied 26 ACT (8M/18F; 28.5±37 months) and 6 normal adrenal tissues. All patients presented hormone excess (16 Cushing's syndrome and virilization, 9 virilization and 1 Cushing's syndrome). Based on the Sullivan modification of the Macfarlane classification, we observed 13 patients at stage I, 2 at II, 6 at III and 5 at IV. The relative expression of each miR was measured by real-time PCR. Relative quantification of miR expression was calculated using the $2^{-\Delta\Delta Ct}$ method. **Results:** 22 out of 26 ACT had the R337H p53 mutation. We found no different expression of miR-16, miR-16.1, miR-143 and miR-145 and a hyperexpression of let-7a (5.4 fold; p=0.006) and miR-449 (5.5 fold; p=0.03) in tumor samples compared to normal adrenal tissue. Higher miR-449 expression was, actually, associated with ACT presenting R337H p53 mutation (3.8 fold; p=0.01). Patients presenting ACT stage III/IV also showed higher expression of miR-449 (5.9 fold; p=0.02) compared to normal adrenal. There was no difference in the expression of any other miRs in ACT with or without R337H p53 mutation. Regarding hormone excess, let-7a and miR-145 were hyperexpressed (1.6 fold; p=0.03 and 2.1 fold; p=0.02, respectively) in ACT producing Cushing's syndrome and virilization. There was no association of miR expression and age of patients or tumor size. However, patients who died due to the disease presented ACT with lower expression of miR-16.1 (5.7 fold; p=0.02). **Conclusion:** The underexpression of miR-16.1, a tumor suppressive miR, in ACT with poor prognosis indicates that this miR might potentially target genes encode proteins with potential oncogenic functions which could have a role in the ACT pathogenesis. Our data on miR-449 hyper-expression in ACT modulated by the R337H p53 mutation confirm the hypothesis that transcription-independent modulation of miR biogenesis is intrinsically embedded in a tumor suppressive program governed by p53(2).

(1)Sandrini R et al., J Clin Endocrinol Metab 1997; 82:2027

(2)Suzuki HI et al., Nature 2009; 460:529

Sources of Research Support: Sao Paulo Research Foundation Grant 07/58365-3.

Nothing to Disclose: LMC, LFL, LMM, CAS, LGT, CEM, SRA, ACM, MC

OR20-6

***FP/TMEM127* is a Novel Pheochromocytoma Susceptibility Gene.**

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Pheochromocytomas are catecholamine-secreting tumors of neural crest origin that are hereditary in approximately one-third of the cases. However, the molecular basis of the majority of these tumors is unknown. We previously mapped a novel Familial Pheochromocytoma (FP) locus at chromosome 2q11. Now we show that the *TMEM127* gene, which maps within this area, is the target of mutations in FP. In a cohort of 103 samples, predominantly truncating germline *FP/TMEM127* mutations were detected in one-third of familial and about 3% of sporadic-appearing tumors without a known genetic cause. The wild-type allele was consistently deleted in tumor DNA, suggesting a classic two-hit mechanism of tumor suppressor gene inactivation. In agreement with a role for this gene in tumor suppression, short hairpin-mediated *FP/TMEM127* knockdown leads to increased cell proliferation, while enforced expression of *FP/TMEM127* leads to cell growth inhibition. Pheochromocytomas with *FP/TMEM127* mutations display a transcription signature distinct from the pseudohypoxic profile characteristic of tumors with *VHL* and *SDH* mutations, but closely related to *RET* and *NF1*-mutant tumors. Similar to tumors with *RET* or *NF1* mutations, *FP/TMEM127* mutants show hyperphosphorylation of mTOR targets. However, in contrast with these tumors, mTOR activation in depleted *FP/TMEM127* cells is PI3K/AKT- and RAS-independent. These results indicate that *TMEM127* is a negative regulator of mTOR that functions by mechanisms distinct from classic pathways. Our studies unveil *FP/TMEM127* as a novel putative tumor suppressor gene and validate the power of hereditary tumors for elucidating cancer pathogenesis

Sources of Research Support: PLMD is a Voelcker Foundation Investigator.

Nothing to Disclose: YQ, LY, EK, KB, EC, JL, NA, MS, FS, GO, RT, ST, CS, RA, PD

OR21-1

Familial Case of 46,XY Disorder of Sex Development DSD and Congenital Heart Defects (CHD) Associated with a Mutation in GATA4.

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GATA4 belongs to the evolutionarily conserved GATA family of six tissue and organ specific vertebrate transcriptional regulators. In the mouse and human, GATA4 is strongly expressed in the somatic cell population of the developing gonad prior to and during the time of sex determination. The critical role for GATA4 in gonadal development is highlighted by Gata4ki mice that have a p.Val217Gly mutation in the N-terminal zinc finger domain. This mutation abrogates the interaction of GATA4 with the cofactor FOG2 and these animals display abnormal testis development. GATA4 cooperatively interacts with several proteins including NR5A1 (encoding steroidogenic factor 1) to regulate the expression of genes involved in sex-determination. In the human, mutations in GATA4 are associated with congenital cardiac defects however; to date no mutations in GATA4 have been reported in association with human disorders of sex development (DSD). We investigated whether mutations in the GATA4 gene might be responsible for 46,XY DSD. We identified a heterozygous missense mutation (p.Gly221Arg) in the conserved N-terminal zinc-finger of GATA4 in a familial case of 46,XY DSD. Female carriers of the mutation had no apparent ovarian phenotype. Several members of the family had septal heart anomalies. This mutation compromised the ability of the GATA4 protein to bind to its site on AMH promoter and thus transcriptionally activate AMH promoter. Although the mutation does not interfere with a direct physical interaction, it disrupts synergism of GATA4 with the NR5A1 protein to activate the AMH promoter. The p.Gly221Arg mutant also lacks the ability to physically interact with a known cofactor, FOG2. Although mouse models of Gata4 mutations associated with abnormal testicular development have been described, this is the first report of mutations in GATA4 associated with 46,XY DSD and the data suggest that GATA4 DNA-binding activity is essential for transcriptional activation of the AMH promoter.

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).

Nothing to Disclose: AB, DL, MR, CN-F, RB, KM

OR21-2

A Novel Exon within the LH/CG Receptor Gene as Transcriptional Regulator of LHCGR Signalling.

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We have recently identified a novel, primate-specific bona fide exon (exon 6A) within the LHCGR gene. It displays composite characteristics of an internal/terminal exon and possesses stop codons triggering nonsense-mediated mRNA decay (NMD) in LHCGR. Transcripts including exon 6A are physiologically highly expressed in human testes and granulosa cells, and result in an intracellular, truncated LHCGR protein of 209 amino acids.

Male pseudohermaphroditism, or Leydig cell hypoplasia (LCH), is an autosomal recessive disorder in individuals with a 46,XY karyotype, characterized by a predominantly female phenotype despite the presence of testicular structures. It is caused by mutations in the luteinizing hormone/chorionic gonadotropin receptor gene (LHCGR), which impair either LH/CG binding or signal transduction. However, molecular analysis has revealed that the LHCGR is apparently normal in about 50% of patients with the full clinical phenotype of LCH.

We sequenced exon 6A in 21 patients with unexplained LCH and detected mutations in four patients. Functional studies revealed a dramatic increase in the expression of the mutated internal exon 6A transcripts, indicating aberrant NMD.

These altered ratios of LHCGR transcripts result in the generation of predominantly nonfunctional LHCGR isoforms, thereby preventing proper expression and functioning.

Using antisense oligonucleotides we were able to introduce exon 6A skipping in COS-7 cells transiently transfected with a LHCGR minigene containing exon 6A. The skipping of a mutated exon 6A at the transcriptional level leads to a putative treatment regimen for aberrant LHCGR transcripts which interfere with proper LHCGR signalling.

The identification and characterization of this novel exon not only identifies a new regulatory element within the genomic organization of LHCGR, but also points toward a complex network of receptor regulation, including the transcriptional level.

Kossack N et al., PLoS Medicine 2009; 5:e88

Sources of Research Support: German Research Foundation (GR 1547/13-1).

Nothing to Disclose: JG, AR-U, NK

OR21-3

CBX2 and Human Sex Development: Insights in the Mechanism of Action.

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CBX2/M33 is a member of the Polycomb group (PcG) proteins, highly conserved regulatory factors initially discovered in *Drosophila*. They act by regulating chromatin structure and chromosome architecture at their target loci. We discovered a loss-of-function double heterozygote mutation state in a 46,XY girl with normal ovaries at histology, normal uterus and external female genitalia, accidentally diagnosed because of a discrepancy between prenatal karyotype and phenotype at birth. Our data identified CBX2 as essential for normal human male gonadal development, suggest that it lies upstream of SRY in the human sex development cascade and identify a novel autosomal recessive cause of defect of sex development. The exact position of CBX2 in sex development cascade is still unknown and the role of CBX2 in ovarian development and maintenance is yet to be explored. In search for the mechanism by which CBX2 regulates gonadal differentiation, we firstly choose to look at the most likely candidate(s), of which SOX9 is an example. CBX2-downregulation (siRNA) and overexpression experiments were performed and in parallel ChIP assays and transactivation studies were designed using the enriched ChIP fragments as targets. The results can be summarized as follows:

In testis derived cells (NT2D1)

1. Downregulation of CBX2 (siRNA) reduces SOX9 expression.
2. Sequences distinct from the testis-specific enhancer core (TESCO) (1) of the human SOX9 promoter are target for specific CBX2 binding. This binding induces enhanced transcription of the reporter (ChIP & Transactivation).
3. CBX2 overexpression induced enhanced expression of endogenous SOX9 (qRT-PCR)

In ovary-derived cells (OvCar3)

1. Downregulation of CBX2 (siRNA) does not influence SOX9 expression.
2. Accordingly, although sequences between -122/+67 of the SOX9 promoter are target for CBX2 in these cells, the binding does not induce change in transcription of the reporter (ChIP & Transactivation).
3. CBX2 overexpression reduces SOX9 endogenous expression (qRT-PCR).

CBX2s mutations abolish all these effects supporting a CBX2- specific phenomenon.

This data suggest that CBX2 in the testis stimulates SOX9 expression directly (and indirectly *via* SRY and SF1).

Intriguingly, CBX2 seems to limit SOX9 expression in the ovary, in accordance with recent models of sex development cascade

(1) Sekido R & Lovell-Badge, Nature 2008; 453:930

Sources of Research Support: Research Grant of the University of Zurich (54181801)to AB-L; Grant 32-116636 of the Swiss National Science Foundation awarded to AB-L.

Nothing to Disclose: AB-L, MM, DK, CdB, EJS

OR21-4

Exposures to Bisphenol A (BPA) Alter Proliferative Activity and Steroidogenic Capacity in Developing Rat Leydig Cells.

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Exposure of the population to the industrial chemical bisphenol A (BPA) is significant because it is used in the manufacture of polycarbonate plastics and epoxy resins, and is a constituent of dental sealants. BPA is known to activate estrogen receptors (ERs), including in testicular Leydig cells, which produce the sex steroid testosterone (T) to support male fertility. Postnatal Leydig cell development is marked initially by intense proliferative activity and later by increased steroidogenesis and is under the control of pituitary luteinizing hormone (LH) and steroid hormones. The present study examined the effects of environmentally relevant doses of BPA on Leydig cell development. Timed pregnant Long Evans dams (n=14) were gavaged with olive oil vehicle or BPA at 2.5 or 25 µg/kg body weight (bw) from gestational day 12 through weaning at postnatal day (PND) 21, i.e., during the perinatal period. Exposure to BPA increased protein expression of LH receptors, androgen receptors and ERα in progenitor Leydig cells (PLCs) isolated from 21 day-old male rats. Also, BPA increased Leydig cell [³H] thymidine uptake (4.1±0.1 and 6.2±0.1 CPM/10³ cells) compared to control (3.6±0.1) (P< 0.01); this effect was linked to enhanced expression of cyclin D3 and proliferating cell nuclear antigen. The mitogenic effects of BPA were replicated *in vitro* because incubation of freshly isolated PLCs (n=3) with BPA at 0.01 nM decreased (3.8±0.2 CPM/10³ cells), whereas 10 nM increased [³H] thymidine uptake (5.6±0.1) compared to control (4.5±0.2) (P<0.01). In contrast to stimulation of cell division, Leydig cell T production at 21 days was decreased from 4.5±0.7 ng/10⁶ cells/3h (control) to 1.9±0.5, and 0.8±0.2 after exposure to 2.5 and 25 µg/kg BPA (P<0.05). To assess Leydig cell division *in vivo*, 60 day-old rats (n=5) that were exposed to BPA only during the perinatal period and their age-mate controls were administered the Leydig cell-specific toxicant ethane dimethanesulphonate (EDS) at 80 mg/kg bw intraperitoneally. Leydig cell regeneration was assessed by measurement of serum T levels at 28 days post-EDS treatment, which were higher in BPA treated rats (5±1.1 and 3.8±0.5 ng/ml) compared to control (2.3±0.5) (P=0.06). Taken together, these results imply that low dose exposures of male rats to BPA during the period of reproductive tract development may alter Leydig cell proliferation and steroidogenesis, which potentially impact serum androgen levels and fertility.

Sources of Research Support: Auburn University Cellular and Molecular Biosciences Program, Alabama EPSCoR Graduate Research Scholars Program and NIH grant ES 15886 (to BTA).

Nothing to Disclose: MKN, IDN, BTA

OR21-5

Epigenetic Regulation of Cyclic-Nucleotide-Mediated Signaling in Rat Neonatally Exposed to Bisphenol A (BPA).

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Fetal exposure to synthetic environmental chemicals compromises one's adaptability to adult life experiences and augments susceptibility to disease such as prostate cancer. Widespread human exposure to bisphenol A (BPA) is mainly through dietary intake, with additional exposure through drinking water, dental sealants and dermal exposure. Studies illustrating distinct pathways leading to the developmental reprogramming of prostate by BPA are in great need. Previously, we reported that the rat prostate epigenome was altered in response to early exposure to an environmentally relevant dose of BPA, which was associated with an increased susceptibility to adult estrogen-induced prostate intraepithelial neoplasia (PIN) with aging. Phosphodiesterase 4 type 4 variant 4 (PDE4D4) that functions in cAMP hydrolysis was found to exhibit persistent promoter demethylation and aberrant gene expression during adult life (1). By employing methylated CpG island recovery assay (MIRA) in combination with promoter array analysis, we herein have further identified over 10,000 differentially methylated sequences in the adult prostate which are susceptible to 10 mg/kgBW BPA exposure on neonatal days 1, 3 and 5. Utilizing in-house LRpath analysis (2), candidates which were annotated by enriched chromosomal loci, GO categories, KEGG pathways and/or transcription factors were chosen for validating their gene expression and promoter methylation status. Specifically, genes associated with cyclic-nucleotide-mediated signaling were found to have higher enrichment in BPA-exposed animals than those exposed to neonatal estradiol. Among them, GHRH, ADRB2, ADCY8, HTR5A, GIPR, GNAI2, INSL3, EIF4EBP2 and RXFP2 were chosen for further studies. These gene candidates may act singularly or conjointly in altering gene expression leading to increased susceptibility to prostate carcinogenesis. Taken together, our data provide support for a unique action of BPA in neonatal reprogramming that is distinct from estrogen. We speculate that BPA exerts its epigenetic effect through non-genomic events such as those associated with cAMP signaling. All-in all, our studies can improve the understanding of how the environmental pollutant, BPA, exerts harmful effects on human health via epigenetic alteration of gene expression.

(1)Ho S et al., Cancer Research 2006; 66(11):5624-32

(2)Sartor M et al., Bioinformatics 2009; 25(2):211-7

Sources of Research Support: NIEHS Grant K99ES16817-1 awarded to W.Tang; NIEHS grant to P30ES006096, ES13071 and ES015905 awarded to S.Ho; NIEHS grant awarded to ES12281 and ES015584 awarded to GS.Prins and S. Ho.

Nothing to Disclose: WT, XZ, JC, SK, LM, YYC, MM, GSP, SH

OR21-6

Alterations in Mammary Gland Function and Reproductive Capacity in CD-1 Mice Exposed Perinatally to Bisphenol-A.

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Bisphenol A (BPA) is used in the manufacture of polycarbonate plastics and epoxy resins; thus, exposure to BPA occurs through the use of many consumer products, dental and medical materials. Pups born to mothers exposed to BPA during gestation present a plethora of health effects that appear long after exposure has ended. Our studies showed that female mice perinatally exposed to low doses of BPA have altered mammary gland (MG) development at all ages. We also observed alterations in reproductive tract, hypothalamus, gonadotropin levels and estrous cyclicity in the exposed offspring. We hypothesize that the first generation (F1) of female mice perinatally exposed to BPA (*BPA daughters*) will develop abnormal MGs during pregnancy and lactation, and their reproductive success will be compromised.

Pregnant CD-1 mice were exposed to 50% DMSO (vehicle-control), 25ng, 250ng or 25µg BPA/kg body weight/day via an osmotic pump. The *BPA daughters* were exposed from the end of embryonic day 8 until postnatal day (PND) 16. At two months of age all F1 mice were mated. MGs were collected 1) on the day of delivery, or 2) on lactation day 15 (N=15-20/treatment). In a cross-fostering experiment, pups born to unexposed dams and nursed by the 250ng *BPA daughters* gained significantly more weight (11.45±0.2g) than pups of *BPA daughters* nursed by unexposed dams (10.56±0.37g). Similar results were observed when the 250ng *BPA daughters* were mated at 6 months of age and allowed to nurse their own pups. The MGs of all *BPA daughters* were studied and subtle changes in their development were observed which suggests the *BPA daughters'* MG development and function is altered during pregnancy and lactation.

Additionally, *BPA daughters* were housed with fertile males for a period of 8 months. *BPA daughters* exposed perinatally to 25ng or 25µg BPA, and DES (positive controls) (N=18-22/treatment), had significantly lower cumulative number of pups compared to unexposed control. *BPA daughters* exposed to 25µg BPA also had significantly fewer pregnancies compared to controls. Furthermore, 40% of *BPA daughters* exposed to 25µg BPA delivered less than 4 times, and 65% had less than 6 deliveries. These preliminary results indicate that the reproductive outcome may be altered in mice exposed perinatally to BPA.

Sources of Research Support: NIEHS Grant 08314.

Nothing to Disclose: PRW, NJC, MVM, BSR, CS, AMS

OR22-1

Serum Thyroglobulin Measurement by LC-MSMS in the Presence of Autoantibodies.

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Background:

Thyroglobulin (Tg), is a 660 kDa non-covalent homodimer glycoprotein. Tg is only produced in the thyroid gland. Tg levels in the blood can be used as a tumor marker for differentiated thyroid carcinoma (DTC). Diverse immunometric, radioimmunometric and immunochemiluminometric methods have been developed for quantitation of Tg [1]. However, these methods are all subject to interference attributable to Tg auto antibodies. The problem of interference attributable to Tg antibodies is particularly troubling for the clinical application of monitoring Tg levels as a tumor marker since up to 20% of thyroid cancer patients have Tg auto antibodies.

Introduction:

We present a novel method for the quantitation of Tg in a serum sample by tandem mass spectrometry which does not suffer from Tg Ab interference. After the serum samples were reduced, alkylated and digested by trypsin, the specific selected peptide from the mixture was attached on the correspondent peptide antibody beads. The enriched peptide was released from the beads and loaded on a LC-MSMS system with MRM mode data acquisition.

Results:

78 patient serum samples were analyzed over multiple experiments. Each set of experiments had its own calibration curve created by adding Tg standard to sera containing Tg < 0.2 ng/mL (by Tg-DPC-Immuplite method) and undetectable Tg auto antibody (TgAb < 20 IU/mL by DPC). The results show that the correlation between the Tg-LC-MSMS vs. Tg-DPC method is good (**n=43, R² = 0.95, b=1.03**) for the undetectable Tg auto antibody serum samples, and poor (**n=35, R² = 0.42, b=2**) for the patient sera with Tg auto-antibodies (TgAb > 20 IU/mL). Tg-LC-MSMS method gave higher Tg values compared to the Tg-DPC method for patient sera positive for Tg auto-antibodies. In order to validate the Tg-LC-MSMS method for Tg auto antibody positive patient serum detection, Tg recovery studies were undertaken. A high Tg patient serum sample was spiked into 16 different patient sera, positive for Tg auto-antibodies plus three control Tg Ab negative sera. The Tg measured by LC-MSMS gave an average recovery of 96.2% while the controls gave an average of 98.3%. These results indicate that the Tg-LC-MSMS method can detect Tg in the presence of Tg auto antibody in patient sera.

Conclusion:

Tg-LC-MSMS (with peptide enrichment) can be applied to measure Tg in Tg Ab positive patient sera and may be useful in the management of patients with DTC.

[1] Spencer, et al., Thyroid, 1999, 9(5): 435-41.

Disclosures: NJC: Employee, Quest Diagnostics. YZ: Employee, Quest Diagnostics. WAS: Employee, Quest Diagnostics. RER: Employee, Quest Diagnostics.

OR22-2

Results of TgAb Kits in Normal Subjects, Hashimoto's Thyroiditis and Papillary Thyroid Carcinoma Are Very Diverse: Practical Implications and Causes of Dissimilarity.

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Serum thyroglobulin (Tg) autoantibodies (TgAb) interfere with measurement of Tg, a marker of differentiated (papillary and follicular) thyroid carcinoma (DTC) and are usually positive in Hashimoto's thyroiditis (HT) patients. We wished to compare TgAb kits in normal subjects (NS) and in patients with HT, papillary thyroid carcinoma (PTC) and papillary thyroid carcinoma associated with HT (PTC-T) and to investigate the reasons of discrepancy. Sera from 150 NS, 160 HT, 105 PTC and 72 PTC-T patients (collected at the time of their first whole body scan) were evaluated by 3 radioimmunoassay (RIA) and 3 immunometric (IMA) TgAb assays. Tg was measured in all sera. To evaluate kit standardizations, we measured the International Reference Preparation (IRP) of TgAb serum (NIBSC 65/093) in the 6 kits. To assess differences in TgAb and Tg in the 3 RIA kits, we measured 4 human monoclonal TgAb-Fab, which identify 4 different, immunodominant Tg epitope regions. In addition, to investigate the influence of serum TgAb epitopes, we compared the TgAb epitope pattern of less frequently detectable (i.e. detectable in 1 to 3 kits) with those of more commonly detectable (i.e. detectable in 4 to 6 kits) sera (discordant and concordant sera, respectively). Results. In the 6 kits, the percent of detectable TgAb was 7-73% (15 ± 25) (median ± SD) in NS, 74-94% (85 ± 8) in HT, 13-74% (23 ± 23) in PTC and 54-83% (71 ± 10) in PTC-T sera. Tg was detectable in all NS, in 71% of HT, in 89% of PTC and in 49% of PTC-T sera. Comparing the titres of the 6 TgAb kits each others, the correlations were extremely variable ($r^2 = 0.03-0.98$). The higher correlations were retrieved comparing the kits of the same (RIA or IMA) group. In the 6 kits, concentration of undiluted IRP resulted 156-1095 (expected: 1000) IU/mL and IRP was detectable at a dilution of 1:16-1:128. In the 3 RIA kits, group A TgAbFab was detectable at a dilution of 1:1 to 1:3, group B TgAb Fab of 1:10 to 1:33 and group C TgAb Fab of 1:3 to 1:10. Group D TgAb Fab was undetectable in all kits. The 4 TgAbFab inhibited the concordant sera at a higher level in comparison with the discordant sera ($p < 0.05$ by Kruskal Wallis test). We confirm that the results of TgAb kits are discordant and that serum TgAb interfere with Tg measurement. Inadequate standardization, differences in Tg and TgAb preparations of the kits and epitope differences of the polyclonal population of serum TgAb cause the discrepancy of TgAb kits.

Nothing to Disclose: FLLF, DRRD, LMML, RRRR, LGGL, CUUC, FBBF, APPA, PVVP

OR22-3

A Randomized Noninferiority Trial To Determine the Optimum Dose of Radioiodine for Remnant Ablation in Differentiated Thyroid Cancer.

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Background: The optimal ¹³¹I activity for remnant ablation in differentiated thyroid cancer (DTC) even after meta-analyses could not conclusively found 100 mCi of ¹³¹I to be superior to lesser doses of radioiodine. Noninferiority trials are more appropriate than superiority trials when comparing small activity of ¹³¹I with higher activities.

Objectives: We conducted a stratified (any effect of histopathology on remnant ablation) randomized noninferiority trial from January 2001 to December 2005 to determine whether smaller doses are as effective as 100 mCi radioiodine for remnant ablation.

Patients & Methods: The sample size calculation used following inputs: $\alpha = 5\%$, power = 80%, ablation rate of 100mCi treatment group = 80% and non-inferiority margin (d) = 0.15. The sample size was found to be 450. We used allocation ratio of 2:2:1, for 25, 50 and 100 mCi group, respectively, as it has been shown that as long as an allocation ratio of 2:2:1 is maintained, reduction in the power of the test is minimal. Randomization with concealment was followed for patient group allotment. All patients underwent WBS with 2 mCi of ¹³¹I along with 48-h neck uptake (RAIU) after keeping patients off L-thyroxin for 4-6 weeks. Post therapy whole body scan was done in all patients to look for any nodal/distant metastases, missed on low dose pre-therapy WBS. The patients were then advised to take L-thyroxin 2 $\mu\text{g}/\text{kg}$ body weight daily as suppressive therapy. Six months later repeat diagnostic studies as described above along with Tg and anti-Tg antibody assays performed. The criteria for ablation were as follows: major criterion of negative ¹³¹I WBS and minor criteria of 48-h RAIU $\leq 0.2\%$ and Tg ≤ 10 ng/ml.

Results: A total of 734 patients were evaluated for their possible inclusion in the RCT. Of these, 312 could not participate in this study due to various reasons. Finally, 422 patients (360 PCT; 62 FCT) could be recruited within the stipulated period of time. First dose ablation was 81.5%, 84.9%, 88.5% and 84.2% in 25, 50, 100 mCi group and overall, respectively. Histology had no effect on ablation rate. The equivalence testing of the hypothesis was performed between 25 and 100 mCi groups, 50 and 100 mCi groups and 25 and 50 mCi groups. Results showed that at the significance level of 5%, null hypothesis was rejected, for each pair.

Conclusion: Ablation rates with 25, 50 & 100 mCi of ¹³¹I are equivalent with pre-specified clinically acceptable noninferiority margin of (d) = 0.15.

Nothing to Disclose: CSB, PC, AK, SND

OR22-4

Role of H₂O₂ in *RET/PTC1* Chromosomal Rearrangement Produced by Ionizing Radiation in Human Thyroid Cells.

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Background: Exposure of the thyroid gland to ionizing radiation (IR), during childhood may lead to papillary thyroid carcinoma (PTC) associated with *RET/PTC* oncogene rearrangement. IR exposure induces a transient "oxidative burst" through radiolysis of water, which can cause DNA damage, including DNA double-strand breaks (DSBs) and consequently chromosomal aberrations and H₂O₂ mediates part of the radiation effects.

Objective: The aim of this study is to investigate the implication of H₂O₂ in the generation of *RET/PTC1* rearrangement after X-Ray exposure.

Results: We developed a highly specific and sensitive nested RT-PCR method to detect *RET/PTC1* rearrangement. We used the human thyroid cell line HTori-3 that produces *RET/PTC1* rearrangement after g-irradiation; H₂O₂ generated during a 5 Gy X-ray irradiation causes DNA DSBs and contributes to *RET/PTC1* formation. Pretreatment of cells with catalase, a scavenger of H₂O₂ significantly decreased *RET/PTC1* rearrangement formation. Finally, *RET/PTC1* chromosomal rearrangement was detected in HTori3.1 cells after exposure of cells to H₂O₂ (25 µM), at a concentration that did not affect the cell viability.

Conclusion: This study demonstrates for the first time that H₂O₂ is able to cause *RET/PTC1* rearrangement in thyroid cells and might play an important role in the initiation of papillary thyroid carcinoma. Any oxidative stress can be responsible for the occurrence of *RET/PTC1* rearrangement found in thyroid lesions even in the absence of radiation exposure.

Rabii Ameziane-El-Hassani and Myriem Boufraquech contributed equally to this study.

Sources of Research Support: Electricité de France (EDF); Association pour la Recherche sur le Cancer (ARC); Ligue Contre le Cancer (comité du Val-de-Marne) and Agence Nationale de la Recherche (ANR).

Nothing to Disclose: RA-E-H, MB, OL-C, UW, MT, DM, FC, J-MB, MEM, MS, CD

OR22-5

CD97 Promotes Cancer Progression in a Murine Model of Follicular Thyroid Cancer.

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CD97 is a member of the adhesion family of G-protein coupled receptors (GPCR) and is normally expressed at high levels constitutively on myeloid cells. In some epithelial cancers CD97 levels have been positively correlated with dedifferentiation and malignant grade. Although CD97 is absent in normal thyroid epithelial cells, it has been demonstrated that CD97 expression increases during thyroid cancer dedifferentiation and progression to anaplasia. However, there is no previous evidence of a direct function of CD97 in cancer progression. To address the question of a potential role of CD97 in thyroid cancer development and progression, we generated a CD97 transgenic mouse model constitutively expressing human CD97 in the follicular epithelial cells of the thyroid and crossed this CD97 transgenic mouse with the TR β ^{PV/PV} mouse. The TR β ^{PV/PV} mouse spontaneously develops follicular thyroid adenoma with pathological progression to carcinoma, which has similarities to human cancer. Studies have indicated that the TR β ^{PV/PV} mouse is a valid preclinical mouse model to identify potential molecular targets for the treatment of thyroid cancer. We showed that constitutive expression of CD97 in the follicular epithelium can promote thyroid cancer progression. TR β ^{PV/PV} mice expressing CD97 exhibited increased occurrence of vascular invasion, a correlate of progression to carcinoma, and lung metastasis with a more aggressive histology than the TR β ^{PV/PV} mice without CD97. Furthermore, we showed that the expression of CD97 in TR β ^{PV/PV} mice increased ERK phosphorylation suggesting that CD97 may drive cancer progression by activating this pathway to increase tumor cell migration and invasion. In addition, constitutive CD97 expression resulted in higher Ki67 indicating that CD97 also plays a role in increasing proliferation. The present study provides in vivo evidence for a direct role of CD97 in thyroid cancer progression, suggesting that this G-protein coupled receptor may be an important target for chemotherapeutic intervention of thyroid cancer.

Nothing to Disclose: YW, RGL, PM, CL, SC, KK

OR22-6

In Vivo Imaging of Mesenchymal Stem Cell Recruitment into the Tumor Stroma of Hepatocellular Carcinoma (HCC) Using the Sodium Iodide Symporter as Reporter Gene.

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The ability of mesenchymal stem cells (MSC) to specifically home to tumors has suggested their potential use as a delivery vehicle for cancer therapeutics. Tracking the delivery and engraftment of MSCs into human tumors is a crucial prerequisite for safety and effectiveness of MSCs as possible gene delivery vehicles. Due to its dual role as reporter and therapy gene the sodium iodide symporter (NIS) allows direct, non-invasive imaging of functional NIS expression by ¹²³I-scintigraphy and ¹²⁴I-PET-imaging, as well as exact dosimetric calculations before proceeding to therapeutic application of ¹³¹I, and thereby provides an ideal means to elucidate the capacity of MSCs for tumor targeting of therapeutic genes.

In the current study we stably transfected immortalized human bone marrow derived CD34- MSCs with a NIS-expressing plasmid (CMV-NIS-pcDNA3) (NIS-MSC) and analyzed functional NIS expression by iodide uptake assay and Western blot analysis. In addition, cytotoxicity of ¹³¹I was examined *in vitro* using a clonogenic assay. In a HCC xenograft model (Huh7) we further investigated distribution and tumor recruitment of NIS-MSCs by ¹²³I scintigraphy.

NIS-MSCs revealed a 12-fold increase in perchlorate-sensitive iodide uptake activity as compared to wild-type MSCs. Further, Western blot analysis confirmed NIS protein expression in NIS-MSCs. In mixed populations of NIS-MSC and HCC cells (ratio 1:1) a clonogenic assay showed a 40% reduction of survival in HCC cells after application of 23.7 MBq ¹³¹I induced by the crossfire effect of ¹³¹I. After establishment of subcutaneous Huh7 xenografts in nude mice, NIS-MSCs were injected via the tail vein and MSC distribution was analyzed by gamma camera imaging after i.p. injection of 18.5 MBq ¹²³I. MSCs were actively recruited into the tumor stroma as shown by tumor-selective iodide accumulation (9.5% ID/g ¹²³I, biological half-life of 4h). Immunohistochemistry and *ex vivo* ¹²³I biodistribution analysis by gamma counter analysis confirmed active recruitment of NIS-MSCs into the tumor stroma while other organs like liver, lung or kidneys showed no significant MSC recruitment and no or only mild iodide accumulation.

In conclusion, our results demonstrate selective recruitment of NIS-expressing MSCs into HCC tumors resulting in induction of tumor-specific iodide accumulation. These results open the exciting prospect of tumor-specific NIS-mediated radionuclide therapy of extrathyroidal tumors using MSCs as gene delivery vehicles.

Nothing to Disclose: KK, MK, CZ, KK, MJW, NW, DD, CZ, FJG, BG, EW, PN, CS

OR23-1

Progesterone Receptor Hinge Region Regulates the Kinetics of Transcriptional Responses through Phosphorylation, Acetylation, and Nuclear Retention.

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Progesterone receptors (PR) are critical regulators of growth during mammary gland development and contribute to breast cancer progression. Post-translational modifications of PR alter receptor function by modulating hormone responsiveness at selected promoters. Recently, an acetylation consensus sequence, KXXX (aa 638-641), was identified in the PR hinge region. Site-directed mutagenesis demonstrated that upon hormone binding, PR is acetylated within this lysine-rich region. Using T47D cells stably expressing either wt or acetylation-deficient (K-A) PR-B, we observed a stark reduction in hormone-induced Ser345 and Ser400 phosphorylation relative to wt PR-B at early time points. However, by 4 hrs, phosphorylation of these sites equaled that of wt PR-B. We thus created mutations that mimic acetylation (K-Q or K-T). Interestingly, similar to K-A PR, PR acetylation mimics (K-Q or K-T) displayed delayed phosphorylation and nuclear entry/retention relative to wt PR-B, indicative of disruption of PR nuclear-cytoplasmic shuttling conferred by the hinge region. Quantitative PCR demonstrated that wt PR-B, but not K-mutant PRs, induced c-myc at 1 hr of progestin treatment. However, at 6 hrs of treatment, c-myc induction was comparable to the levels induced by wt PR-B, coinciding with the lag-time for hormone-dependent nuclear retention. These data demonstrate that the precise timing of PR phosphorylation and nuclear retention are critical for cells to rapidly initiate robust transcriptional programs in response to hormone. In contrast to c-myc, SGK displayed sensitivity to PR acetylation, but not nuclear entry. Namely, acetylation-deficient (K-A) mutant PR-B up-regulated SGK mRNA relative to wt in response to progestin. However, K-Q and K-T acetylation mimics of PR only weakly induced SGK expression, well below the levels induced by wt PR-B and independently of nuclear retention. These data reveal the ability of PR acetylation to alter the magnitude of transcriptional response at selected (slow-response) promoters (SGK), while the hinge region appears to dictate the kinetics of the transcriptional response to hormone at other (rapid-response) promoters (c-myc). In sum, the PR hinge region is multifunctional. Understanding the ability of this region to couple acetylation, phosphorylation events, and nuclear entry/retention may provide clues to the mechanisms of differential regulation of hormone responsiveness.

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Nothing to Disclose: ALG, ARD, LMC, CJH, CAL

OR23-2

Dual Function of FOXA1 in Regulation of Androgen Receptor Binding to Chromatin.

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Androgen receptor (AR) regulates distinct transcriptional programs in a cell- and tissue-specific fashion and is of prime importance in both androgen-dependent and castration-resistant prostate cancer (1). AR action is mediated in collaboration with other transcription factor networks. Our genome-wide analysis of AR-binding sites (ARBs) in androgen-responsive prostate cancer (LNCaP-1F5) cells by ChIP-sequencing revealed FOXA1-binding site as the most over-represented cis-element in close proximity to the ARBs. FOXA1 acts as a pioneer factor in the recruitment of ER α binding to chromatin and facilitates the ability of estrogens to activate transcription (2, 3). On the other hand, FOXA1 is also known to recruit Groucho/TLE/Grg corepressor complexes to genomic sites to generate compact chromatin (4). To understand the relationship between ARBs and FOXA1 occupancy, we used ChIP-sequencing to map FOXA1 binding to LNCaP-1F5 cell chromatin and identified some 22,000 high-confidence FOXA1-binding sites. Most of the ARBs (72%) overlapped with FOXA1-binding sites, lending credence to the role of FOXA1 as pioneer factor in androgen signaling. However, depletion of cells from FOXA1 by siRNA resulted in a dramatic increase (>2-fold) in the number of ARBs. The overlap of the ARBs in parental cells to those in FOXA1-depleted cells was about 55%, i.e., close to one-half of the original ARBs disappeared subsequent to FOXA1 depletion. These results imply that there are three classes of ARBs in LNCaP-1F5 cells: (i) the sites that need FOXA1 for binding to chromatin, (ii) the sites that are independent of FOXA1, and (iii) the sites the accessibility of which is masked by FOXA1 and that appear upon FOXA1 depletion. Therefore, FOXA1 is envisioned to act both as a pioneer factor and as a repressor generating compact chromatin structure that is inaccessible to AR binding. Our expression profiling results support this conclusion, in that FOXA1 depletion both modulated the magnitude of androgen response by a number of genes (e.g., KLK3, FKBP5, TMPRSS2, and SGK1) and permitted expression of a new set of genes upon androgen exposure. Androgen regulation of these latter genes was commensurate with the appearance of new ARBs in their regulatory regions in FOXA1-depleted cells. Collectively, the present work has revealed important new insights into the role of FOXA1 in androgen signaling and AR function.

(1) Wang Q et al., Cell 2009; 138:245

(2) Carroll JS et al., Cell 2005; 122:33

(3) Lupien M et al., Cell 2008; 132:958

(4) Sekiya Y, Zaret KS, Mol Cell 2007; 28:291

Sources of Research Support: Academy of Finland, Sigrid Juselius Foundation, and European Union (CRESCENDO).

Nothing to Disclose: BS, ML, SH, OAJ

OR23-3

Adipose-Specific Knockout of Androgen Receptors in Male Mice Results in Altered Adipose Gene Expression, Hyperinsulinemia and Hypertriglyceridemia without Obesity.

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BACKGROUND: Visceral fat is a key factor underlying type 2 diabetes. The amount and distribution of body fat is strongly influenced by sex steroids. Androgen receptors (ARs) are present in adipose tissue and are more abundant in the more detrimental visceral bed than other adipose depots (1). Here, we sought to determine the contribution of AR in adipose tissue to the pathophysiology of visceral obesity and type 2 diabetes.

METHODS: Male fat-specific AR-knockout (fARKO) mice (12 weeks; n=12 each group) were created by breeding floxed-AR mice (2) with adipose-specific FABP4-Cre mice. Glucose homeostasis was assessed by intraperitoneal glucose tolerance test (IPGTT, 2mg glucose/g body weight) following a 6hr fast. At cull, tissues were weighed and snap-frozen. mRNA expression was assessed by real-time PCR. Data are mean \pm S.E.M and were analysed by Student's t-tests.

RESULTS: AR mRNA expression was specifically and significantly decreased (by 80%) in fARKO adipose tissue compared to floxed-AR controls. Knockout of AR in adipose tissue did not alter plasma testosterone levels. fARKO mice were lighter (27.9 ± 0.38 vs 30.1 ± 0.28 g, $p < 0.001$), had decreased epididymal fat (9.9 ± 0.5 vs 12.0 ± 0.8 mg/g body weight, $p < 0.05$) and increased intrascapular brown adipose tissue (5.1 ± 0.27 vs 4.1 ± 0.28 mg/g body weight, $p < 0.05$) compared to floxed-AR controls. fARKO mice had normal fasting plasma glucose levels and NEFA suppression but showed elevated plasma insulin after fasting (0.7 ± 0.06 vs 0.5 ± 0.04 ng/ml, $p < 0.05$) and 90-minutes post-IP glucose bolus (0.8 ± 0.07 vs 0.6 ± 0.06 ng/ml, $p < 0.05$). Liver triglycerides were unchanged in fARKO mice (11.8 ± 0.4 vs 11.3 ± 0.4 nmol/mg), however plasma triglycerides were significantly increased (1.5 ± 0.2 vs 1.1 ± 0.1 mg/dL, $p < 0.05$). Plasma and adipose tissue leptin levels were not different in fARKO mice however the mRNA profile of fARKO white adipose tissue compared to floxed-AR mice revealed significant increases in resistin (2-fold, $p < 0.005$), TNF α (2-fold, $p < 0.005$) and hormone sensitive lipase (4-fold, $p < 0.005$).

CONCLUSIONS: Ablation of adipose AR in mice results in altered adipose gene expression and produces hyperinsulinemia suggesting that fARKO mice are insulin resistant in the absence of obesity. These findings highlight a specific role for androgen action in adipose in the pathophysiology of insulin resistance and we anticipate that therapeutic manipulation of androgen action at the level of the adipose tissue may improve insulin sensitivity.

(1) Bjorntorp P, International Journal of Obesity 1991; 15: 67-81

(2) De Gendt et al., Proceedings of the National Academy of Sciences, U.S.A 2004; 101: 1327-1332

Sources of Research Support: KJM is the recipient of a Diabetes UK RD Lawrence Fellowship.

Nothing to Disclose: KJM, LBS, PTKS, RA, BRW

OR23-4

Expression of Glucocorticoid Receptor β in Mice and Its Potential Role To Increase Lipid Storage and Insulin Sensitivity.

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Glucocorticoids (GC) are well-known antagonists of insulin and long-term GC treatment results in insulin resistance. These GC effects are mediated by the glucocorticoid receptor (GR), specifically the GR α isoform. Sequencing of human GR cDNAs revealed two GR isoforms, α and β , that result from alternative splicing of exon 9. Humans are the only mammals known to produce GR β . Human GR β exhibits a degenerate amino acid sequence in helix 12 that abrogates ligand-binding and acts as a dominant-negative inhibitor of hGR α . Therefore, the quantity of hGR β expressed can determine sensitivity of cells and tissues to glucocorticoids. Most studies of hGR β have used the immune system and indicate that ascending levels of hGR β correlate with severity of disease, especially states of GC-resistant inflammation. To date, efforts to establish a mouse model of GR β based on alternative splicing of exon 9 have been unsuccessful. In this study, we show that mice generate GR β as a result of alternative splicing of intron 8. All functional properties of mGR β so far tested are shared with hGR β , including structure and antagonism of GR α . We also demonstrate hormonal and dietary control of mGR β expression. In vitro, upregulation of mGR β was observed in response to dexamethasone, inflammatory cytokine TNF α and insulin. In vivo studies to confirm the involvement of mGR β in metabolism showed significantly elevated levels of mGR β in the livers of mice subjected to fasting-refeeding. Mice on a long-term high-fat diet developed insulin resistance with a significant increase in hepatic and visceral adipose mGR β and TNF α . We propose that inflammatory states in obesity are a result of GC-resistance, and that mGR β may work to increase lipid storage in both liver and visceral adipose by inhibiting the actions of GR α . This study has generated the much-needed mammalian model of GR β and has uncovered a potential new role for GR β in metabolism and insulin sensitivity.

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Nothing to Disclose: TDH, SR, HAC, SMN, ERS

OR23-5

Mice Lacking FXR and SHP Accumulate Less Body Fat and Are Resistant to Diet Induced Obesity.

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Genetic makeup, feeding habits and physical activity are key determinants for obesity. Nuclear receptors, Farnesoid X Receptor (FXR) and Small Heterodimer Partner (SHP), play important roles in cholesterol and lipid metabolism. To examine the combinatorial impact of FXR and SHP in body weight maintenance we generated FXR/SHP double knockout (DKO) mice. DKO mice consistently weigh 10-15% less than the WT or individual FXR or SHP knockout mice. We examined the DKO mice and determined two key reasons for their lean phenotype. One, they utilize more fat than the WT or individual knockout mice as determined by calorimetry and by the observed increase in in vivo lipolysis. This is strengthened by increases in adiponectin, a hormone that inversely correlates with fat levels, and decreases in leptin levels. The increase in CPT1 and PGC1 alpha expression strongly supports increased fatty acid oxidation. These changes are consistent such that DKO mice accumulate much less visceral fat over their lifetime, averaging about 50% of WT mice. Second, their food intake is about 30% lower but monitored physical activity is higher than WT animals setting up an ideal lean phenotype environment. Additionally, 45% high fat diet did not cause obesity in DKO animals. In fact DKO gained 70% less weight than the WT animals. These data for the first time uncover a critical combined role for FXR and SHP in holistic regulation of body weight.

Nothing to Disclose: SA, MJF, DDM

OR23-6

Estrogen Modulates Metabolic Pathway Adaptation to Available Glucose in Breast Cancer Cells.

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In cancer cells and tumors ATP production occurs predominantly via glycolysis unlike normal cells which utilize mostly oxidative phosphorylation. Altered metabolic phenotype is one of the key features associated with breast cancer progression and little to no information exists on the role of estrogen in the metabolic adaptation of these cells. Differential glucose concentrations within the tumor microenvironment can cause a shift towards glycolysis or oxidative phosphorylation. We hypothesized that breast cancer cells switch between metabolic processes depending on glucose availability and that estrogen may modulate this transition. MCF-7 breast carcinoma cells were cultured in DMEM supplemented with high glucose (normal culture media concentration - 4500 mg/L) or low glucose (1000 mg/L). Cells were switched to low glucose media one week prior to assays in order to study chronic effects. The low glucose environment resulted in an increase in the basal activity levels of pyruvate dehydrogenase (PDH) and an increase in citrate levels, both evidence of enhanced activity of the citric acid (TCA) cycle. Conversely an increase in basal lactate levels occurred in high glucose cells compared to the low glucose cells indicating enhanced glycolysis. Estrogen (15 minutes) stimulated PDH in a dose dependent (0.01 - 10 nM) manner in low glucose cells only. Estrogen treatment (15 minutes) of low glucose exposed cells produced a dose dependent decrease in lactate production but an increase in high glucose cells. Increased lactate production in the high glucose environment correlated to a shift towards glycolysis augmented by estrogen. Estrogen drives oxidative phosphorylation as a compensation for low glucose availability seen as an increase in PDH activity. Estrogen induced a rapid increase (1 nM, 2 hours) in ATP production in the low glucose environment blocked by the ER antagonist ICI 182,780, with no change in high glucose cells. ATP induction was increased in response to the specific ER α /ER β agonists PPT and DPN demonstrating involvement of both receptor types. In summary, estrogen differentially targets metabolic components in high and low glucose environments in ER positive breast cells to ensure substrate for fatty acid oxidation or ATP availability. Compensation by estrogen may be a rescue effect in overcoming a decrease in available glucose.

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Nothing to Disclose: FO, MR, AP, BJH, ERL

OR24-1

Achieving Tight Glycemic Control in Critical Care without Precipitating Life-Threatening Hypoglycemia: The VA Pittsburgh Experience.

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The question of mortality benefit from aggressive glycemic intervention in critical care cannot be answered until and unless it becomes possible to achieve and maintain tight glycemia control without causing life-threatening hypoglycemia (Blood Glucose[BG]<40mg/dl). Although it is not known if this seemingly inevitable accompaniment contributes to a failure of aggressive intervention to show a significant mortality benefit in critical care, a five-fold increase in incidence of BG<40mg/dl (13.7% for tight vs 2.5% for conventional control[1]) is a major deterrent to such aggressive intervention.

We have used a Glycemic Expert System for Nurse Implemented Euglycemia (GENIE©) for the past 5 years to maintain BG between 80-110mg/dl in 980 patients undergoing cardiac surgery. In contrast to other algorithms, most of which are two-dimensional linear or curvilinear models of dose vs BG, and/or change in BG, GENIE© calculates and fractionates total insulin dose into bolus and infusion doses from a multidimensional decision-surface that uses a concept we term "excess glucose" and is a composite of several variables, including BG level, observed BG response, "distance" to target, and desired change. GENIE© also calibrates retest times to observed response and has a seamlessly integrated hypoglycemia prevention algorithm.

Mean BG on GENIE© was 110mg/dl±0.3[SE], with a median of 109mg/dl[95%CI of 2], and BG<110mg/dl was achieved in 95% of patients within 4h. Stability of glycemic control is reflected in the time-weighted excess glucose above 140 (TWEG₁₄₀, calculated as the integrated area under the curve of BG>140mg/dl over time). Mean TWEG₁₄₀ was 32±8 mg.dl⁻¹.hr (corresponding to, on average, 3 readings of 151 mg/dl over the mean time on protocol of 31h), while median TWEG₁₄₀ was only 3 mg.dl⁻¹.hr[CI 13], indicating that BG never exceeded 143mg/dl on GENIE© in 50% of patients, and did not exceed 156mg/dl in 95%.

Despite such aggressive maintenance of euglycemia, there was not even one instance of BG<40mg/dl in 5 years, contrasting with a prior incidence of 6%. In addition, since implementation of GENIE©, there have been no deaths from deep sternal wound infections (historically ~3/yr). We conclude that it is possible to safely achieve and maintain euglycemia in patients after cardiac surgery without precipitating life-threatening hypoglycemia, thus making it feasible to determine if aggressive control is truly beneficial in the absence of such hypoglycemia.

[1] Wiener RS et al., JAMA 2008; 300:933

Nothing to Disclose: RHR, CAC, PLP

OR24-2

Roux-En-Y Gastric Bypass Surgery in T2DM Patients Is Superior to Low Calorie Diet in Improving Insulin Sensitivity and Beta-Cell Function Despite Equivalent Weight Loss.

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Background: Improved glycemic control after RYGB may be due to changes in food-associated gut hormone signals that occur independent of weight loss. This study investigates whether effects unique to RYGB improve insulin sensitivity and beta cell function independent of simultaneous enteral nutrient passage.

Methods: Insulin-supplemented fsIVGTT was performed before and after equivalent weight reduction achieved by either low calorie liquid diet of 800 kcal/day (LCD, n=7) or RYGB (n=7) in obese T2DM subjects. MINMOD analysis included glucose effectiveness (Sg), insulin sensitivity (Si), and insulin secretion normalized to the degree of insulin resistance (DI). Acute insulin response to glucose (ACRg) was assessed by the relative C-peptide increase 3-5 min after glucose injection. Fasting plasma levels of gut hormones and adiponectin were quantified.

Results: Baseline weight and glucostatic parameters were comparable in both groups; HbA1c was 7.8±1.4 and 7.5±1.1% in the LCD and RYGB group, respectively. Weight loss (8.1±0.7%) occurred faster after RYGB (3.4±0.3 vs. 8.1±0.5 wks in RYGB vs. LCD; P<0.001). All antidiabetic medication was discontinued in RYGB patients by the time of the second fsIVGTT, contrasting with 55% reduction of medication in the LCD group (P<0.05). While Sg was unaltered in either group, Si increased significantly in the RYGB group from 1.22±0.35 to 1.86±0.33 (mU/L)⁻¹×min⁻¹ (P<0.01), but not in the LCD group (1.38±0.50 to 1.8±0.37 (mU/L)⁻¹×min⁻¹; P=n.s.). DI significantly improved in both groups, but the absolute increase was greater in the RYGB group (258.2±86.6 vs. 55.9±19.9 in the LCD group; P<0.05). ACRg was significantly improved in RYGB from 8.4±4.3 to 57.3±19.9 (P<0.05), with only a trend in the LCD group (5.0±4.5 to 17.9±7.9; P=n.s.). Neither intervention significantly affected fasting gut hormone (ghrelin, GLP-1, PYY) levels. In line with increased insulin sensitivity, there was a significant increase in adiponectin only after RYGB (2548±252 to 3811±444 ng/ml; P<0.01), and not after LCD (2530±443 to 3105±480 ng/ml; P=n.s.).

Conclusion: Insulin sensitivity and adiponectin levels, as well as beta cell function in diabetic RYGB patients showed better improvement compared with LCD patients, despite equivalent weight loss and cessation of anti-diabetic medications. These data reaffirm a beneficial glucostatic effect of RYGB in T2DM patients that occurs independent of acute enteral nutrient passage and fasting gut hormone levels.

Sources of Research Support: NIH Grant DK072011 awarded to JK; NIH RR00645.

Disclosures: MB: Clinician, Ethicon, Covidien; Consultant, Covidien. WI: Investigator, Covidien; Consultant, Covidien. JK: Investigator, Covidien.

Nothing to Disclose: LP, GF, LA, EK

OR24-3

On the Resolution of Diabetes Following Bariatric Surgery: The Role of Incretins.

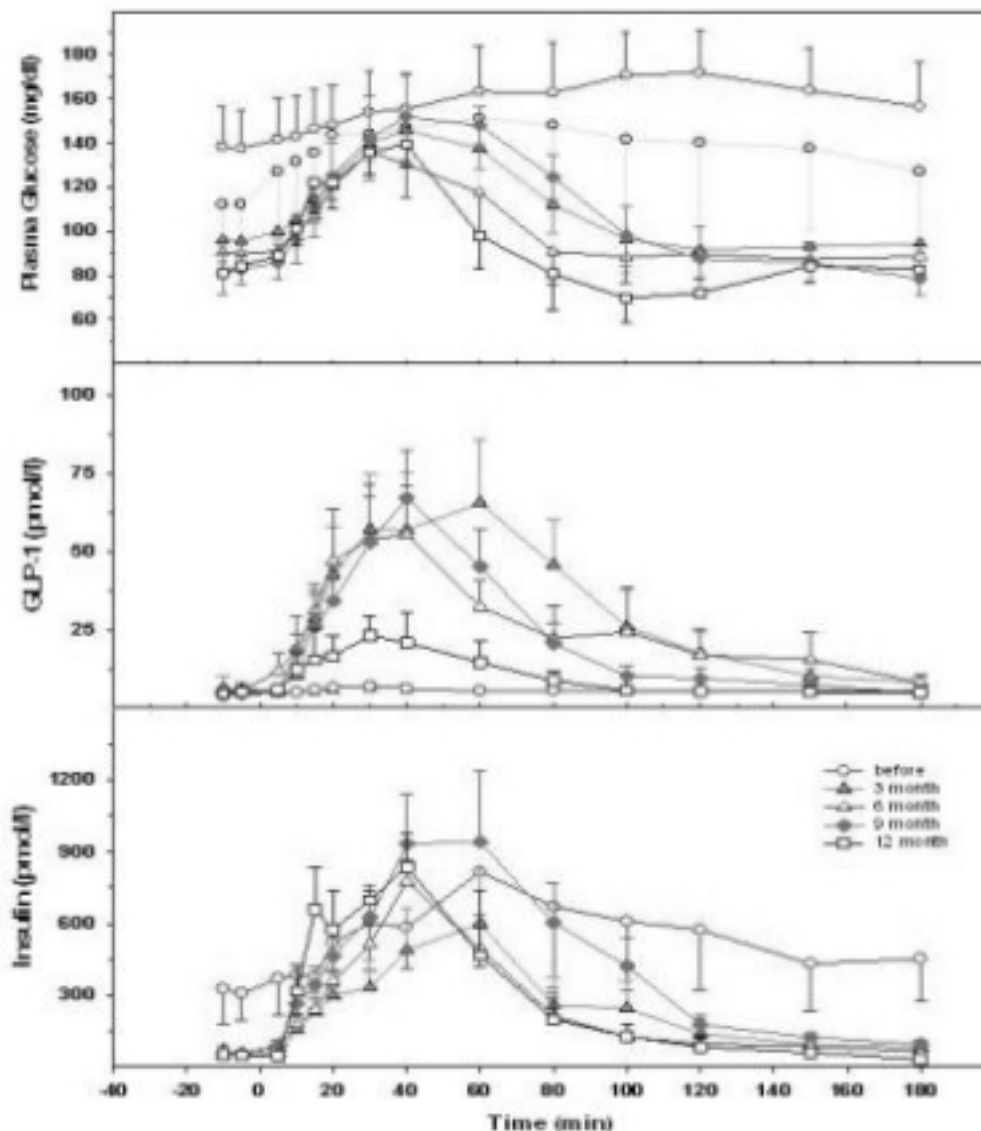
A Rabiee MD¹, JT Magruder BS¹, TH Magnuson MD¹, AO Lidor MD¹, KE Steele MD¹, MD Schweitzer MD¹, DK Andersen MD¹ and D Elahi PhD¹.

¹Johns Hopkins Univ Sch of Med Baltimore, MD.

Introduction: The mechanism of the prompt resolution of Type 2 diabetes (T2DM) after bariatric surgery remains unclear.

Methods: We evaluated 8 patients before and at 1, 3, 6, 9, and 12 months after Roux-en-Y gastric bypass (RYGB) with a standardized test meal (STM) to assess incretin responses, a euglycemic clamp to assess sensitivity to insulin, a hyperglycemic clamp with glucagon-like peptide-1 (GLP-1) infusion to assess beta-cell sensitivity, and body composition measurements.

Results: Fasting plasma glucose was reduced from 136 ±15 mg/dl pre-op to 114±41 at 1 month, to 96±3 at 3 months. Fasting insulin levels decreased from 326±149 pmol/l pre-op to 67±20 at 3 months but the insulin response to STM did not resume a normal pattern of release until 6 months after RYGB. GLP-1 responses to STM were negligible pre-op, but increased dramatically by 3 months to levels which were 3-4 fold normal, and remained high for at least 9 months.



Insulin sensitivity was impaired pre-op, and did not improve until 3 months, when % body fat began to decline. Beta-cell sensitivity to glucose and GLP-1 remained low for 9 months after RYGB. **Conclusion:** GLP-1 hypersecretion, and not fat loss, is largely responsible for early improvements in glucose metabolism after RYGB. Over time, insulin sensitivity and beta-cell sensitivity normalize and GLP-1 responses return toward normal. Both insulinotropic as well as insulinomimetic actions of GLP-1 likely contribute to the normalization of glycemia. The resolution of T2DM is best served by procedures such as RYGB which result in early enhanced incretin responses.

Nothing to Disclose: AR, JTM, THM, AOL, KES, MDS, DKA, DE

OR24-4

The Combination of a Long Acting GLP-1 Analog (E-XTEN; VRS-859) and Long Acting Glucagon (Gcg-XTEN; AMX-808) for Superior Weight Loss and Glycemic Control in Treatment of Type 2 Diabetes and Obesity.

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¹Versartis, Inc Mountain View, CA and ²Amunix, Inc Mountain View, CA.

Previous studies of a VRS-859, exenatide-XTEN (E-XTEN), demonstrated comparable weight loss and glycemic control to exenatide in mouse models. The long half-life of VRS-859 (60 hr in monkeys) provides a monthly dosage option in humans. Agonism of both the GLP1 and glucagon receptor may increase weight loss and glycemic control in type 2 diabetes and obesity. A novel glucagon-XTEN (Gcg-XTEN; AMX-808) construct was developed to allow weekly dosing of glucagon. The unique properties of XTEN, a hydrophilic tail of natural amino acids, allow these products to be combined and their doses and pharmacokinetic profiles to be adjusted to optimize the efficacy and safety. In diabetes induced obese (DIO) mice, VRS-859 was administered either every 2 days or 4 days along with AMX-808 administered BID or QD, respectively. Combination treated DIO mice had an approximate 25% weight loss (Fig 1) and 40% decrease in fasting blood glucose (Fig 2) after 28 days of treatment, and these changes were significant compared to the single agents or placebo. The combination treatment groups achieved glycemic control, increased insulin sensitivity, and significant reduction in cholesterol and triglycerides at the end of the study. The data suggest that the combination of the two products may represent a convenient and effective treatment for type 2 diabetes and obesity.

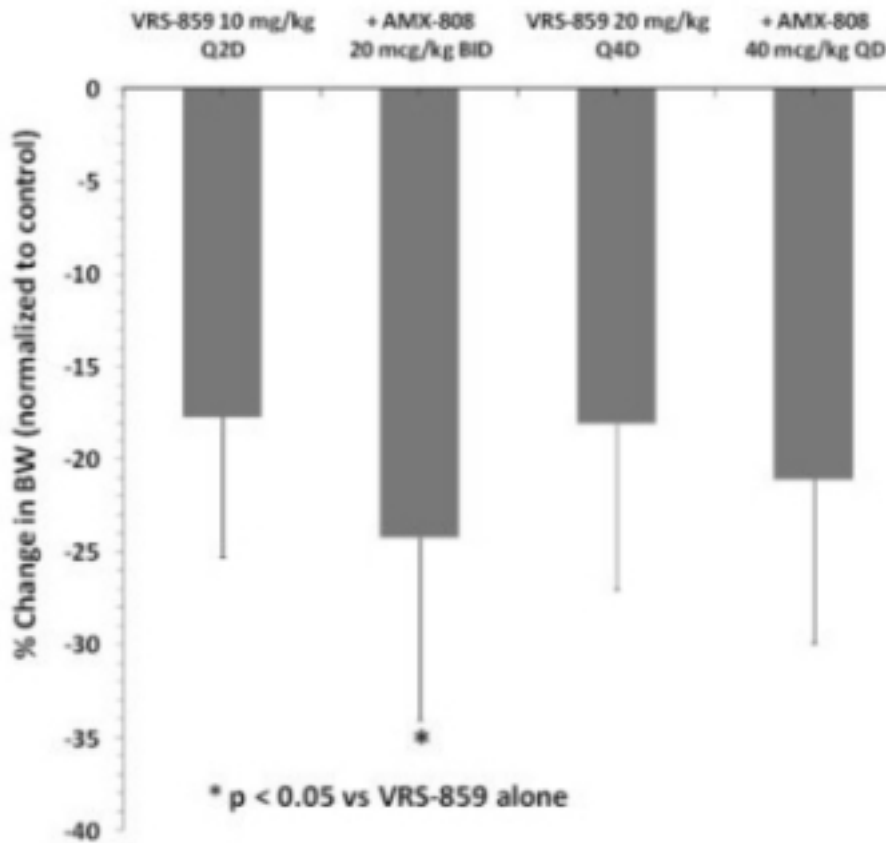


Figure 1. Weight loss due to combination therapy

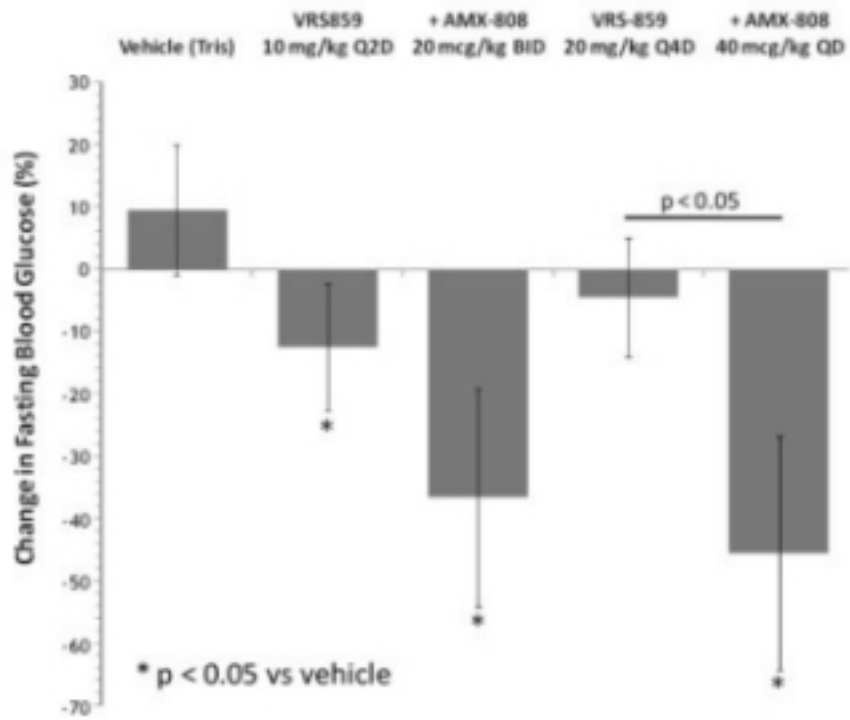


Figure 2. Improvement in glycemic control due to combination therapy

Nothing to Disclose: JLC, NG, WT, BS, LL, YY, JS

OR24-5

Effectiveness of GSK1362885, a Novel Glycogen Phosphorylase Inhibitor, Demonstrated in Single Dose Suppression of a Glucagon Stimulated Glucose Rise in Healthy Volunteers.

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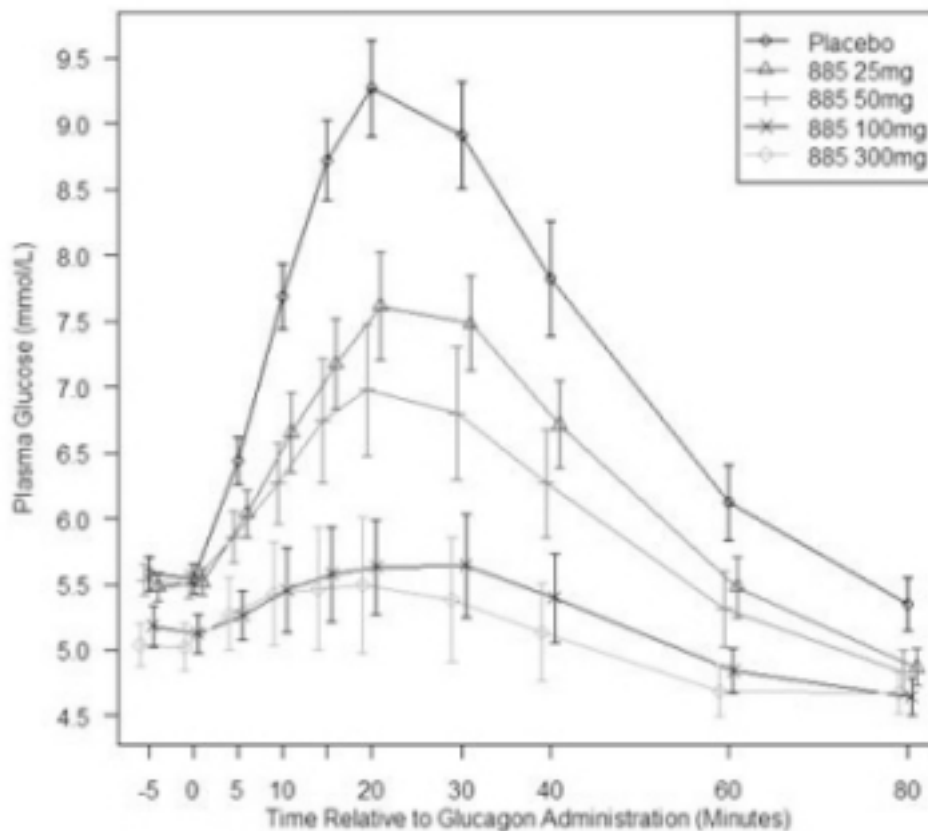
Background: GSK1362885 is a novel glycogen phosphorylase inhibitor that may reduce hepatic glucose output (HGO) by inhibiting glycogenolysis. Preclinical studies showed evidence of significant suppression of glycogenolysis with reduced glucose following glucagon challenge (GC) in rats, and reduced fasting glucose over 3 weeks in marmosets. Glucagon is a rapid activator of glycogenolysis in humans (1). Reduction of glycogenolysis may offer an approach to reduce HGO that is distinct from metformin, which inhibits gluconeogenesis.

Objectives: This study was designed to evaluate *in vivo* efficacy and PK/PD relationships by administering glucagon to healthy subjects after dosing with GSK1362885 (2).

Methods: Initial dose selection for the GC was based on safety and PK/PD data from previous single dose escalation cohorts. Doses were continually updated by fitting exposure-response models to emerging data. GC methodology was based on a study by Lockton (1). Healthy subjects (n=12) were randomized to receive 4 treatments once weekly over 4 weeks, placebo or a single oral dose of GSK1362885 (25, 50, 100, or 300mg), followed by a 0.5mg IV bolus of glucagon, dosed 100 minutes post-oral dosing to coincide with the T_{max} of GSK1362885. PK and glucose profiles were obtained, and baseline-corrected AUC(0-20min) values were computed to determine PK/PD relationships.

Results/Conclusions: GSK1362885 was safe and generally well-tolerated across all groups. Adverse events were mild to moderate. A clear dose-dependent suppression of glucose profiles was observed, with near complete suppression, median > 90%, of the baseline-corrected glucose AUC(0-20min) at doses of 100 and 300mg compared to placebo (Figure 1). The full exposure-response curve was described well by a sigmoidal E_{max} PK/PD model. These data clearly demonstrated *in vivo* efficacy of GSK1362885. Thus, the novel use of GC methodology in this study allowed for efficient demonstration of GSK1362885 efficacy and supports dose selection for future studies in T2DM subjects.

Figure 1: Dose-dependent suppression of mean glucose profiles with GSK1362885 following glucagon administration



(1)Lockton J et al., J Pharm and Tox Methods 2007;55:86-90

(2)ClinicalTrials.gov Identifier:NCT00823940

Disclosures: LSV: Employee, GlaxoSmithKline. JL: Employee, GlaxoSmithKline. JSS: Employee, GlaxoSmithKline. RLB: Employee, GlaxoSmithKline. SKS: Principal Investigator, GlaxoSmithKline. SAT: Employee, GlaxoSmithKline. FT: Clinical Researcher, GlaxoSmithKline. CC: Clinical Researcher, GlaxoSmithKline. RVC: Employee, GlaxoSmithKline.

OR24-6

LX4211, an SGLT2 Inhibitor, Shows Rapid and Significant Improvement in Glycemic Control over 4 Weeks in Patients with Type 2 Diabetes Mellitus.

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In patients with type 2 diabetes mellitus (T2DM), maintenance of lower blood glucose concentrations is associated with fewer microvascular complications. LX4211 is a once-daily, orally-administered small molecule inhibitor of the sodium glucose cotransporter type 2 (SGLT2). SGLT2 inhibitors provide improvement in glycemic parameters by blocking glucose reabsorption by the kidney, which results in glucose loss in the urine. Since SGLT2 inhibitors do not rely on insulin secretion or action, they would be expected to spare marginally functioning pancreatic beta cells, thus potentially postponing the time when patients require insulin therapy. A Phase 1 clinical study with LX4211 showed dose-dependent increases in 24-hour urine glucose excretion.

In the Phase 2 study, patients (N=36) with T2DM received one of two oral doses of LX4211 monotherapy, given as 150 mg or 300 mg once daily, or matching placebo, for 28 days. Preliminary data showed significant and sustained glucosuria, with rapid and significant improvements in multiple parameters of glycemic control over the 28-day dosing period for both dose levels when compared to placebo (Table). Adverse events were generally mild and evenly distributed across all dose groups, including placebo, with no evidence of dose-limiting toxicities observed.

Table

| Mean Change from Baseline, Treatment Group vs Placebo (*p ≤ 0.01) | LX4211 150 mg (n=12) | LX4211 300 mg (n=12) | Placebo (n=12) |
|---|----------------------|----------------------|----------------|
| Plasma Fructosamine (μmol/dL) | -24.5* | -24.9* | +18.2 |
| 2-Hour Postprandial Test - Glucose (mg/dL) | -50.6* | -57.6* | -8.0 |
| HOMA R-Value | -0.7* | -1.1* | +2.2 |

In addition, 50% of patients treated with LX4211 achieved fasting plasma glucose (FPG) < 120 mg/dL, compared to 0% placebo-treated (p = 0.006) and 33% of patients treated with LX4211 achieved FPG < 105 mg/dL, compared to 0% placebo-treated (p = 0.037).

These results demonstrate that LX4211-induced glucosuria is accompanied by significant improvements in multiple assessments of glycemic control after only 4 weeks treatment in patients with T2DM. The improvements in glycemic control with LX4211 may reduce the risk of development and progression of hyperglycemia-associated complications in patients with T2DM and warrant further evaluation of LX4211 in this population.

Disclosures: JF: Employee, Lexicon Pharmaceuticals, Inc. DAR: Employee, Icon Development Solutions; Investigator, Lexicon Pharmaceuticals, Inc. KF: Employee, Lexicon Pharmaceuticals, Inc. KC: Employee, Lexicon Pharmaceuticals, Inc. AT: Employee, Lexicon Pharmaceuticals, Inc. MS: Employee, Lexicon Pharmaceuticals, Inc. DP: Employee, Lexicon Pharmaceuticals, Inc. JB: Employee, Lexicon Pharmaceuticals, Inc. BZ: Employee, Lexicon Pharmaceuticals, Inc. PB: Employee, Lexicon Pharmaceuticals, Inc.

OR25-1

A Negative Interference of Pregnant Serum in a GH Immunometric Assay Results in Marked Suppression of Growth Hormone Concentrations during both Normal and Acromegalic Pregnancies.

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GH levels decline progressively during normal pregnancy along with increasing concentrations of hPL and placental GH (pGH). The high homology between these hormones frequently cause GH assays to detect falsely high GH levels in pregnancy. Using a highly sensitive/specific GH immunometric assay (<0.01% crossreactivity with pGH and hPL), we have recently found, as expected, that GH levels progressively suppressed during normal pregnancy. Surprisingly, GH suppression was also observed in six acromegalic pregnancies (1). Our findings were in disagreement with previous data by RIA showing no GH suppression in two pregnant acromegalics (2). The interpretation was that both tumoral and normal GH-secreting cells exhibited the same sensitivity to the negative feedback of pregnancy. An alternative explanation is that a negative interference of pregnant serum resulted in falsely low GH levels. To test this hypothesis, GH was measured in samples from acromegalic men diluted with late normal pregnancy serum, and two standard curves with 22K GH with and without pGH at high concentration (4,000 µg/L) were compared. All dilutions of acromegalic sera with normal pregnancy serum resulted in marked suppression (range: -91.5% to -96.8%) of GH concentrations, (table below). Addition of excess 22K pGH to the standard curve decreased assay sensitivity but did not significantly change GH measurements. In conclusion, lack of crossreactivity of a GH immunometric assay for GH-related placental hormones (pGH and hPL), as previously demonstrated in our assay (1), is necessary but not sufficient to validate its use in pregnancy. The interference of pregnant serum in our assay resulted in falsely low GH values in acromegaly. This negative interference is probably due a GH-simile placental hormone that binds to the first but not to the second antibody, preventing the "sandwich" formation. Although 22K pGH was ruled out as the interfering hormone in this assay, other pGH isoforms and hPL remain to be tested.

Expected and measured GH values

| [GH] in acromegalic man serum (µg/L) (1) | [GH] in normal late pregnancy (µg/L) (2) | Dilution (1) + (2) V:V | Expected [GH] (µg/L) | Measured [GH] (µg/L) |
|--|--|------------------------|----------------------|----------------------|
| 8,22 | 0,02 | 1:1 | 4,11 | 0,29 |
| | | 1:2 | 2,74 | 0,16 |
| | | 1:4 | 1,64 | 0,11 |
| [GH] in acromegalic man serum (µg/L) (3) | [GH] in normal late pregnancy (µg/L) (4) | Dilution (3) + (4) V:V | Expected [GH] (µg/L) | Measured [GH] (µg/L) |
| 22,2 | 0,02 | 1:1 | 11,1 | 0,73 |
| | | 1:2 | 7,4 | 0,35 |
| | | 1:4 | 5,5 | 0,17 |

(1) ML Dias, J Abucham et al. Marked Growth Hormone Suppression during pregnancy in acromegaly. Proceedings of 91st Meeting of the Endocrine Society, 2009, P1-689.

(2) Beckers, A et al. Placental and pituitary growth hormone secretion during pregnancy in acromegalic women. JCEM, 1990, 71: 725-31

Nothing to Disclose: MLD, LHO, JGHV, JA

OR25-2

Clinical Characteristics of the Glucagon Stimulation Test (GST) in the Evaluation of Growth Hormone (GH) Reserve and Hypothalamic-Pituitary-Adrenal (HPA) Axis in Adults: A Multi-Centered US Experience.

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¹Oregon Hlth and Sci Univ Portland, OR ; ²Massachusetts Gen Hosp Boston, MA ; ³Stanford Univ Sch of Med Stanford, CA and ⁴Univ of Mississippi Med Ctr Jackson, MS.

Rationale: With the unavailability of recombinant GHRH in the US in 2008, the GST has been proposed as the alternative to the ITT when the latter is contraindicated for evaluating adult GH deficiency (GHD) (1, 2). Until now, the GST is underutilized in the US and reliability of its evaluation on the HPA axis is unclear. We describe experience with GSTs from 4 US centers and explored its potential in testing the HPA axis.

Methods: Patients who underwent GSTs at 4 US centers from Dec 2008 to Jan 2010 ($n = 143$, 41 M, age 45.6 ± 1.2 yrs, BMI 31.5 ± 0.7 kg/m²) were retrospectively studied. IM glucagon (1-1.5 mg) was administered and blood was collected at baseline and every 30 min for 240 min. Peak GH < 3 ng/mL and peak cortisol < 18 µg/dL defined GH and cortisol deficiencies. For patients with cortisol levels measured during the GST and underwent the 250 µg ACTH stimulation test (CST) ($n=18$), ROC analysis was applied to peak cortisol levels of 18 µg/dL as the CST cutpoint.

Results: Most peak and nadir glucose levels occurred between 30-60 min (87.5%) and 180-240 min (58.8%). Most peak GH levels occurred between 120-180 min (77%). Main side-effects were nausea (41.3%, occurred mainly between 90-150 min), fatigue, headaches, weakness and hunger (12%, occurred mainly between 180-240 min). Nadir glucose levels ranged from 44-200 mg/dl, with rescue juice needed in 5 (3.5%) patients. Most side-effects resolved by 240 min without intervention. There was a negative correlation between fasting glucose ($r=-0.24$, $P<0.01$), peak glucose ($r=-0.32$, $P<0.01$), nadir glucose ($r=-0.29$, $P<0.05$) and BMI ($r=-0.37$, $P<0.01$) with peak GH levels. Of the 67 patients (46.9%) who failed the GST, 21 patients had fasting hyperglycemia (glucose ≥ 100 mg/dL) and 46 patients had normal fasting glucose levels. Peak cortisol levels with GSTs were lower ($P<0.02$), had higher failure rates (44.4% vs 33.3%) and the 120-min peak cortisol of 16.5 µg/dL achieved 83.3% sensitivity and 75% specificity with the CST cutpoint of 18 µg/dL.

Conclusion: The GST is well-tolerated and can be performed as an out-patient. Samples at 120, 150 and 180 min yielded the most information. Further studies are needed to determine whether GSTs may falsely diagnose GHD in patients with fasting hyperglycemia and high BMIs. Until then, a second GH stimulation test should be considered for such patients. Lower cortisol cutpoints may also be needed if the GST is used to evaluate the HPA axis, but requires further clarification.

1. Yuen KCJ, Biller BM, Molitch ME, Cook DM. Is lack of recombinant GH-releasing hormone (GHRH) in the United States a setback or time to consider glucagon testing for adult growth hormone deficiency? (J Clin Endocrinol Metab. 2009; 94: 2702-2707).

2. Cook DM, Yuen KCJ, Biller BM, Kemp SF, Vance ML on behalf of American Association of Clinical Endocrinologists. American Association of Clinical Endocrinologists medical guidelines for clinical practice for growth hormone use in growth hormone-deficient adults and transition patients - 2009 update: executive summary of recommendations. Endocr Pract. 2009 Sep-Oct;15(6):580-6.

Sources of Research Support: KCJY has received research grants from Novo Nordisk. BMKB has received research grants and consulting honoraria from Novo Nordisk. CK, LK and DMC have received consulting honoraria from Novo Nordisk.

Nothing to Disclose: KCJY, BMKB, SEL, SAR, THD, MHG, OC, GIU, CK, LK, DMC

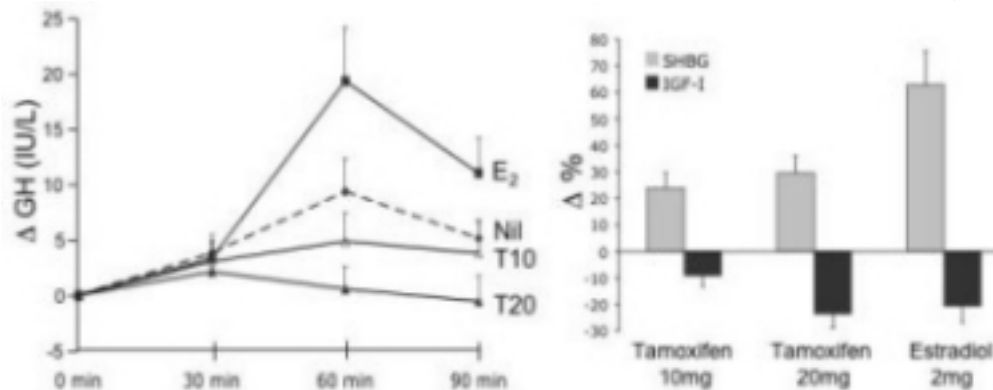
OR25-3

Evidence for a Paracrine Mechanism in the Central Regulation of GH Secretion by Estrogen in Women.

V Birzniece¹, A Sata¹, S Sutanto¹ and KKY Ho¹.

¹Garvan Inst of Med Res and Dept of Endocrinology, St Vincent's Hosp Sydney, Australia.

There is emerging evidence that paracrine mechanisms play a major role in the regulation of the endocrine system. In men, stimulation of GH secretion by testosterone is blunted by tamoxifen, a central estrogen receptor antagonist, indicating an action mediated by prior aromatisation to estradiol (1). In women, estrogen replacement via physiological non-oral route fails to enhance GH secretion (2). While this observation does not support a role of circulating estrogen, it does not exclude the possibility that estrogens produced locally from aromatisation regulate GH secretion in women. We compared the effects on GH secretion of tamoxifen with 17 β -estradiol, in a model of estrogen deficiency. Ten healthy postmenopausal women were treated with tamoxifen (T; 10 and 20 mg/d) and 17 β -estradiol valerate (E2; 2 mg/d) orally for two weeks each, in an open-label sequential cross-over study. We measured the GH response to arginine, and circulating levels of IGF-I and SHBG, markers of hepatic estrogen action. The GH response to arginine was reduced by 10 and 20 mg tamoxifen in a dose-dependent manner (Δ -36% and -88%, respectively), and potentiated significantly by E2 (Δ 84%; $p < 0.05$; left panel). Mean IGF-I concentration fell significantly ($p < 0.05$) with high dose tamoxifen and E2 treatments, while mean SHBG levels increased with both ($p < 0.05$; right panel). The effects on IGF-I and SHBG levels between tamoxifen and E2 treatments were not significantly different.



In summary, tamoxifen reduced but E2 enhanced GH response to arginine in postmenopausal women. Both tamoxifen and E2 reduced IGF-I and increased SHBG levels, indicative of a hepatic estrogen-like effect of tamoxifen. The finding of a blunted GH response to stimulation despite reduced IGF-I feedback inhibition indicates central suppression of GH output by tamoxifen. The contrasting effects on GH status between systemic estrogen supplementation and receptor blockade suggest a paracrine mechanism and an important role of aromatase in the neuroregulation of GH secretion in women.

1) Weissberger et al., JCEM 1993; 76:1407

2) Weissberger et al., JCEM 1991; 72:374

Sources of Research Support: NHMRC of Australia. We thank Alphapharm for providing tamoxifen.

Nothing to Disclose: VB, AS, SS, KKYH

OR25-4

The Growth Hormone Receptor Exon 3 Deleted/full-Length Polymorphism TagSNP Rs6873545 Is Associated with Obesity-Related Parameters in a Randomly Selected Population.

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¹The Sahlgrenska Academy at Gothenburg Univ Gothenburg, Sweden ; ²Chalmers Univ of Technology Gothenburg, Sweden ; ³Sahlgrenska Univ Hosp Gothenburg, Sweden and ⁴Hosp de Clins da Univ Fed do Paraná Curitiba, Brazil.

Introduction: Growth hormone (GH) is a powerful metabolic hormone regulating growth, metabolism and body composition. The GH receptor (GHR) exon 3 deleted/full-length (d3/fl) polymorphism has been suggested to affect sensitivity to GH therapy and might therefore also influence the response to endogenous GH. We hypothesized that carriers of the d3-allele could be more sensitive to GH and therefore as adults be taller and have more lean body mass and less body fat, particularly abdominal. We have investigated the putative link between the GHR d3/fl polymorphism and aspects of GH action (including anthropometric measures).

Methods: The Swedish Obese Subjects (SOS) reference study comprises 1135 subjects (46% men; mean age 49.5±7.0 yrs, mean BMI 25.3±3.8 kg/m²). Study participants were randomly selected from a Swedish population registry to constitute a reference group to the SOS study. The polymorphism was analyzed using the d3/fl tagging single nucleotide polymorphism (tagSNP; $r^2=1$ in the HapMap CEU Panel) rs6873545.

Results: The frequency of the d3-allele was 24.0% (d3/d3 5.6%, d3/fl 36.5% and fl/fl 57.1%) and no deviation from the Hardy Weinberg equilibrium was found (χ^2 test $p=0.769$). In multiple regression analyses adjusting for gender and age, homozygosity of the d3-allele was associated to higher anthropometric measures. In particular, association to weight ($p=0.011$), BMI ($p=0.049$), waist ($p=0.016$), waist-to-hip ratio ($p=0.036$), sagittal diameter ($p=0.035$) and fat free mass measured by potassium 40 ($p=0.026$) was found. In addition, d3/d3 subjects (as compared to grouped d3/fl and fl/fl subjects) were on average 1.3 cm taller, although this was not significant ($p=0.115$).

Conclusion: The findings in this study support the notion that the GHR d3/fl polymorphism is of functional relevance, and present for the first time an effect of the polymorphism on obesity-related parameters in a cross-sectional, population-based study. Interestingly, the results are not entirely in line with our original hypothesis and we conclude that further studies are needed in order to further elucidate the impact of this polymorphism in the normal population.

Nothing to Disclose: CAMG, LMSC, LS, SN, IL, EJLB, PAS, GJ

OR25-5

Effects of Tesamorelin, a Growth Hormone Releasing Analogue, over 52 Weeks in HIV-Infected Patients with Excess Abdominal Fat: A Pooled Analysis of 2 Multicenter, Randomized, Placebo-Controlled Phase III Trials.

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Background: HIV patients treated with antiretroviral therapy (ART) often develop increased visceral adipose tissue (VAT). We report a pooled analysis from 2 randomized, placebo-controlled Phase 3 studies of tesamorelin in ART-treated HIV patients with excess abdominal fat.

Method: Patients were randomized to tesamorelin 2 mg (n=543) or placebo (n=263) sc daily. At Week 26, patients initially on tesamorelin were re-randomized to 2 mg tesamorelin (T-T group, n=246) or placebo (T-P, n=135) for an additional 26 weeks, whereas patients on placebo switched to tesamorelin (P-T, n=197). The primary endpoint was the percent change in VAT by CT scan at Week 26. At Week 52, endpoints were safety and maintenance of effect on VAT.

Results: Baseline age was 48±7 (mean±SD) yrs and WC 105±9 cm. At Week 26, VAT decreased significantly in tesamorelin-patients [-24±41 vs. 2±35 cm², tesamorelin vs. placebo, P<0.001, a -15.4% treatment effect]. No significant changes were observed in abdominal SAT [-2±32 (0.7%) vs. 2±29 cm² (1.3%), P=0.08]. Treatment with tesamorelin resulted in significant decreases in TG [-37±139 vs. 6±112 mg/dL, P<0.001 vs. placebo], cholesterol [-4±33 vs. 1±27 mg/dL, P=0.01], cholesterol/HDL [-0.18±1.00 vs. 0.18±0.94, P<0.001], and non-HDL [-5±31 vs. 2±26 mg/dL, P=0.001] and improvements in belly appearance distress (P=0.002), patient rating (P=0.003), and physician rating of belly profile (P<0.001). Mean IGF-I level increased within the physiological range for young adults in tesamorelin-treated patients (108±112 vs. -7±64 ng/mL, P<0.001, an 84% increase). IgG antibodies to tesamorelin were detected in 50% of T-T patients and unrelated to effects on VAT and IGF-I. At Week 52, improvements in VAT [-35±50 cm² (-17.5%), P<0.001 vs. baseline], TG (-48±182 mg/dL, P<0.001 vs. baseline), cholesterol (-8±38 mg/dL, P<0.001 vs. baseline), and non-HDL (-7±38 mg/dL, P<0.01 vs. baseline) were maintained in the T-T group, while SAT was preserved and not different from baseline. Patients switching to placebo (T-P) regained VAT at Week 52. Treatment with tesamorelin was overall well tolerated, with relatively few adverse events and without clinically meaningful differences between groups in glucose parameters at Weeks 26 and 52.

Conclusion: Treatment with 2 mg tesamorelin daily reduces VAT and maintains the reduction for up to 52 weeks, preserves SAT, improves body image and lipids, and is overall well tolerated without clinically meaningful changes in glucose parameters.

Sources of Research Support: Theratechnologies, Inc.

Disclosures: JF: Consultant, Tibotec, Theratechnologies, Abbott Laboratories, Boehringer Ingelheim; Speaker, Gilead, Bristol Meyers Squibb, Abbott Laboratories, Roche Pharmaceuticals; Research Funding, Theratechnologies. JM: Employee, Theratechnologies. DP: Employee, Theratechnologies. GM: Research Funding, Theratechnologies, Bristol Meyers Squibb, GlaxoSmithKline, Gilead, Merck & Co., Ardea Biosciences, Abbott Laboratories; Consultant, Theratechnologies, Bristol Meyers Squibb, GlaxoSmithKline, Gilead, Merck & Co., Ardea, Pfizer, Inc., Tobira, Johnson & Johnson. GS: Employee, Theratechnologies. HL: Employee, Theratechnologies. CM: Employee, Theratechnologies. RT: Employee, Phase V Technologies; Research Funding, Theratechnologies. SG: Research Funding, Bristol Meyers Squibb, Theratechnologies, GlaxoSmithKline, Amgen; Consultant, Theratechnologies, Serono.

OR25-6

Two Year of Growth Hormone Therapy Improves Body Composition in Adults with Prader-Willi Syndrome.

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Introduction: Prader-Willi syndrome (PWS) presents clinically with a multitude of findings, including abnormal body composition and partial growth hormone (GH) deficiency. Until now three studies have reported beneficial effects upon body composition of GH treatment in adults with PWS. However, only one of these studies had the optimal randomised controlled design.

Aim: The aim of this study was to confirm and substantiate the results from previous studies.

Patients and methods: 46 patients, 25 women, 21 men, age 29 years (16-41) (median and range) with genetically verified PWS participated in a multinational Scandinavian study. The patients were randomised to treatment with GH (0.6 - 0.8 mg daily) (Norditropin SimpleXx®) or placebo for 12 months, the following 12 months all patients were treated with GH according to their IGF-I value. Body composition was measured yearly by dual x-ray absorptiometry. The study was conducted in accordance with Note for Guidance on Good Clinical Practice and approved by the local Ethical Committees.

Results: Body fat changed -2.05 vs. +2.60 kg (P<0.001) and lean body mass +2.39 vs. -0.00 kg (P=0.006) the first year. During the second year non significant changes was found in body fat +0.70 kg (P=0.37) and lean body mass 1.10 kg (P=0.15) in the group primarily randomised to GH, whereas the change in body fat was -4.51 kg (P<0.001) and lean body mass +2.15 kg (P<0.001) in the group primarily randomised to placebo.

Conclusion: In this first large scale, long-term placebo-controlled study the improvement in body composition by GH treatment in adults with PWS was confirmed. No side effects were observed. Based on our two years results, findings persist during long-term therapy.

Disclosures: RS-C: Clinical Researcher, Novo Nordisk. SF: Clinical Researcher, Novo Nordisk. KFRR: Clinical Researcher, Novo Nordisk. JB: Investigator, Novo Nordisk. TS: Clinical Researcher, Novo Nordisk. JSC: Investigator, Novo Nordisk; Speaker Bureau Member, Novo Nordisk; Advisory Group Member, Novo Nordisk. CH: Investigator, Novo Nordisk; Advisory Group Member, Novo Nordisk.

OR26-1

Coronary Microvascular Dysfunction in Primary Hyperparathyroidism: A Study Performed with Transthoracic Doppler Echocardiography.

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Background: Current evidence suggests a potential link between primary hyperparathyroidism (PHPT) and increased cardiovascular risk. However, data on the incidence of cardiovascular abnormalities in untreated PHPT and the potential mechanisms for this relationship are limited.

Objective: To assess the coronary flow reserve (CFR) by transthoracic Doppler echocardiography, as an index of coronary microvascular function, in PHPT patients.

Design and methods: 43 patients with PHPT (34 female, aged 58±11 years) without clinical evidence of ischemic heart disease and normal left ventricular function, and 43 control group subjects matched for age and sex were studied. Coronary flow velocity in the left anterior descending coronary artery was detected by transthoracic Doppler echocardiography at rest and during adenosine infusion. CFR was obtained as the ratio of hyperaemic diastolic flow velocity (DFV) to resting DFV. A CFR ≤2.5 was considered abnormal. The median time from diagnosis of PHPT was 7 months (range 5 to 79 months).

Results: In patients with PHPT, CFR was lower than in controls (2.8±0.7 vs. 4±0.8, p<0.0001). CFR was ≤2.5 in 17 (39.5%) patients with PHPT compared with controls (4.3%) (p<0.0001). CFR was significantly lower in these patients compared with the remaining PHPT patients (2.1±0.3 vs. 3.3±0.6, p<0.0001). CFR was inversely related to parathyroid hormone (PTH) levels (r = -0.421, p=0.01). Moreover in patients with CFR ≤2.5, PTH was higher (5.6±0.9 vs. 5.0±0.3 pmol/L, p=0.02) while calcium levels was similar (1.04±0.09 vs. 0.99±0.05 mmol/L, p=0.07). Prevalence of diabetes, hypertension, left ventricular hypertrophy and dyslipidemia was comparable. At multivariable linear regression analysis adjusted for age, gender, smoke, hypertension, diabetes, calcium levels and dyslipidemia, PHT was the only determinant of CFR (β=-0.485, p=0.003).

Conclusions: Microvascular function, as assessed by CFR, is impaired in PHPT patients. This alteration seems to be correlated with high PTH levels independently of calcium levels. Our findings suggest that PTH concentrations have a negative effect on coronary microcirculatory function and may contribute to explain the increased risk of cardiovascular mortality in PHPT.

Nothing to Disclose: EO, FT, AM, SI, NS, FT, MRP, FF

OR26-2

The Effect of Body Mass Index on the Relationship between Serum Intact Parathyroid Hormone and 25-Hydroxyvitamin D in Women.

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Obesity has been found to be associated with lower serum 25-hydroxyvitamin D (25OHD) levels and higher levels of serum intact parathyroid hormone (iPTH). Vitamin D insufficiency is likely due to the decreased bioavailability of Vitamin D₃ from cutaneous and dietary sources because of its deposition into body fat compartments (1). A secondary hyperparathyroidism then occurs. In this study, it was hypothesized that the physiologic inverse relationship between iPTH and 25OHD would be significantly greater in obese women with body mass index (BMI, kg/m²) ≥30 compared to nonobese women. A retrospective analysis was performed on 383 women with a wide range of body weights (43-185 kg) recruited in the Nutrition & Bone Lab at Rutgers University between 1994-2009. The mean age was 53.4 ± 10.6 years, 70% were postmenopausal, 46% were obese, the mean BMI was 31.4 ± 7.7, 71% were recruited in the winter/spring, and 29% were recruited in the early fall. A non-linear exponential equation (iPTH = A + B exp (C x 25OHD)), was fit to the data for obese and all subjects, but the data for women with BMI < 30 did not fit this equation. This may be due to higher serum 25OHD levels in women with BMI < 30 (28.9 ng/mL) compared to those with BMI ≥ 30 (22.6 ng/mL) (p <0.001). Maximal suppression of iPTH for all women occurred at a 25OHD level of 38 ng/mL (95% CI=28-48). For the women with BMI ≥ 30, the maximal suppression of iPTH was at a 25OHD level of 25 ng/mL (95% CI=15-34). Others have found that maximal suppression of iPTH occurs at 25OHD levels of 31 ng/mL, though BMI was not reported (2). Multiple linear regression revealed that BMI was the most important explanatory variable for iPTH and 25OHD with r²% ranging from 11.4-15.6%.

Multiple Linear Regression

| dependent variable | explanatory variable | beta coefficient | r2% | p value | model r2 |
|--------------------|----------------------|------------------|-------|---------|----------|
| iPTH | BMI | 0.99 | 15.58 | <0.0001 | 26.49 |
| | 25OHD | -0.53 | 10.91 | 0.0009 | |
| 25OHD | BMI | -0.32 | 11.44 | <0.0001 | 22.35 |
| | iPTH | -0.1 | 10.91 | 0.0009 | |

These data have important implications for the effect of BMI on the relationship between iPTH and 25OHD. While these data confirm lower 25OHD levels in the obese, it is interesting that iPTH is maximally suppressed at lower serum levels of 25OHD compared to the entire population of women.

(1) Wortsman et al., Am J Clin Nutr 2000;72:690-3

(2) Chapuy et al., Osteoporos Int 1997; 7:439-443

Nothing to Disclose: EJL, DS, RD-A, SAS

OR26-3

Vitamin D Deficiency Is Associated with Subclinical Carotid Atherosclerosis.

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Introduction: Indices of mineral metabolism have recently been linked to cardiovascular disease. We assessed the association of calcium, phosphorus, vitamin D, and parathyroid hormone (PTH) with subclinical carotid markers and early predictors of coronary and cerebrovascular events.

Methods: 203 consecutive community-dwelling stroke free multi-ethnic adults from the Northern Manhattan Study [age: 68±11 (SD), range: 50-93 yrs] had biochemical measurements (serum calcium, phosphorus, vitamin D and PTH) and carotid ultrasound measures of plaque presence, maximal carotid plaque thickness (MCPT) and intima-media thickness (IMT).

Results: Carotid measures were highly correlated with traditional vascular risk factors, with age as the strongest predictor (IMT: $r=0.41$, $p<0.0001$; plaque number: $r=0.44$, $p<0.0001$; MCPT: $r=0.48$, $p<0.0001$). Over half had plaque present (N=116; 57%). In an analysis controlling for age, race, sex, renal function, BMI, and vascular risk factors, inverse associations were found between 25(OH)D and maximal carotid plaque thickness ($r=-0.20$; $p<0.05$) and carotid IMT ($r = -0.18$; $p=0.056$). Calcium-phosphorus product also emerged as an independent predictor of carotid plaque number ($r=0.23$; $p<0.05$), and IMT ($r=0.19$; $p<0.05$). Although no independent association was found with serum calcium levels, serum phosphorus levels were positively correlated with carotid plaque number ($r=0.24$, $p<0.05$), maximal carotid plaque thickness ($r=0.19$, $p<0.05$) and a trend with IMT ($r=0.18$, $p=0.07$). No independent associations between PTH or 1,25(OH)₂D and carotid measures were found. A final model tested the interdependence of the associations discovered. Indices of mineral metabolism were simultaneously entered into a model with carotid measures adjusted for the presence of known cardiovascular risk factors. The inverse associations of 25(OH)D with MCPT ($r=-0.19$; $p<0.05$) and IMT ($r = -0.19$; $p<0.05$) persisted. The positive relationship of serum phosphorus with MCPT ($r= 0.18$; $p<0.05$) also persisted, while there was no evidence for an independent relationship of carotid measures with calcium-phosphorus product.

Conclusion: There is an independent association between low 25(OH)D levels and subclinical markers of carotid vascular disease. Further research will be needed to elucidate the precise nature of this association and to determine the optimum levels of vitamin D for vascular health.

Nothing to Disclose: ALC, HL, MW, DJM, TR, RLS, SJS

OR26-4

Reduced Serum Total Osteocalcin Is Associated with Metabolic Syndrome in Older Men Via Waist Circumference, Hyperglycemia and Triglyceride Levels.

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Objective

Bone-derived undercarboxylated osteocalcin regulates insulin secretion and sensitivity in mice, and reduced serum total osteocalcin (TOC) is associated with Type 2 diabetes in humans. However, the relationship between osteocalcin and other cardiovascular risk factors is uncertain. We sought to determine whether TOC is associated with metabolic syndrome and its components in older men.

Participants and Methods

We conducted a cross-sectional analysis from the Health In Men Study, a population-based cohort of men aged ≥ 70 years resident in metropolitan Perth, Western Australia. Early morning sera were assayed for TOC. Insulin resistance was estimated using a homeostatic model (HOMA2-IR). Metabolic syndrome was defined according to NCEP-ATPIII criteria.

Results

TOC was assayed in 4,047 men. Men not fasted and those reporting bone fractures, Paget's disease, or bisphosphonate, glucocorticoid or warfarin use were excluded, leaving 2,765 men with metabolic syndrome present in 797 (28.8%). TOC was lower in men with metabolic syndrome compared to those without (mean \pm SE: 20.1 \pm 0.4 vs 21.4 \pm 0.2 μ g/L, $p=0.002$). TOC was inversely associated with waist circumference, glucose and triglyceride levels and HOMA2-IR (all $p<0.001$). After adjusting for age, smoking, alcohol use and serum creatinine, men with TOC of 13.25-16.55 and <13.25 μ g/L had 1.5 to 2-fold increased risk of metabolic syndrome compared to men with levels ≥ 30 μ g/L. Osteocalcin <13.25 μ g/L remained associated with metabolic syndrome after further adjustment for each individual component, but not after adjusting for both waist circumference and glucose.

Conclusions

Lower total osteocalcin levels are associated with metabolic syndrome in older men. Increased waist circumference, reduced osteocalcin, elevated glucose and triglyceride levels are inter-related. This may reflect an underlying common factor, alternatively, osteocalcin may lie in the causal pathway between central adiposity and insulin resistance. Further research is required to clarify the direction of causality and determine whether interventions which raise osteocalcin levels could improve glucose metabolism and decrease cardiovascular risk in ageing men.

Sources of Research Support: Hormone assays were funded by research grants from the Fremantle Hospital Medical Research Foundation, Fremantle Hospital, Western Australia, and the Ada Bartholomew Medical Research Trust, University of Western Australia. The Health In Men Study was funded by Project Grants 279408, 379600, 403963 and 513823 from the National Health and Medical Research Council of Australia (NHMRC). BBY is recipient of a Clinical Investigator Award from the Sylvia and Charles Viertel Charitable Foundation, New South Wales, Australia. PEN is supported by NHMRC Practitioner Fellowship 458505.

Nothing to Disclose: BBY, SAPC, LF, KM, PRE, JPB, PEN

OR26-5

Aortic Calcification, vBMD and Vertebral Fracture in Asian Women - Is Age Dependent Linkage?.

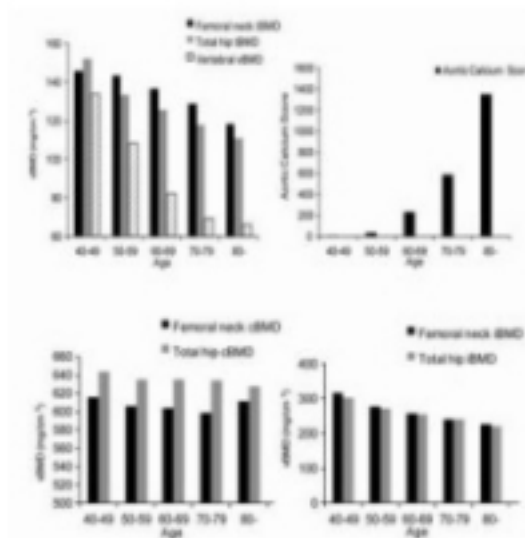
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¹Yonsei Univ Coll of Med Seoul, Korea.

Backgrounds: The aim of this study is to investigate the relationship between volumetric BMD (vBMD), vertebral fracture, and abdominal aortic calcification score (ACS) computed by quantitative computed tomography (QCT) in **Asian women**.

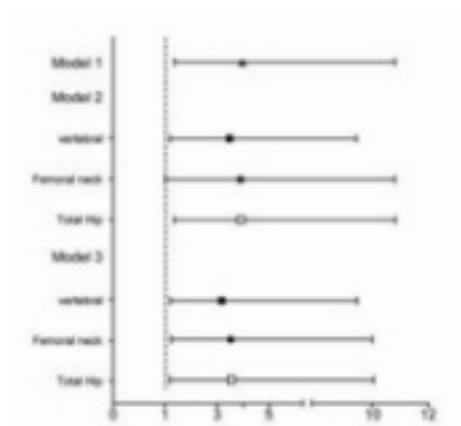
Results: Among 569 women subjects, 96 patients (17%) had at least one vertebral fracture, and 345 patients (60%) were found to have aortic calcification in QCT.

Figure 1. Trabecular vBMD, Aortic calcification, cortical vBMD and integral vBMD (according to the age groups)



Overall, quantified ACS measured by Agatston scoring method correlated as follows: Moderate correlation with age ($r = 0.57$; $P < 0.001$), total hip tBMD ($r = -0.36$; $P < 0.001$), femur neck tBMD ($r = -0.38$; $P < 0.001$) and vertebral vBMD ($r = -0.56$; $P < 0.001$). Neither total hip cortical nor femur neck cortical vBMD correlated with ACS. After age adjustment, ACS was not correlated with any vBMD. High ACS was directly related to high prevalence of vertebral fractures. We divided subjects into 2 different groups based on the presence of AC. Multivariate logistic regression analysis showed that age, history of HTN, history of osteoporosis treatment, vertebral vBMD, femoral neck trabecular vBMD, total hip trabecular vBMD, and ACS independently associated with vertebral fractures. After adjusting for age, body mass index (BMI), vertebral vBMD, femoral neck trabecular vBMD, total hip trabecular vBMD, and other confounding factors, patients with AC had more than a 3 fold increased risk of vertebral fracture. (OR 3.29-3.57; $P < 0.05$ according to the site).

Figure 2. Odd ratio
(vertebral fracture as dependent variables and odd ratio of aortic
calcification after adjusting BMD according to the site)



Conclusion: Our findings support an age-dependent inverse correlation between AC and trabecular vBMD. However, cortical vBMD of the femoral neck and total hip were not associated with either age or ACS. We also found that AC is significantly associated with vertebral fracture in Asian women regardless of age, BMI, vBMD, and other confounding factors.

Nothing to Disclose: KJK, KMK, HSC, YMR, SKL

OR26-6

Effects of a Single Oral High Dose Vitamin D3 Supplementation in Critically Ill Vitamin D Deficient Patients: A Randomized, Double-Blind, Placebo-Controlled Pilot Study at a Medical Intensive Care Unit.

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Introduction

Vitamin D deficiency is frequently encountered in critically ill patients. Because vitamin D is thought to exert pleiotropic effects among which are those on immune function, the metabolic as well as musculoskeletal system, the idea of a fast correction of low vitamin D status in critically ill patients is attractive.

Objectives

Our first intention before initiation of a larger trial was to evaluate the safety and efficacy of a single oral high dose vitamin D3 supplementation in an intensive care setting over a one-week observation period because the medical situation of such patients may warrant special considerations.

Methods

In this pilot RCT 540.000 IU of cholecalciferol dissolved in an herbal oil volume of 45 ml (VitD) or matched placebo (PBO) were given enterally to 20 patients (mean age 62±16y) with 25-hydroxyvitamin D deficiency [25(OH)D <20 ng/ml]. Results were calculated using non-parametric tests.

Results

Simplified Acute Physiology (SAPS) as well as Therapeutic Intervention and Severity Scores (TISS) were balanced between the two groups. Mean serum 25(OH)D levels at baseline were 14±4ng/ml in both groups. 25(OH)D levels in the VitD group increased progressively over the first 3 days to a mean of 37±13ng/ml and remained at this level until day 7 (38±16). In the PBO group 25(OH)D levels did not change. The mean difference in 25(OH)D levels between the two groups became significant at day 2. In 8/10 patients 25(OH)D levels increased to ≥30ng/ml, 2 patients showed a low (+7ng/ml) or no response (+1ng/ml) over the 1-week observation period. Serum calcium levels significantly increased by 0.11 (PBO) and 0.15 mmol/L (VitD) in both groups, whereas parathyroid hormone (PTH) levels significantly decreased by 21 (PBO) and 23pg/ml (Vit D) respectively, compared to baseline. Hypercalcemia did not develop in any patient and the highest 25(OH)D level reached was 63ng/ml.

Conclusions

This pilot study suggests that a single oral ultra-high dose of 540.000 IU cholecalciferol is able to restore normal 25(OH)D levels within 2 days in most patients. Hypercalcemia was not seen. Independent of 25(OH)D status mean serum calcium levels rise and PTH levels decrease in critically ill patients. Further research is necessary to establish whether changes in 25(OH)D or calcium status are causally related to important clinical outcomes of these patients.

Nothing to Disclose: KA, HS, GW, TRP, AH, CS, CS, TS, HD

OR27-1

Primary Versus Secondary Polycystic Ovary Syndrome (PCOS): A Novel Approach to Classifying Women with PCOS.

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Rationale: PCOS is common among women of reproductive age and is associated with metabolic disturbance. The literature suggests most women with PCOS date symptom onset to the early adolescent period. In clinical practice, we have noted two distinct groups of women. Primary PCOS (PP) includes women who report irregular menstruation since menarche. Secondary PCOS (SP) includes women who initially report regular cycles followed by irregular cycles later in life. The study objective was to determine the existence and prevalence of these two populations, and to compare menstrual, clinical and biochemical parameters between the groups.

Methods: Retrospective cross-sectional cohort of 189 women from Women's College Hospital with PCOS(1). Women were classified as PP or SP according to menstrual history. Differences between groups were compared using 2-sample t-tests or Pearson Chi-square analyses for clinical, social, metabolic and biochemical parameters. Regression analyses were undertaken to determine whether group predicted these variables.

Results: The prevalence of SP was 38.1%. Weight gain preceding PCOS onset was noted in 65.3% of SP women. The SP group had significantly higher BMI (36.7 vs. 31.6 kg/m², $p=0.001$), waist circumference (119.1 vs. 95.4 cm, $p=0.003$), Ferriman-Gallwey score (9.9 vs. 7.5, $p=0.009$), 2-hour glucose (6.8 vs. 5.7 mmol/L, $p=0.014$), prevalence of acanthosis nigricans (37.5 vs. 19.7%, $p=0.029$), diastolic blood pressure (80.2 vs 76.3 mmHg, $p=0.023$), triglycerides (1.81 vs 1.34 mmol/L, $p=0.003$), and greater number of pregnancies (2.50 vs. 1.75, $p=0.024$) compared with the PP group. A family history of PCOS symptoms was more frequent (44.4% vs. 29.2%, $p=0.040$) and HDL-cholesterol was higher (1.46 vs. 1.28 mmol/L, $p=0.010$) in the PP group. In stepwise linear regressions, group assignment (PP or SP) was a significant predictor of 2-hour glucose ($p=0.004$), total cholesterol ($p < 0.001$) and LDL cholesterol ($p=0.002$).

Conclusion: Two distinct groups of women with PCOS were found to exist. Women with SP have a more severe metabolic profile, but less frequent family history of PCOS or related symptoms. These findings suggest women with PP may have a stronger genetic predisposition for ovulatory dysfunction, whereas SP onset is more commonly associated with insulin resistance parameters. This novel classification suggests different mechanisms of PCOS for the two groups and may have important implications for diagnosis and management.

(1) Azziz R et al., J Clin Endocrinol Metab 2006; 91(11):4237-45.

Nothing to Disclose: SEL, BJNR, SAS

OR27-2

TNF α Resistance in PCOS Theca Cells: Can the Lack of TNF Inhibition of Ovarian Androgen Biosynthesis Be Explained by Differential MAPK Signaling?.

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Although there are data to support that tumor necrosis factor α (TNF) inhibits thecal 17 α -hydroxylase (CYP17A1) gene expression and androgen synthesis, these data conflict with clinical data demonstrating that PCOS women have elevated circulating plasma and FF levels of TNF. In these studies we compared the dose response effects of TNF on androgen synthesis and CYP17A1 gene expression in normal and PCOS theca cells. TNF was observed to be less effective in inhibiting basal and forskolin stimulated CYP17 mRNA accumulation and DHEA synthesis. Deletion analysis of the CYP17A1 promoter in theca cells showed that sequences within -188 bp to -61 bp are required for TNF dependent inhibition. TNF inhibited CYP17A1 promoter function 75% in normal cells, and negligibly in PCOS cells. These data imply that the lack of ability to respond to TNF, or TNF resistance, in PCOS theca cells results in increased androgen synthesis and CYP17A1 gene expression.

To examine the molecular mechanisms for TNF resistance, EMSA analysis showed that Sp3, NF-1, and SF-1 bind within -180/-150 bp of the CYP17A1 promoter, and that TNF treatment differentially alters the binding pattern of these factors in normal and PCOS cells. ChIP analysis further demonstrated that in PCOS theca cells, ~30% less NF-1, and ~50% less Sp3 occupies the CYP17A1 promoter. TNF treatment results in a 90% reduction in NF-1 occupancy to the CYP17A1 promoter in normal cells, whereas in PCOS cells there is only a slight ~10% reduction in occupancy. In addition, TNF treatment results in a 3-fold increase in Sp3 occupancy of the CYP17A1 promoter in normal cells and only a 2-fold increase in PCOS theca cells. Combined these data further demonstrate that normal theca cells are more sensitive to TNF than PCOS theca cells.

To examine whether upstream MAPK-signaling components might be associated with TNF resistance in PCOS theca cells, both cell types were infected with varying doses of control, MEKK3, or MEKK1 adenovirus then treated with TNF. These studies demonstrated that both MEKK3 and MEKK1 infection increased TNF sensitivity in PCOS theca cells while having little effect on normal cells. MEKK1 and MEKK3 also converted normal cells to a PCOS phenotype of increased CYP17A1 expression and androgen synthesis. Therefore, defects in TNF action is a characteristic phenotype of PCOS theca cells that not only results in hyperandrogenism, but may also be related to other defects in MAPK-signaling.

Sources of Research Support: NIH-HD33852.

Nothing to Disclose: BD, YX, RSL, JMM

OR27-3

The Effect of Anti-Müllerian Hormone (AMH) on Follicle Development: Do the High Levels Produced by PCOs Contribute to Anovulation?.

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Polycystic ovary syndrome (PCOS) is the commonest cause of anovulatory infertility. Symptoms include hyperandrogenaemia, obesity, insulin resistance and anovulation. Granulosa cell production of AMH in women with anovulatory PCOS is, on average, more than seventy times higher than in women with normal ovaries and may have an inhibitory role on folliculogenesis. We have previously shown that in GCs from small and large follicles, AMH significantly inhibited FSH/LH-stimulated aromatase mRNA expression which was mirrored by a similar reduction in oestradiol production. Our aim was to determine whether this effect is on aromatase promoter activity, or an effect on FSHr. In addition, we investigated whether, in parallel with the inhibitory effects on aromatase, AMH affected other factors produced by the ovary. This work was carried out in the granulosa cell line, KGN.

Granulosa cells were exposed to AMH at a range of concentrations (2-200ng/ml) in the presence of forskolin for 48hrs. RNA was extracted and aromatase and FSHr mRNA expression measured using real time qPCR. Oestradiol, inhibin A and B and VEGF production were measured in the conditioned medium by ELISA. Aromatase promoter II activity was measured using luciferase assay.

AMH decreased forskolin-stimulated aromatase and this effect was via promoter II which was inhibited in a dose-dependent manner. Surprisingly, AMH also reduced FSHr mRNA expression, but had no effect on inhibin or VEGF production.

In summary, AMH decreased aromatase mRNA and oestradiol levels as a result of a decrease in PII activity. Interestingly, AMH also decreased FSHr expression either directly or indirectly. AMH overproduction in anovPCOs may therefore restrict normal follicle development by an overall inhibitory effect on follicle sensitivity to FSH, therefore contributing to anovulation.

Sources of Research Support: Medical Research Council UK.

Nothing to Disclose: HDM, ND, AH, RG, SR, MB, LJP

OR27-4

Continuous Positive Airway Pressure Treatment of Obstructive Sleep Apnea Improves Cardiometabolic Function in Obese Women with Polycystic Ovary Syndrome.

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Background: Women with polycystic ovary syndrome (PCOS) are insulin resistant and at increased risk for early-onset cardiometabolic disease. Obstructive sleep apnea (OSA), a treatable disorder with adverse cardiovascular and metabolic consequences, is present in about 50% of women with PCOS. Whether treatment of OSA can reduce cardiometabolic risk in PCOS is not known.

Objective: To determine whether continuous positive airway pressure (CPAP) treatment of OSA has beneficial effects on cardiometabolic function in PCOS.

Design: Clinic-based interventional study

Setting: University of Chicago Hospitals

Patients: Nineteen obese, normotensive PCOS women with newly diagnosed OSA

Intervention: CPAP treatment at home for 8 weeks, with built-in monitoring of daily usage

Measurements: Insulin sensitivity and secretion (from an intravenous glucose tolerance test), 24-hour profiles of catecholamines, cortisol, and leptin, and daytime blood pressure. Changes in cardiac autonomic activity were estimated from analyses of heart rate variability (HRV) and cardiac autonomic activity (heart rate variability).

Results: CPAP treatment improved insulin sensitivity (adjusted $p=0.013$) after controlling for BMI. The change in insulin sensitivity correlated positively with CPAP use (adjusted $p=0.027$) and negatively with BMI (adjusted $p=0.003$). Both daytime and nighttime plasma norepinephrine levels were decreased after CPAP ($p=0.002$). Increased CPAP use was associated with greater reductions in norepinephrine levels ($p=0.03$). Epinephrine, cortisol, and leptin levels, and daytime blood pressure were not significantly affected by CPAP use. The spectral power in the high frequency band (HF) derived from EKG analysis served as a measure of vagal activity and the spectral power in the low frequency band (LF) as a measure of sympathetic activity. The LF to HF ratio (a marker for cardiac sympathovagal balance) was 44% lower ($p=0.007$) after CPAP, reflecting a shift toward lower sympathetic activity.

Conclusions: In obese women with PCOS, CPAP treatment of OSA improves insulin sensitivity, decreases sympathetic output, and thus may reduce cardiometabolic risk. The magnitude of these beneficial effects is modulated by the hours of CPAP use and the degree of obesity.

Sources of Research Support: PO1- AG11412, RO1-HL-075079, P50-HD057796, and P60-DK20595), and a gift from the Blum-Kovler Foundation. ClinicalTrials.gov Identifier: NCT00203996.

Nothing to Disclose: ET, FC, RL, EVC, HW, DAE

OR27-5

Incidence of Diabetes Mellitus Following Gestational Diabetes in Women with Polycystic Ovarian Syndrome.

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BACKGROUND: Polycystic ovary syndrome (PCOS) is associated with insulin resistance, glucose intolerance and diabetes mellitus (DM). Several studies have also shown a higher incidence of gestational diabetes (GDM) in women with PCOS. This study examines the prevalence of GDM in PCOS women, association with race/ethnicity, family history, pre-pregnancy BMI, and the subsequent incidence of DM.

METHODS: Within Kaiser Permanente Northern California, we identified non-diabetic pregnant women with PCOS (Rotterdam criteria) who delivered during 2002 - 2006 and underwent screening for GDM during pregnancy. GDM was defined by standard 3-hr glucose tolerance test (GTT) criteria or by a 50g screening glucose >180 mg/dL in patients treated presumptively as GDM without GTT (N=17 only). Detailed chart review was conducted to obtain demographic factors, family history, clinical factors and pharmacologic treatment of GDM. The diagnosis of DM was established by laboratory criteria (glucose \geq 126 mg/dL or HbA1C \geq 6.5%) or treatment with a hypoglycemic agent (insulin or glyburide).

RESULTS: 979 pregnant women with PCOS (41.1% White, 4.1% Black, 25.9% Hispanic, 26.8% Asian and 2.2% other race) underwent GDM screening, and 19.1% were found to have GDM during the current pregnancy. Of these 187 women, 30.4% required pharmacologic treatment for GDM (insulin or glyburide). Multivariable predictors of GDM included older age (adjusted OR 1.1), Asian race (OR 3.8), higher BMI (OR 2.7 for BMI 30-39.9, OR 4.0 for BMI \geq 40), multiple gestation (OR 2.0) and family history of DM (OR 1.6). Among the 162 (86.6%) GDM women who remained health plan members for 3 or more years post delivery, 31 (19.1%) developed DM, of whom 5 met criteria by HA1C only and 2 met criteria by treatment only. However, considering only the subset who received pharmacologic GDM treatment, 36.4% developed subsequent DM during follow-up.

CONCLUSION: Among women with PCOS, the incidence of GDM is high compared to the background rate of about 7-8%. Among PCOS women who develop GDM, the subsequent risk of DM is also high, particularly if pharmacologic treatment of GDM is required. Understanding the predictors of subsequent DM in PCOS women with GDM pregnancy will be important in guiding additional screening efforts in this high risk population.

Sources of Research Support: NIH/ NICHD Grant R01 HD052966 awarded to JCL.

Nothing to Disclose: JCL, GAL, SYP, SLF, JY, MKH, EPG, AF

OR27-6

Hyperandrogenism of Significant Adrenal Contribution Persists after Menopause in Women with Polycystic Ovary Syndrome.

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Context: Hyperandrogenism of ovarian and adrenal origin has been documented in premenopausal women with polycystic ovary syndrome (PCOS). Ovarian and adrenal androgen secretion declines with age in both healthy women and patients with PCOS. Aim of our study was to investigate ovarian and adrenal hyperandrogenism in PCOS after menopause at baseline as well as after hypothalamic-pituitary-adrenal axis stimulation and suppression tests.

Patients and Methods: Twenty postmenopausal women with PCOS and twenty age- and body mass index-matched controls from the endocrinology unit of a university hospital were included in the study. All subjects had serum cortisol, 17-hydroxyprogesterone (17-OHP), Δ_4 -androstenedione (Δ_4 A), dehydroepiandrosterone sulfate (DHEAS), total testosterone (T) and free androgen index (FAI) levels measured at baseline, after ACTH stimulation and after 3-day dexamethasone suppression. ACTH and cortisol levels were also measured during a CRH test.

Results: Postmenopausal PCOS women had higher ($p < 0.05$) 17-OHP, Δ_4 A, DHEAS, T, FAI and lower ($p < 0.05$) SHBG baseline levels. ACTH and cortisol responses after CRH test did not differ significantly between PCOS and control women. ACTH stimulation resulted in greater ($p < 0.05$) area under the curve responses for Δ_4 A, DHEAS and T in PCOS than in control women. After 3-day dexamethasone suppression, 17-OHP, Δ_4 A and FAI levels remained higher ($p < 0.05$) in PCOS than in control women, while cortisol, DHEAS and T levels did not differ significantly between the two groups.

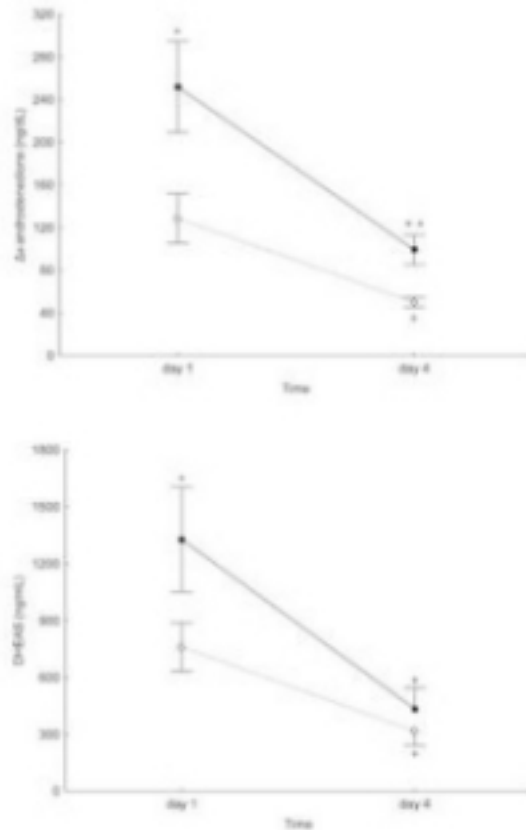


Figure 1: Δ_4 A and DHEAS levels after dexamethasone suppression in PCOS women (straight line) and controls (dotted line). Asterisk (*) indicates statistically significant difference ($p < 0.05$) from the respective baseline. Cross (+) indicates statistically significant difference ($p < 0.05$) from controls at the same time-point.

Conclusions: Baseline androgen levels (Δ_4 A, DHEAS, T and FAI) are higher in postmenopausal PCOS than in control women. Adrenal androgen production is enhanced after ACTH stimulation in postmenopausal PCOS women, without a centrally originating HPA axis dysfunction. Three-day dexamethasone suppression reveals the adrenal origin of elevated T and DHEAS levels in postmenopausal PCOS women. Ovarian Δ_4 A production persists after menopause in PCOS women. Thus, postmenopausal PCOS women are exposed to higher levels of bioavailable androgens than non-PCOS women resulting to multiple possible adverse clinical effects.

Nothing to Disclose: MCM, DR, GV, OG, GPC, GC, GM

P3-1

Exploring the Direct Estrogen-Regulated Transcriptome in Breast Cancer Cells Using GRO-Seq.

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Estrogens play crucial roles in regulating the expression of hundreds, perhaps thousands, of genes in normal physiological states, such as the development of reproductive organs, as well as disease states, such as breast cancers. Although recent genomic analyses of estrogen-dependent transcription have provided the vast amount of information, many aspects of the estrogen-dependent gene regulatory network, however, have not yet been elucidated. Foremost among these is the set of genes comprising the set of direct or primary estrogen-regulated targets. This information is critical because the direct estrogen-regulated transcriptome is likely to define a useful set of biomarkers with prognostic value and utility as therapeutic targets in breast cancers.

To define the direct estrogen-regulated transcriptome with greater sensitivity than existing genomic analyses, we have applied an innovative new method called Global Nuclear Run-On and Massively Parallel Sequencing (GRO-seq) to explore the estrogen-regulated transcriptome in MCF-7 breast cancer cells. GRO-seq is a deep sequencing-based genomic approach that provides a genome-wide map of the location and orientation of transcriptionally engaged RNA polymerase II (Pol II). Using GRO-seq as a direct measure of transcriptional output, we have produced libraries from MCF-7 breast cancer cells in response to short treatments with 17-estradiol (E2) that reveal the location of actively transcribing Pol II in human breast cancer cells. Our goals are to (1) identify in an unbiased manner the annotated and unannotated transcripts that change expression during estrogen signaling, (2) obtain a time-course of activation for each transcript, and (3) identify the direct estrogen target genes. Our preliminary results have allowed us to detect E2-regulated transcripts with greater sensitivity and at much earlier time points than has been achieved with conventional expression microarray platforms. Collectively, these studies are revealing many unexpected novel features of E2-regulated gene expression. We expect that genome-wide inferences based on the direct estrogen-regulated transcriptome will be useful for understanding the molecular mechanisms driving the development and progression of breast cancers.

Sources of Research Support: Grants from the NIH/NIDDK to W.L.K.

Nothing to Disclose: NH, CGD, LJC, ACS, JTL, WLK

P3-2

GATA4 Is a Pioneer Factor for Estrogen Receptor α in Osteoblasts.

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Estrogens are important in the development of the skeleton and maintenance of bone mineral density. To understand how estrogen receptor α (ER α) determines tissue specific effects, we identified GATA4 as a transcription factor expressed in bone, but not in breast, both classical estrogen-responsive tissues. GATA4 co-localizes with RUNX2 in osteoblasts, as visualized by immunofluorescence of mouse femurs and GATA4 expression is reduced in ER α knockout mice as compared to wildtype mice. ER α binds to chromatin near GATA4 at five different enhancers. GATA4 also binds to these same enhancer elements to control its own expression in response to estradiol (E2) treatment. In fact, GATA4 and ER α both are recruited to many ER α binding sites that are specific to osteoblasts. Knockdown of GATA4 reduced the binding of ER α to these sites. As a result, E2-mediated induction of FasL, an important osteoblast-specific direct target of E2, is reduced. Furthermore, we demonstrate that GATA4 is necessary for histone 3 lysine 4 dimethylation (H3K4me2) at ER α binding sites and recruitment of ER α to those sites. This work shows that GATA4 is a pioneer factor for ER α recruitment to osteoblast-specific enhancers.

Sources of Research Support: K12 BIRCWH grant to SAK from the NIH/ORWH (HD001400-08).

Nothing to Disclose: SAK

P3-3

Proinflammatory Cytokines and NFκB Activation Alter Estrogen Receptor Occupancy on DNA in a Gene-Specific Manner and Lead to Enhanced Gene Expression.

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The anti-inflammatory role of estradiol (E2) is mediated by estrogen receptor (ER) repression of the pro-inflammatory transcription factor NFκB. However, little is known about how NFκB affects ER activity. In breast cancer cells, we find that the NFκB pathway enhances E2 action on numerous genes, including two important for drug resistance and cell survival - ABCG2 and BIRC3. Both genes have an ERE in their proximal promoters but these sites are influenced differently by NFκB. The previously described ERE of ABCG2 recruits ER and drives transcription, both of which we find are enhanced by NFκB. In contrast, BIRC3 has a near-consensus ERE that is not accessible to ER unless NFκB is activated, indicating that NFκB is required for ER recruitment to the BIRC3 ERE. Mutation of each ERE to consensus shows that the sequences of these EREs are not responsible for their distinct responses to NFκB. Instead it appears that the flanking regions of the two EREs are important. Both genes have at least one NFκBRE located near the ERE. Additional mutagenesis studies demonstrated that these NFκBREs and their position relative to neighboring EREs, are crucial for enhanced expression of the two genes. For ABCG2, the NFκBRE is capable of recruiting p50, which results in H3 acetylation. Although NFκB cannot drive transcription of ABCG2, it is required for enhanced ER occupancy, which is accompanied by a switch from p50/p50 homodimer to p65/p50 heterodimer recruitment. In contrast, the NFκBREs of BIRC3 recruit p65/p50 heterodimers directly in response to cytokines, which leads to an increase in H3 acetylation and local nucleosome instability, as demonstrated by H3 loss in ChIP assays. Thus, NFκB activity on chromatin remodeling of this region promotes accessibility of the ERE and allows ER to be recruited. The net result for both genes is the formation of an ER/p65/p50 complex at adjacent REs, further increase in H3 acetylation, and a substantial increase in transcription. These findings indicate that inflammation can enhance estrogen activity in a gene-specific manner by increasing ER occupancy at known EREs, as well as facilitating ER recruitment to novel, inflammation-dependent EREs that were previously inaccessible for ER binding. Our findings of positive crosstalk between ER and NFκB, in combination with widely reported transrepression, emphasize the complex interaction between estrogen and inflammation in multiple pathologies.

Sources of Research Support: NIH R01 CA130932-01A2.

Nothing to Disclose: MAP, LAB, JMF

P3-4

E6-AP Facilitates p300 Recruitment to an Estrogen Responsive Promoter To Allow Efficient Transcription.

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The Estrogen Receptor alpha (ER) is a multi-domain transcription factor that has been extensively studied due to its known involvement in breast cancer treatment and progression. Subsequent studies have shown coregulators are extensively involved in modulating the transcriptional activation of ER and many of these proteins possess enzymatic functions. Coregulators are divided into two categories, coactivators which enhance transcriptional output and corepressors which decrease transcriptional output. One protein responsible for Angelmans syndrome, E6-associated protein (E6-AP) was also found to be a coactivator of ER and possessed ubiquitin ligase activity; however, the ubiquitin ligase activity has been shown not to be essential to E6-AP coactivation ability.

The current work was undertaken to explore the mechanism by which E6-AP acts as a coactivator of ER target genes. TFF1 (pS2) was examined as a model ER target gene. In T47D cells, a time course ChIP over a 150 minute period after hormone (estradiol) addition was used to look at recruitment of various proteins known to be involved in transcription. T47D cells were treated with control scrambled siRNA or siE6-AP in order to determine what effect loss of E6-AP would have on ER-mediated transcription. P300 is a well studied histone acetyl transferase (HAT) that can change chromatin structure associated with actively transcribing genes. It has also been shown p300 and E6-AP interacts on the promoter of TFF1. P300 recruitment was decreased with the knockdown of E6-AP in the presence of estrogen. ER possesses a DNA binding domain and can bind directly to the TFF1 ERE near the promoter. ER was still recruited to the promoter with knockdown of E6-AP. Phosphorylated RNAPol II binding was decreased with knockdown of E6-AP. Transcriptional output from TFF1 was decreased with knockdown of E6-AP as measured by rtPCR. The current work provides a new role for E6-AP as a coactivator in the form of a scaffold allowing creation of fully functional transcription complexes.

Nothing to Disclose: HWC, ZN

P3-5

Role of Cellular Kinases in Estrogen-Dependent Transcription in Human Breast Cancer Cells.

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Growing evidence supports both enzymatic and structural roles for signal-regulated kinases in the regulation of transcription through interactions with chromatin-associated complexes. In estrogen receptor alpha (ER)-positive human breast cancer cells, several mitogen-activated protein kinases have been implicated in functional crosstalk with the estrogen signaling pathway, which play key roles in regulating gene expression patterns that have implications for breast cancer progression and responses to endocrine therapy. Elevated kinase activity and/or expression levels are observed in human breast cancers, and these kinases phosphorylate key transcription factors involved in the ER-mediated signaling pathway. For examples, JNK1 phosphorylates c-Jun, a major AP1 protein that tethers ER to some estrogen-responsive gene promoters. Moreover, RSK1 phosphorylates ER and facilitates ER-mediated transcriptional activation. We propose that such kinases act as ER coregulators at target gene promoters to regulate estrogen-dependent gene expression. They may do so by tethering to chromatin via ER and/or additional DNA-binding transcription factors and by phosphorylating components of these chromatin-associated complexes. Using chromatin immunoprecipitation coupled with hybridization to a promoter microarray (ChIP-chip), we observed estrogen-induced genomic binding and relocalization of kinases, including JNK1 and RSK1. We identified sets of estrogen-regulated genes with promoter binding of both the kinases and ER. In addition, we showed that the catalytic activity of these kinases is important in achieving the full extent of estrogen-dependent changes in gene expression. We also identified subsets of genes that show estrogen-dependent, but ER-independent recruitment of kinases to their promoters, suggesting a different mechanism of action. We are applying bioinformatic methods to these gene sets to determine which DNA-binding transcription factors might recruit the kinases to their target gene promoters in the absence of ER binding in response to estrogen signaling. We are also exploring the effects of crosstalk between the kinase and estrogen signaling pathways on breast cancer cell growth.

Sources of Research Support: Grants from the NIH/NIDDK to W.L.K., and predoctoral fellowships from the AHA to M.S. and DOD BCRP to G.D.I.

Nothing to Disclose: MS, GDI, WLK

P3-6

Activation of Estrogen Receptor- α by Nitrite.

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Recent studies show that the anion nitrite binds to and activates estrogen receptor-alpha (ER-alpha). Mutational analysis and molecular modeling identified three potential nitrite binding sites in the ligand binding domain (LBD) of the receptor. Site A is formed by lys529 on helix H11 and asn532 in the loop between helices H11 and H12; site B is formed by his516 on helix H10 and lys520 on helix H11; and site C is formed by cys381 on helix H4 and his547 on helix H12. To determine the mechanism by which nitrite activates the receptor, wild-type and mutants forms of ER-alpha were tested for their ability to dissociate from the heat shock protein 90 (hsp90) complex, translocate into the nucleus, dimerize and bind DNA, interact with coactivator, and recruit SRC-1 and RNA polymerase II. Preliminary results demonstrate that, upon treatment with 1uM nitrite, wild-type ER-alpha dissociated from hsp90, was recruited to DNA, bound coactivator, and recruited SRC-1 and RNA polymerase II to DNA. Site A mutants, K529A and N532A, did not dissociate from the hsp90-receptor complex, bind to DNA or bind coactivator. The site B mutants, H516A and K520A, dissociated from hsp90. However, H516A did not bind to DNA or recruit coactivator, while K520A bound to DNA, but did not recruit coactivator. The site C mutants, C381A and H547A, dissociated from hsp90. C381A failed to bind to DNA, while H547A was recruited to DNA but failed to bind coactivator. The results suggest a model whereby the interaction of nitrite with site A results in a conformational change at the interface of helix H11 and loop 11-12 that is necessary for the dissociation of hsp90. Site B is involved in the formation of a continuous helix between helices H10 and H11 that is necessary for binding to DNA. Site C, formed by amino acids located on helices H4 and H12, is involved in the recruitment to DNA as well as the formation of the coactivator binding site. Together, these findings represent a novel role for the anion nitrite in the activation of ER-alpha.

Sources of Research Support: National Institutes of Health R21ES015160-02 awarded to MBM; DOD BCRP Predoctoral Traineeship Award BC073599 awarded to GBS.

Nothing to Disclose: GBS, KLK, MBM

P3-7

Estradiol and Body Composition in Men.

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Background: The relationship between sex steroids and body composition in men has received considerable attention. While testosterone levels have been shown to increase lean mass and decrease fat mass in men, the association between estradiol (E2) and body composition is still uncertain.

Objective: To examine the association between E2 and body composition in men.

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. Age and race/ethnicity were obtained via self-report, and weight, body mass index (BMI), and waist-to-hip ratio (WHR) were measured during study examinations. Body composition parameters including percent lean/fat mass, percent trunk lean/fat mass were measured by dual x-ray absorptiometry. E2 was measured by liquid chromatography-tandem mass spectrometry and free E2 was calculated by using mass action equations. Partial Pearson correlation coefficients (adjusted for age, physical activity, and smoking pack years) were used to estimate the linear relation between the body composition parameters and E2.

Results: Of the 760 men with complete data included in this analysis, the mean age was 46.6 years and the mean (SD) of free E2 concentration was 2.41 (0.91) pmol/l and that for total E2 was 85.04 (31.9) pmol/l. Percent lean mass, trunk lean mass, and appendicular lean mass were negatively and significantly correlated with total E2 levels after multivariate adjustment (partial correlations ranged between -0.10 and -0.14; all $P \leq 0.03$). Conversely, percent fat mass, weight, BMI, waist, and WHR were significantly positively associated with free E2 levels (partial correlations ranged between 0.11 to 0.18; all $P \leq 0.04$) after multivariate adjustment. Percent trunk fat mass, but not appendicular fat mass, was significantly positively associated with free E2 ($r=0.13$, $P < 0.001$). Further adjustment for race/ethnicity in the multivariate models did not change the results. The results for total E2 were qualitatively similar.

Conclusions: In our diverse population, free E2 levels in men are negatively correlated with lean mass and positively correlated with measures of fat mass. Prospective studies are needed to establish the directionality of the association between E2 and the various body composition parameters.

Sources of Research Support: NIH Grant R01AG020727.

Nothing to Disclose: RAM, GRC, ABA

P3-8

Raloxifene Induces Nucleolar Translocation of the Estrogen Receptor.

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Raloxifene (RLX), a selective estrogen receptor modulator (SERM), is widely used for the prevention and treatment of postmenopausal osteoporosis. RLX binds to the estrogen receptor alpha (ER α) and acts as an estrogen agonist in some tissues, and as an antagonist in others. However, the molecular mechanism underlying the tissue specificity of SERMs has not been clarified. In this study, we examined the intracellular localization of ER α using a green fluorescent protein (GFP)-tagged protein in the presence of RLX in culture cells from various tissues. Although ER α formed intranuclear foci in the presence of estradiol (E2), RLX translocated ER α into the nucleoli in breast cancer cell lines. This phenomenon was not observed in cells from other tissues. Immunofluorescence staining revealed that endogenous ER α was also translocated into the nucleoli in the presence of RLX. Other ER α antagonists such as ICI 182,780 and tamoxifen did not induce this nucleolar translocation of ER α in the breast cancer cells. These results suggest that nucleolar translocation of ER α is a specific phenomenon of RLX treatment. Fluorescence recovery after photobleaching (FRAP) analyses showed that RLX-bound ER α was much less mobile than E2-bound ER α . Immunofluorescence staining revealed that endogenous ER α was also translocated into the nucleoli after exposure to RLX in breast cancer cells, whereas there was no significant accumulation of ER α in the nucleoli in cells from other tissues. As two ER α mutants carrying amino acid substitutions in the coactivator-binding domain of helix 12 (L536A or L539A) failed to be translocated into the nucleoli in the presence of RLX, these residues were clearly essential to the nucleolar translocation of ER α . These results suggest that translocation of ER α into the nucleoli is RLX-specific and is a key event for RLX-induced growth repression of mammary gland cells. This RLX-induced nucleolar translocation of ER α might explain the tissue specificity of SERMs.

Nothing to Disclose: MG, HK, KO, MN, RT

P3-9

Transcription Factor Krüppel-Like Factor 9 (KLF9) as Potential Predictor of Dysfunctional Estrogen Receptor- α Signaling in the Uterus.

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Krüppel-like factors (KLFs) are zinc finger-containing transcription factors that are implicated in diverse physiological processes in the reproductive tract. We previously showed that KLF9, a 33kDa protein family member, influenced estrogen receptor- α (ER α) expression and activity in mouse and human uterine cells, suggesting its potential role as a nuclear receptor co-regulator. KLF9 knockdown in Ishikawa endometrial cancer cells with siRNAs impaired ER α autoregulation, leading to enhanced ER α signaling. Further, we and others have reported the loss of KLF9 expression in human endometrial tumors. To determine the contribution of KLF9 to ER α signaling *in vivo*, we superimposed *Klf9* null mutation with the diethylstilbestrol (DES) model of carcinogenesis in mice and evaluated uterine morphological and molecular indices as predictors of dysfunctional estrogenic stimulation. Wildtype (WT) and *Klf9* null (KO) female mice were injected with sesame oil or DES (2 μ g/pup/day) on postnatal (PND) days 1-5, and uteri were collected at PND84. Compared to WT, KO mice had larger and reduced numbers of endometrial glands; DES exposure exacerbated these differences. KLF9 loss resulted in increased glandular ER α immunoreactivity with DES, without affecting serum estradiol levels. QPCR analyses of WT and KO uterine RNAs indicated altered expression of genes commonly dysregulated in endometrial cancers (*Akt1*, *Mmp9*, *Slpi*, *Tgf β 1*) and those involved in growth regulation (*Fos*, *Myc*, *Tert*, *Syk*), alone and in concert with DES. Since early DES exposure leading to aberrant adult uterine phenotypes has been linked to epigenetic mechanisms involving changes in chromatin modification enzyme expression, uterine transcript levels of DNA methyltransferase-1 (*Dnmt1*) and histone deacetylase (*Hdac*) 3, 6, and 9 were also evaluated. *Hdac9* transcript levels were increased with loss of *Klf9* and early DES exposure. Collectively, these data support a functional KLF9/ER α molecular network in the uterus and suggest that KLF9 and its downstream targets may constitute 'molecular fingerprints' for early diagnosis of endometrial carcinoma arising from dysregulated ER α signaling. The predictive significance of these altered genes in clinically relevant tissues will be addressed by analyses of endometrial tumors and adjacent non-tumor tissues collected from women undergoing surgery.

Sources of Research Support: NIH-HD21961, Arkansas Biosciences Institute Grants Program, Arkansas Children's Hospital Research Institute Student and Clinical Staff Research Intramural Grant, and UAMS Committee for the Allocation of Graduate Student Research Funds.

Nothing to Disclose: CDS, JMPP, AFB, TF, MTS, DG, FAS, RCMS

P3-10

Role of ER α in Prostate and Urological Disorders – Using In Vivo Cre-LoxP Knockout Strategy.

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Estrogen receptors were reported to be associated with disorders of several urologic organs, including prostate, testis, and bladder. However, the detailed mechanisms and how ER functions in a selected type of cells in these organs remain largely unclear. There are two types of Estrogen Receptors, ER α and ER β . Results from conventional ER α and ER β knockout (ER α KO and ER β KO) mice indicated that ER α and ER β could play differential roles in the urological diseases. We recently applied the cre/loxP system to generate total ER α KO mice by mating ACTB-cre with floxed ER α mice (ACTB-cre ER α KO). Our results showed that the model could ablate ER α protein expression without any truncated ER α remaining and had more severely defected phenotypes as compared with conventional neo-ER α KO mice. Our data demonstrate that ER α is important for branch morphogenesis of the prostate and this is possibly mediated by the regulation of expression of BMP4, a critical factor in regulating the branch morphogenesis. In addition to studying prostate ER α roles, it was reported that antiestrogen treatment could have beneficial effects for bladder cancer patients. To understand ER α roles in bladder carcinogenesis, we uses the UPII-cre ER α KO mice to characterize the ER α roles in bladder cancer. Meanwhile, we found that mice with 3 week-implantation of DES, a synthetic estrogen, show increased proliferative signal and enlargement of bladder size, increased thickness of urothelial layer and stromal compartment. These effects are mediated by ER α since ER α KO mice cannot respond to DES exposure. Higher expression of ER α in bladder cancer cells could increase the cell viability and colony forming capacity of bladder cancer cells. Furthermore, we and others found ER α is positively expressed in testicular germ cells, Leydig cells, and epididymis. ACTBcre-ER α KO male mice are infertile and the sperms had less motility. To initiate our understanding of ER α functions in the epididymis and sperm mobility, we recently generated a transgenic mouse with GPX5 promoter-driven Cre (GPX5-Cre) where GPX5 promoter is reported to be specifically expressed in epididymis. We are breeding the GPX5-Cre ER α KO mice to test whether ablation of epididymal ER α could affect sperm motility. Together, our findings of ER α roles in selective type of cells in prostate, bladder and epididymis could potentially lead to developing alternative treatments for prostate cancer, bladder cancer or fertility disorders.

Sources of Research Support: NIH Grant CA137474.

Nothing to Disclose: MC, IH, SS, SY

P3-11

Estrogen Receptor Alpha Suppresses the Expression of Testicular Steroidogenic Enzyme Genes through the Crosstalk with Orphan Nuclear Receptor Nur77.

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Estrogen receptor alpha (ER α) is one of the important factors in male reproductive track development and fertility. The exposure of animals to excess amount of estrogenic compounds induces abnormal fetal Leydig cell development and testicular steroidogenesis. Although the role of ER α in testicular Leydig cells has been confirmed with deletion mutants of ER α in mice, the mechanism by which ER α controls the function of Leydig cells has not been elucidated. Here, we investigated the effect of estrogen and its receptor on the expression of steroidogenic enzyme genes and the molecular mechanism for such an effect in MA-10 Leydig cells. Estrogen treatment of Leydig cells repressed the expression of steroidogenic enzyme genes in a dose-dependent manner. Further studies revealed that ER α suppressed the transactivation of Nur77, resulting in the repression of steroidogenic enzyme gene expressions. Nur77 is one of major transcription factors that regulate the expression of steroidogenic enzyme genes in Leydig cells. Interestingly, ER α suppressed Nur77 transactivation irrespective of its ligand, although the degree of suppression was much larger in the presence of estrogen. The ER α -mediated suppression of Nur77 transactivation was due to the inhibition of DNA binding activity of Nur77 by ER α , based on the analysis of electrophoretic mobility shift assays. In addition, co-expression of ER α decreased the protein level of Nur77 both in the presence and absence of estrogen. All together, these results suggest that ER α /estrogen represses the expression of steroidogenic enzyme genes through the suppression of Nur77 transactivation and the down-regulation of Nur77 protein stability, resulting in the inhibition of testicular steroidogenesis. These finding may provide a mechanistic explanation for the abnormal testicular steroidogenesis by estrogenic compounds.

Nothing to Disclose: S-YL, KL

P3-12

Primary Role of the Estrogen Receptor Alpha and the RAR α Apoproteins in Maintaining the Basal Cycling State of Hormone Depleted or Tamoxifen-Treated Breast Cancer Cells.

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The development of resistance to hormonal adjuvant therapies in breast cancer implies persistence of a cycling fraction of tumor cells during the treatment for which the underlying causes need to be elucidated. Estrogen-sensitive MCF7 breast cancer cells depleted of hormone for an extended period in conditioned media exhibited basal proliferation that was not abrogated by 4-Hydroxy tamoxifen (OH-Tam) at therapeutically relevant concentrations (0.1 μ M to 0.5 μ M). Knocking down ER inhibited the basal proliferation by promoting G1 arrest and modestly (from 6.8% to 8.3%) increased the rate of apoptosis. OH-Tam did not affect the expression of most genes supported by ER in the absence of hormone including the basal expression of retinoic acid receptor α (RAR α) and that of ER repressed genes, which were primarily growth inhibitory. Knocking down basal apo-RAR α recapitulated the effects of the ER knockdown. Restoration of the basal levels of apo-RAR α in the ER knockdown cells by ectopic expression rescued basal cell cycling. The results indicate that in hormone-sensitive breast cancer cells, ER can maintain a basal fraction of cells in S-phase by mechanisms unaffected by hormone depletion or OH-Tam primarily through maintenance of the basal expression of apo-RAR α . ER may, in this manner, limit the long-term adjuvant effects of either hormone depletion or tamoxifen in breast cancer enabling the eventual development of resistance to those treatments.

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Nothing to Disclose: MdS, IK, RT, MR

P3-13

Sp1 Response Elements within the MAPK Phosphatase-1 (MKP-1/DUSP1) Promoter Mediate Progesterone Receptor (PR) Induced MKP-1 Expression in Breast Cancer Cells.

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The roles of progesterone (P₄) and of PR in development and pathogenesis of breast cancer remain unclear. Previously, we observed that P₄/PR had anti-inflammatory effects in breast cancer cells (1). We recently found that treatment of T47D breast cancer cells with P₄ antagonized effects of fetal bovine serum (FBS) and IGF-I to stimulate cell proliferation, while siRNA-mediated knockdown of endogenous PR in T47D cells abrogated these anti-proliferative effects of P₄. To begin to define mechanisms for this anti-proliferative action of P₄/PR, we considered the role of MKP-1, which catalyzes dephosphorylation of MAPKs, resulting in their inactivation. In T47D cells, treatment with P₄ or R5020 induced both MKP-1 mRNA and protein expression. R5020 induction of MKP-1 mRNA was repressed by the PR antagonist RU486 and by siRNA knockdown of PR, suggesting a role of endogenous PR in MKP-1 upregulation. Importantly, P₄ induction of MKP-1 was associated with reduced levels of phosphorylated ERK-1/-2. Further, with siRNA knockdown of MKP-1, P₄ lost its ability to dephosphorylate ERK-1/-2 and to repress IGF-1- or FBS-induced cell proliferation. This suggests that induction of MKP-1 by P₄ mediates the anti-proliferative action of P₄/PR in T47D breast cancer cells. Using luciferase reporter constructs and deletion mapping of the *hMKP-1* promoter and first exon, we found that the region between +18 to +113 mediated P₄ induction of *MKP-1* promoter activity in transfected T47D cells. Site-directed mutagenesis of three potential Sp1 response elements at +20, +54 and +86 abrogated P₄ induction of luciferase activity, suggesting their functional role. Furthermore, using chromatin immunoprecipitation, we observed that P₄ treatment enhanced PR recruitment to these putative Sp1 response elements. Treatment of T47D cells with mithramycin A, an inhibitor of Sp1 binding to DNA, reduced the induction of MKP-1 mRNA expression by P₄, suggesting that PR may interact with Sp1 bound to these elements. Collectively, these findings suggest that MKP-1, a critical mediator of anti-proliferative and anti-inflammatory actions of P₄ in breast cancer cells, is induced by PR interaction with Sp1 bound to response elements within its first exon.

(1) Hardy DB et al., Mol Endo 2008; 22:1812

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Nothing to Disclose: C-CC, CRM

P3-14

A Progesterone Responsive Gene, 14-3-3 Tau, Upregulates the Transcriptional Activity of Progesterone Receptor B (PR-B) in Uterine Endometrium.

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[Objectives]14-3-3 proteins have been known to modulate signal transduction. In the present study, we investigated the regulation and expression of 14-3-3 tau, and determined its function on transcriptional activity of progesterone receptor (PR) in uterine endometrium.[Materials and Methods]Ovariectomised rats were treated with 17-beta estradiol (E2) and/or progesterone (P), and the uteri of these rats were used for quantitative RT-PCR and immunohistochemistry. The amount of 14-3-3 tau mRNA was investigated in cultured human endometrial stromal cells (ESCs), and cultured human endometrial epithelial cells (EECs) treated with E2 and/or P. ESCs and EECs were also treated with cAMP and MPA for *in vitro* decidualization. RNAs were extracted and used for quantitative RT-PCR. The localization of 14-3-3 tau in human endometrium during menstrual cycle was detected by immunohistochemistry. Endometrial tissues were collected from patients undergoing hysterectomy for benign gynaecological conditions. The experimental procedures were approved by the institutional review board of the University of Tokyo. Flag tagged 14-3-3 tau expressing vector, HA tagged PR-B expressing vector, and luciferase reporter plasmid were transfected into endometrial adenocarcinoma Ishikawa cells, in order to analyze the function of 14-3-3 tau on the transcriptional activity of progesterone receptor B (PR-B).[Results]The amount of 14-3-3 tau mRNA significantly increased in rat uteri 12h after the administration of P4, but not after the administration of E2. Immunohistochemistry revealed that the 14-3-3 tau protein was detected in endometrial stromal cells and epithelial cells 24h after the administration of P4. The amount of 14-3-3 tau mRNA significantly increased in both EECs and ESCs 12h after the treatment of P4. The amount of 14-3-3 tau mRNA also significantly increased in ESCs by the stimulation of *in vitro* decidualization. By immunohistochemistry the strong positive signals of 14-3-3 tau were detected in human endometrial epithelial cells and stromal cells during the mid secretory phase of menstrual cycle. The transcriptional activity of PR-B significantly increased in Ishikawa cells overexpressing 14-3-3 tau, with P4, and without P4.[Conclusion]This study revealed that 14-3-3 tau is one of the progesterone responsive genes in uterine endometrium. 14-3-3 tau could increase the transcriptional activity of PR-B.

Nothing to Disclose: MI, HH, TU, MM, YH, RT, MK, TY, SI, YT

P3-15

Cell Cycle Dependent Regulation and Association of Progesterone Receptors with Cyclin D in Breast Cancer Cells.

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Activation of progesterone receptors (PR) by progestins initiates breast cancer cell cycle progression via sequential expression and activity of cyclins and cyclin-dependent kinases (CDKs). While proliferating cells in the normal breast are devoid of steroid hormone receptors, cyclin D and PR are often co-upregulated in breast cancer cells. Progestins rapidly activate CDK2; full length PR (PR-B) contains 8 CDK2 consensus phosphorylation sites and 17 Cy motifs for direct cyclin binding. Additionally, PR or cyclin D knock-out mice display identical delays in lobeolaveolar development, while transgenic mice overexpressing either of these molecules exhibit mammary hyperplasias. Herein, we hypothesized functional linkages between PR and cyclin D1. To model cell cycle-dependent regulation of PR, T47D breast cancer cells were synchronized to induce cell cycle progression in the absence of progestins. Mimosine-induced synchronization (in late G1/early S phase) resulted in phosphorylation of PR at CDK2 and casein kinase 2 (CK2) consensus sites, Ser400 and Ser81, respectively. Nocodazole-synchronized cells (in G2M phase) contained PR phosphorylated on Ser400, and the MAP kinase sites, Ser294 and Ser345, but not Ser81. PR phosphorylation events are associated with hypersensitive or ligand-independent PR transcriptional activity and promoter selectivity. PR interactions with cyclins E and A have been described. We found that PR-B associated with cyclin D in co-immunoprecipitation experiments. Mutants of PR and cyclin D1 demonstrated that this interaction did not require PR Ser400 phosphorylation or CDK2 activity or cyclin D interaction with CDK4/6, suggestive that PR and cyclin D1 associate in a constitutive manner. Most notably, selected genes upregulated in breast cancer and first identified as associated with cyclin D overexpression are also highly responsive to PR. These results underscore the need for a clearer understanding of PR regulation as part of cyclin/CDK complexes in rapidly dividing breast cancer cells and suggest that in addition to ER-alpha, improved breast cancer therapies should include PR as a valuable endocrine target.

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Nothing to Disclose: GED, CAL

P3-16

Differential and Interactive Effects of Ligand-Bound Progesterone Receptor A and B Isoforms on Tyrosine Hydroxylase Promoter Activity.

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Progesterone receptors (PRs) are expressed in hypothalamic and brainstem catecholaminergic neurons. Progesterone differentially alters neuronal activity in a brain region-specific and reproductive cycle-dependent manner. PR expression shows cyclic rhythms during the reproductive cycle, which has the potential to change both PR content as well as the ratio of A and B isoforms in specific brain nuclei. In particular, PRs are highly expressed in tuberoinfundibular dopamine neurons and ovarian steroids influence prolactin release in part by regulating dopamine synthesis. Tyrosine hydroxylase (TH) is the rate-limiting enzyme and progesterone can exert both stimulatory and inhibitory actions on TH activity and TH mRNA levels in these dopamine neurons. The aims of this study were to: 1) determine the independent transcriptional effects of PRA and PRB on the TH promoter, 2) map the location of progesterone's action using TH promoter deletion constructs and 3) evaluate interactions of PRA and PRB on TH promoter activity. TH positive neuronal mouse CAD cells were transiently transfected with 1) CMV-PR-A and/or CMV-PR-B, 2) TH-9000 luciferase, TH-2400 luciferase or TH-272 luciferase and 3) CMV-Renilla luciferase (transfection efficiency control) constructs. Transfected cells were treated for 20 hours with vehicle (0.1% ethanol) or 200 nM progesterone and then TH promoter activity was assessed using the Promega dual luciferase assay. When PRA was transfected alone, progesterone treatment did not alter TH-9000 promoter activity above basal levels. In contrast, when cells were transfected with PRB alone, progesterone treatment increased TH-9000 and also TH-2400 promoter activity 7 fold and 6 fold, respectively, compared to vehicle-treated cells, but did not alter TH-272 promoter activity. When PRA and PRB were transfected at a 1:1 ratio, the fold activation of the TH-9000 promoter was about 50% of the activation of PRB alone after 20 hours of progesterone treatment. These data indicate that ligand bound PRB may act as a transcriptional activator of the TH gene and that its site of action lies between the -272 and -2400 positions of the TH promoter, whereas PRA may inhibit the activities of PRB on the TH promoter. Taken together, these data suggest that progesterone actions on the TH promoter are determined by the PR isoform expressed, and changes in the ratio of PRA to PRB may affect the ability of progesterone to increase TH expression.

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Nothing to Disclose: PJJ, LAA

P3-17

Evidence that Activation of the mTOR Pathway by Human Chorionic Gonadotropin Leads to Stimulation of Steroidogenic Enzyme Expression in Theca-Interstitial Cells.

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Theca-Interstitial (T-I) cells secrete androgens in response to luteinizing hormone (LH) and play a central role in the maintenance of ovarian integrity and function. In pathological conditions such as polycystic ovarian syndrome (PCOS), the T-I cells are hyperactive leading to hyperandrogenism and anovulation. Although LH induces androgen synthesis by triggering cAMP/PKA-dependent signaling cascades, the roles of other intracellular signaling pathways are not well characterized. In the present study, we examined the possible role of LH/hCG-mediated activation of mammalian target of rapamycin (mTOR) on the expression of steroidogenic enzymes. T-I cells isolated from 25 day-old rat ovaries by collagenase digestion were cultured in the presence of hCG (50 ng/ml) for 0, 12 and 24 h and whole cell lysates were analyzed for the activation of downstream targets of mTOR and steroidogenic enzymes by Western blot analysis. The results revealed that hCG treatment resulted in a two-fold increase in ribosomal protein S6 kinase (S6K1) and eukaryotic initiation factor 4E (eIF4E), downstream targets of mTOR signaling. In addition, the expression of Cyp11A1 and Cyp17A1 were also increased in a time-dependent manner. Furthermore, hCG treatment significantly reduced TSC2 protein expression, thereby reducing its role as a repressor of mTOR signaling. In order to further examine the role of mTOR signaling in steroidogenesis, cells were pretreated with the mTOR inhibitor, rapamycin (20 nM) for 1 h, followed by stimulation with hCG for additional 6 h. Total RNA was isolated and Cyp11A1, 3 β -HSD and Cyp17A1 mRNA levels were examined by quantitative PCR. The results showed that hCG causes a three-fold increase in the expression of mRNA encoding all three steroidogenic enzymes. Pretreatment with rapamycin significantly reduced this hCG-induced response. Additionally, treatment with rapamycin for 24 h reduced the hCG and forskolin-induced increases in Cyp11A1 and Cyp17A1 protein expression. However, StAR protein expression, which was induced by hCG or forskolin, was not affected by rapamycin treatment. These results suggest that hCG-mediated mTOR signaling specifically regulates the expression of steroidogenic enzymes in T-I cells. Taken together, our data suggest that hCG stimulates the mTOR signaling pathway, which in turn increases the translational machinery leading to up-regulation of steroidogenic enzymes contributing to stimulation of androgen synthesis in T-I cells.

Sources of Research Support: NIH Grant HD-38424.

Nothing to Disclose: MP, KMJM

P3-18

Polyunsaturated Fatty Acids Modify Prostaglandin and Steroid Output from Bovine Ovarian Cells *In Vitro*.

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Polyunsaturated fatty acids (PUFAs) form ~7% of the total energy in a typical daily diet in the modern western world¹. Consisting of multiple carbon double bonds, they are classified as n-3, n-6 and n-9 according to the position of the first double bond. PUFAs physiological function far exceeds that of an energy source². They act as substrates to the cyclooxygenase enzymes (COX-1/2) for the synthesis of prostaglandins (e.g. prostaglandin E₂:PGE₂ and prostaglandin F_{2α}:PGF_{2α}), which are important regulators of granulosa cell steroidogenesis³. PUFAs are also known to affect steroidogenesis through actions on steroidogenic acute regulatory protein (StAR). Hence in this study we have assessed the ability of either n-3 or n-6 PUFAs to modify ovarian steroid synthesis and attempted to establish whether these actions are exerted directly or indirectly through altered prostaglandin synthesis.

Bovine granulosa cells from antral follicles (2-8mm) were isolated and cultured at a density of 3x10⁶/ml for 96h in McCoy's 5A medium containing supplements including; 0.1% fatty acid free bovine serum albumin. At 48h, cells were treated with 100μM PUFA (linoleic acid LA; n-6), linolenic acid (LNA; n-3) arachidonic acid (AA; n-6) and eicosapentaenoic acid (EPA; n-3) with or without 100μM indomethacin (IMC; COX inhibitor). Spent culture media was assessed for PGE₂ and progesterone (P₄) by radioimmunoassay. COX-1/2 and StAR mRNA were analysed by qPCR. Data was expressed as % change from control, statistical significance were determined by a one-way ANOVA (p<0.05). The n-6 PUFAs, LA and AA, stimulated P₄ secretion. AA (but not LA) stimulated expression of StAR, COX-2, and secretion of PGE₂ (p<0.05). As expected IMC inhibited basal PG levels but surprisingly increased basal P₄ secretion (P<0.05). Neither n-3 PUFA had any effect on P₄, StAR, COX-2 or PGE₂. However, in the presence of IMC, both EPA and LNA stimulated P₄ secretion (p<0.05).

These data suggest that n-6 PUFAs affect P₄ synthesis through different mechanisms: LA stimulates steroidogenesis independently of StAR and PGE₂ whereas AA stimulates both PGE₂ and StAR in inducing steroidogenesis. As the n-3 PUFAs increased P₄ in the presence of IMC without affecting either COX expression or PGE₂, it is likely that the PUFA stimulation of steroid synthesis is independent of PGs. However, we have yet to establish whether the PG-independent effects are mediated directly through actions on StAR expression.

(1) Grundy SM. Am J Clin Nutr. 1997 Oct;66(4 Suppl)

(2) Zachut M et al. Reproduction, 2008, 135, 5, 683-692.

(3) Richardson MC. Reproductive Biology, 1986, 8, 321-378.

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Nothing to Disclose: HJLR, ZC, RF, DCW, DREA

P3-19

Identification of Hormone-Induced Bioactive Mitochondrial Protein Complexes in MA-10 Leydig Cells.

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Cholesterol transfer from the outer mitochondrial membrane (OMM) to the inner mitochondrial membrane (IMM) is the rate-limiting and hormone-sensitive step in the acute regulation of steroid hormone synthesis. The hormone-induced transfer of cholesterol to the OMM is accomplished by the formation of a multimeric protein complex anchored at the OMM by the translocator protein (18 kDa; TSPO) and composed of the cytosolic proteins; steroidogenic acute regulatory protein, TSPO-associated protein PAP7 and PAP7-associated regulatory subunits of the cAMP-dependent protein kinase, as well as the TSPO-associated voltage-dependent anion channel. To evaluate how upon hormonal stimulation cholesterol reaches the IMM enzyme CYP11A1, which initiates steroid synthesis, we analyzed the presence of such complexes in control and hormone-treated MA-10 Leydig cells. We used Blue Native- Polyacrylamide Gel Electrophoresis (BN-PAGE) followed by immunoblot analysis and mass spectrometry to identify protein complexes in control cells, in which TSPO was found in 66 and 800 kDa protein complexes. A 140 kDa complex containing CYP11A1 was identified as well. Upon hormone treatment, however, CYP11A1 was identified in the 800 kDa complex. Many proteins that belong to both the OMM and IMM were found in the 800 kDa complex suggesting that it may be part of the OMM/IMM contact sites. To investigate where cholesterol is associating with the mitochondria for conversion to pregnenolone radiolabeled photo-activatable azido-cholesterol was incubated with the isolated mitochondria, solubilized and run on a BN-PAGE gel. Cholesterol was found to bind at the 66kDa complex where TSPO was also identified to be present. To determine whether the previously identified CYP11A1-containing complexes are functional we devised a method using a chemically synthesized CYP11A1 probe, cholesterol resorufin in which cleavage of cholesterol to form pregnenolone by CYP11A1 releases the fluorescent resorufin. The results obtained demonstrated that under basal conditions the 140 kDa complex contains a functional CYP11A1. Upon hormonal stimulation, this activity moves to the 800 kDa complex and steroidogenesis begins. These results suggest that acute hormone-induced steroidogenesis begins at the 66kDa TSPO monomer site where cholesterol initially binds and is delivered to the 800 kDa mitochondrial OMM/IMM contact site complex for conversion into pregnenolone.

Sources of Research Support: NIEHS; CIHR.

Nothing to Disclose: MBR, GR, JB, DAP, TV, VP

P3-20

A Genome-Wide Expression Profile of Steroidogenic Cells Selectively Derived from Adrenal Glands of Knockout Mice Lacking Steroidogenic Acute Regulatory Protein by Targeted Expression of Enhanced Green Fluorescent Protein.

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Steroidogenic acute regulatory protein facilitates cholesterol transfer from the outer to the inner mitochondrial membrane in the rate-limiting step of steroidogenesis. Both human individuals having *STAR* mutations (congenital lipoid adrenal hyperplasia, lipoid CAH) and knockout mice lacking *Star* (*Star*^{-/-} mice) show defective synthesis of steroid hormones and diffuse accumulation of lipid droplets in adrenal glands and gonads. It has been well described that the onset of steroid hormone deficiency is variable among adrenal cortex, testis, and ovary, leading to a two-hit model for the pathogenesis of lipoid CAH that steroidogenic cells initially retain some capacity for STAR-independent steroidogenesis, but ultimately accumulate cholesterol esters that abrogate the residual capacity (1). However, the molecular mechanism for the two-hit model remains unclear.

In order to define specific patterns of molecular changes with disruption of *Star*, we performed a transcriptome analysis of steroidogenic cells in adrenal glands. We harvested adrenal glands at E17.5 or 18.5 from *Star*^{+/+} males (N=4) or *Star*^{-/-} males (N=4) having the transgene expressing enhanced green fluorescent protein under the control of the *Star* regulatory sequences (2). After the dissociation of adrenal glands into single cells, we collected steroidogenic cells selectively by fluorescent-activated cell sorting. We generated the gene expression profile of the fluorescence-positive cells by Agilent Whole Mouse Genome Microarray G4122F and confirmed the relative expression levels of the selected genes by real time RT-PCR. We considered genes of interest as having significantly differential expression by fold difference ≥ 2 and *p* value < 0.05 .

We identified 591 and 508 transcripts that were significantly up-regulated and down-regulated, respectively, in adrenal steroidogenic cells derived from *Star*^{-/-} mice compared with *Star*^{+/+} mice. In *Star*^{-/-} mice, expression levels of genes involved in antigen processing and presentation or immune response were significantly increased, whereas those of genes related to steroid biosynthetic pathway or lipid transport were significantly decreased. These data suggest *Star*^{-/-} mice-derived adrenal steroidogenic cells exhibit characteristic changes, which could result from cholesterol ester accumulation in the cytosol. The transcriptome analysis of *Star*^{-/-} mice-derived steroidogenic cells would provide a robust approach to clarify the pathogenesis of lipoid CAH.

(1) Bose H, Sugawara T, et al., N Engl J Med 1996; 335: 1870

(2) Sasaki G, Ishii T, et al., Mol Endocrinol 2007; 22 : 951

Nothing to Disclose: TI, MH, MT, AS, SN, SS, YM, KLP, TH

P3-21

Glucocorticoid Synthesis Pathways in Pre- and Post-Natal Lungs.

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Glucocorticoids are regulators of male and female lung development. In previous experiments, we showed adrenal-like expression of mRNA encoding for the enzymes involved in glucocorticoid synthesis from cholesterol in gestational day (GD) 15.5 murine lungs (1). We present here a study testing the sexual and temporal specificity and functionality of this pathway during cannalicular, saccular and alveolar murine lung development. Expression of P450_{scc}, 3 β -hydroxysteroid dehydrogenase (HSD), 11 β -hydroxylase (cyp11b1) and 21-hydroxylase (cyp21) mRNAs was measured by QPCR in mouse lung samples of both sexes from GD 15.5 to postnatal day (PN) 30. Expression of cyp11b1 was restricted to GD 15.5, which confirms previous data (1). On the other hand, p450_{scc}, 3 β -HSD and cyp21 mRNA expression was maintained during the saccular (GD 17.5 - PN 5) and alveolar (PN5 - PN30) stages. Presence of cyp21 and cyp11b1 proteins was assessed by western blots. Cyp21 protein was detected in all the samples, whereas cyp11b1 was restricted to GD15.5. Finally, lung explants were cultured in the presence of ³H-progesterone (PROG), ³H-deoxycorticosterone (DOC) and ³H-corticosterone (CORTICO), and the conversion pattern of each steroid was studied by thin layer chromatography (TLC). Lungs at GD 15.5 transformed PROG, DOC and CORTICO at a much faster rate than saccular or alveolar stage lungs. Addition of unlabeled DOC to ³H-PROG-containing media increased accumulation of a product corresponding to DOC, which suggests cyp21 activity. Nevertheless, we were unable to observe CORTICO formation using DOC or PROG precursors. However, the conversion pattern of ³H-PROG by GD 15.5 lungs was distinct from that seen in saccular, alveolar or adult samples. Indeed, the GD 15.5 pattern shows two specific bands of retention factors lower than that of CORTICO. Our results strongly suggest that an adrenal-like glucocorticoid synthesis pathway exists in the developing mouse lung for both sexes at GD 15.5, but that an incomplete pathway exists in saccular and alveolar lungs in which only the production of DOC is observed. Biological significance of DOC production by the lung is still unclear, but could constitute a latent source of glucocorticoid or mineralocorticoid action as suggested by luciferase assays done in other studies (2) and confirmed in our laboratory.

[1]. Provost, P.R. and Tremblay, Y. *Endo* 2005. 14(5): p. 2239-45.

[2]. Lu, N.Z., et al., *Pharmacol Rev* 2006. 58(4): p. 782-97.

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Nothing to Disclose: EB, AD, PRP, YT

P3-22

Expression and Regulation of CYP17 in the Human Placenta.

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It has long been assumed that the human placenta has significantly reduced CYP17 expression that limits its capacity to produce estrogens without the continuous supply of androgenic precursors from the fetus. Immunohistochemical studies from our laboratory have demonstrated immunoreactivity for CYP17 in the cytoplasm of human syncytiotrophoblasts, which we have confirmed by western blot analysis. OBJECTIVES: To assess the biosynthetic capacity of the human placenta to express CYP17 and generate androgens *de novo*, and to elucidate under which signaling pathways these may be regulated. METHODS: Two cell types were utilized in all experiments, human primary placental cells extracted from mid-trimester placentas and Jeg3 from a human placental line. Cells in baseline experiments were grown in media without the addition of steroid precursors. The media was exchanged and collected daily for steroid measurement via radioimmunoassay. To optimize the maximal enzymatic activity, experiments were duplicated in the presence of 22-hydroxycholesterol (5 μ M). CYP17 expression was quantified by RT-PCR. Protein kinase A pathway activator and inhibitor, forskolin (10 μ M) and H89 (10 μ M), respectively, were used to assess this pathway's role in placental androgen modulation. RESULTS: 17-hydroxyprogesterone, androstenedione and estradiol were all easily detected and quantified in both cell types. The daily production of 17-hydroxyprogesterone and androstenedione ranged from 0.05-0.5 ng/mg of cellular protein, with a higher production in primary placental cells. Estradiol generation was similar in both cell types, 100-200 pg/mg of protein per day. Dehydroepiandrosterone was undetectable throughout. No discrete trends in the rate of steroid production were noted over five days of cell culture. In the presence of 22-hydroxycholesterol, 17-hydroxyprogesterone and androstenedione production increased 5-8 fold, to a rate similar to that seen in non-pregnant premenopausal women. CYP17 mRNA was easily detected by standard RT-PCR. When treated with forskolin, CYP17 mRNA levels increased 10-fold from baseline, meanwhile H89 suppressed its expression. Co-treatment with forskolin and H89 completely inhibited the inductive effect of forskolin. CONCLUSION: Human placental cells are capable of producing androgens and estradiol *de novo* in the absence of a fetal compartment. CYP17 mRNA is easily detected and its expression is regulated at least in part by the PKA pathway.

Nothing to Disclose: JCE, SSP, VEB, BRC

P3-23

Evidence for the Existence and Significance of an Alternative Pathway towards Androgen Synthesis in the Human Fetal Adrenal.

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P450 oxidoreductase deficiency (ORD) is a unique congenital adrenal hyperplasia variant in that it can be associated with undervirilization in boys and virilization in girls, 46,XY and 46,XX disordered sex development (DSD). POR has a pivotal role as electron donor to all microsomal CYP enzymes, including CYP17A1, which catalyzes the synthesis of the crucial androgen precursor dehydroepiandrosterone (DHEA). Consequently, ORD results in low circulating androgens, which explains 46,XY DSD but not 46,XX DSD. This paradox has been proposed to be explained by a novel pathway to androgens in the fetus, which generates 5 α -dihydrotestosterone (DHT) from 17-hydroxyprogesterone without the need of DHEA synthesis, though also requiring CYP17A1 for converting 17-hydroxyallopregnanolone (5 α 17HP) to androsterone (An). To determine the existence and significance of this pathway we firstly performed longitudinal urinary steroid excretion analysis by GC/MS in ORD (n=3) and healthy controls (n=8) from birth to day 90 of life. This revealed significantly increased 5 α 17HP and An excretion in ORD, ceasing 3 weeks after delivery, suggestive of enhanced early neonatal alternative pathway activity. Secondly, we carried out *in vitro* yeast microsomal co-expression of CYP17A1 with two distinct POR mutants, A287P and H628P, associated with 46,XX DSD and 46,XY DSD, respectively. Assays showed that the conversion of 5 α 17HP to An was differentially affected, with significant residual activity in the presence of A287P (65% of WT) whereas co-expression of H628P reduced CYP17A1 activity to only 16% of WT. Thirdly, we performed *ex vivo* human fetal adrenal and external genital skin organ cultures with tissues from gestational weeks 7-9, the key period of sexual differentiation. Organ incubation with radiolabeled steroids was followed by product identification with two-dimensional TLC and tandem mass spectrometry. Results showed that the fetal adrenal is capable of all conversions within the proposed alternative pathway, except for the final step, the conversion of androstenediol to DHT, which was active in fetal genital skin. Taken together, our results provide conclusive evidence for the existence of a novel androgen pathway in early human life, exacerbated in ORD. This reveals cooperation of the fetal adrenal and genital skin to generate 46,XX DSD, mirroring the seminal discovery of the cooperation of gonads and genital skin to cause 46,XY DSD in 5 α -reductase type 2 deficiency.

Nothing to Disclose: NR, VD, AAB, NK, AET, EFN, EMM, PCH, SAW, NFT, PMS, CHLS, NAH, WA

P3-24

Genetic Variants in P450 Oxidoreductase (POR) Affect Activities of CYP2D6 and CYP3A4.

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POR is the single flavoprotein that donates electrons to all microsomal P450 enzymes, including steroidogenic 17 α -hydroxylase/17,20 lyase (P450c17), 21-hydroxylase (P450c21) and aromatase, and hepatic drug-metabolizing enzymes. POR mutations, typically A287P and R457H, decrease steroidogenesis in Antley-Bixler syndrome (ABS) (1); the polymorphism A503V is found on 28% of human alleles (2) decreases P450c17 and cytochrome c activities to ~60% (3) but effects of POR variants on drug metabolism are unclear. We assessed the effects of POR variants on the activities of human CYP2D6 and CYP3A4, which metabolize over 50% of clinically used drugs. Plasmids for N-27 forms of wild type (WT), Q153R, A287P, R457H and A503V POR, and WT CYP2D6 and CYP3A4 were expressed in *E. coli* and the encoded proteins were purified. Each POR protein was reconstituted with each CYP enzyme in vitro. The effects of POR variants on CYP2D6 were examined with EOMCC and dextromethorphan (dextro) as substrates; effects on CYP3A4 were determined using testosterone, midazolam, quinidine and erythromycin. Metabolites were measured by TLC (testosterone), fluorescence (EOMCC) or LC/MS/MS (all others). Km and Vmax was determined in triplicate for each reaction; catalytic efficiency is expressed as Vmax/Km. Compared to WT POR, A287P and R457H had no detectable activity with CYP2D6 (EOMCC) and CYP3A4 (quinidine, erythromycin) but A287P retained 27% of WT activity with CYP2D6 for dextro and less than 20% with CYP3A4 for testosterone and midazolam. Q153R, which causes ABS and has poor activity with both cytochrome c and P450c17, resulted in gain-of-function with CYP2D6 (128% with EOMCC; 187% with dextro) and variable function with CYP3A4 (150% with quinidine; 129 % with testosterone; 92% with midazolam; 77% with erythromycin). The A503V polymorphism supported CYP2D6 at 85% for EOMCC and 63% for dextro and supported CYP3A4 at 77 % for testosterone and 74 % for midazolam but 89% for quinidine and 98% for erythromycin. These data show that: 1) individual POR variants can have different effects on a single P450 enzyme depending on the substrate metabolized; 2) R457H and A287P dramatically reduces the activities of CYP2D6 and CYP3A4; this is probably important for patients with ABS; 3) the A503V POR variant has mildly impaired activity with CYP2D6 and CYP3A4, possibly contributing to genetic variability in drug and xenobiotic metabolism.

(1) Fluck CE et al., Nat Genet 2004; 36:228

(2) Huang N et al., PNAS 2008; 105:1733

(3) Huang N et al., Am J Hum Genet 2005; 76:729

Nothing to Disclose: DS, VA, JHC, KMM, KMG, WLM

P3-25

Structural Analysis of the Change-of-Function E120H Mutation in Human Liver Δ^4 -3-Ketosteroid 5 β -Reductase (AKR1D1).

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Human liver aldo-keto reductase 1D1 (AKR1D1) and the AKR1C enzymes catalyze sequential steps in steroid hormone and bile-acid metabolism. AKR1D1 catalyzes a unique 5 β -double bond reduction of Δ^4 -3-ketosteroid, while the AKR1C enzymes reduce the corresponding saturated 3-ketosteroid to yield 5 β -tetrahydrosteroids containing either a 3 α - or 3 β -alcohol. AKR1D1 and the AKR1C enzymes perform these different functions despite the fact that they share high amino acid sequence identity. Previous studies revealed a conserved catalytic tetrad crucial for hydride transfer in the AKR1C enzymes composed of Tyr55, Lys84, Asp50, and His117. The tetrad is preserved in AKR1D1 except His117, which is substituted by a glutamate. This residue has been speculated to be pivotal in discriminating between steroid double bond and ketone reduction. For example, the histidine-to-glutamate substitution in AKR1C9 introduced 5 β -reductase activity (1). We have previously reported the construction of a corresponding E120H mutation in AKR1D1 (2). Our results revealed that the mutation completely abolished the 5 β -reductase activity while introducing 3-ketosteroid reductase activity. The mutant stereospecifically converted 5 α -dihydrotestosterone to 5 α -androstane-3 β ,17 α -diol and reduced 5 β -dihydrotestosterone primarily to 5 β -androstane-3 α ,17 α -diol. To investigate the structural basis of this mutation, we have now determined the crystal structures of the AKR1D1 E120H mutant complexed with NADP⁺ at 1.90-Å resolution, and with NADP⁺ and 5 α -dihydrotestosterone at 1.64-Å resolution. The structure of the E120H•NADP⁺•5 α -dihydrotestosterone complex clearly demonstrates that steric hindrance from the histidine bulky side chain prevents the steroid from reaching the bottom of the active site. This dislocation places the steroid C3 carbon within feasible hydride transfer distance to the 4-pro-R hydride on the NADPH nicotinamide ring. The steroid binding orientation also explains the observed stereospecific production of the 3 β -equatorial alcohol. We are currently working on the structure of E120H•NADP⁺•5 β -dihydrotestosterone complex.

(1) Jez, J. M., and Penning, T. M. (1998) Engineering steroid 5 β -reductase activity into rat liver 3 α -hydroxysteroid dehydrogenase, *Biochemistry*. 37, 9695-9703.

(2) Drury, J. E., Di Costanzo, L., Christianson, D. W., and Penning, T. M. (2009) Mutation and Inhibitor Analysis of Human 5-Reductase (AKR1D1). Abstract for poster presentation., in The 91st Endocrine Society Annual Meeting, Washington, DC.

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Nothing to Disclose: MC, JED, TMP, DWC

P3-26

Metabolism of Natural and Synthetic Glucocorticoids by Aldo-Keto Reductase 1 Family Enzymes.

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The natural active glucocorticoid in human cortisol is metabolized predominantly in liver to result in the excretion of inactive metabolites. The metabolic conversions involve the sequential reductions of the Δ^4 -3-oxo functionality by human steroid 5 α - or 5 β -steroid reductase to form 5 α - or 5 β -dihydrocortisol (DHF) and subsequently by ketosteroid reductases to form 3 α / β ,5 α / β -tetrahydrocortisol (THF) as the major metabolites. Members of the aldo-keto reductase (AKR) superfamily, namely AKR1D1 (5 β -steroid reductase) and AKR1C1-4 (ketosteroid reductases) are implicated, however this has not been proven. In particular, the individual role of the four AKR1C isoforms in cortisol metabolism remains unknown. To directly investigate the role of AKR enzymes in cortisol metabolism, purified recombinant AKR1D1 and AKR1C enzymes were used in the following metabolizing reactions: 1) reduction of cortisol by AKR1D1 (Δ^4 -reduction), 2) reduction of 5 α - and 5 β -DHF by AKR1C1-4 (3- and/or 20-keto-reduction), and 3) reduction of cortisol by AKR1C1-4 (20-keto-reduction). Reaction products were identified using TLC by reference to authentic standards and kinetic constants were determined by continuous fluorimetric assays. AKR1D1 was found to reduce cortisol to 5 β -DHF with a catalytic efficiency of $0.2 \text{ min}^{-1}\mu\text{M}^{-1}$. The reaction of 5 α - and 5 β -DHF with all AKR1C enzymes yielded the respective 3 α -reduced steroid, i.e. 3 α ,5 α / β -THF, as the main product. AKR1C4 displayed excellent catalytic efficiencies of 16 and 22 $\text{min}^{-1}\mu\text{M}^{-1}$ with 5 α -DHF and 5 β -DHF, respectively. In contrast, the k_{cat} values of AKR1C1-3 for the same reactions were 20-100 fold lower than those of AKR1C4. Low turnover rates were observed for the 20-keto reduction of cortisol by AKR1Cs. These results are consistent with AKR enzymes playing a critical role in hepatic cortisol metabolism. The liver specific expression pattern and superior reactivity of AKR1C4 identify this enzyme as the principal AKR1C isoform for the formation of THFs in this organ. In addition, activities of AKR1D1 on two synthetic glucocorticoids were examined, which yielded catalytic efficiencies of 0.016 and 0.039 $\text{min}^{-1}\mu\text{M}^{-1}$ for budesonide and flunisolide, respectively. These data suggest that AKR1 family members may also be involved in the systemic metabolism of synthetic glucocorticoids.

Sources of Research Support: Pilot project fund from NIH grant P30 ES015857 and grant R01-DK47015.

Nothing to Disclose: YJ

P3-27

Novel 20beta-Hydroxysteroid Dehydrogenase of Zebrafish Plays a Role in Glucocorticoid Catabolism.

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Hydroxysteroid dehydrogenases (HSD) represent a protein family which members are mostly involved in the biosynthesis of steroid hormones and in the regulation of their biological activity. The HSDs catalyze a stereospecific reaction at different positions on the steroid backbone. For example, 11beta-HSDs are involved in the control of glucocorticoid action and 17beta-HSD type 1 and type 3 are known for their ability to synthesize estradiol and testosterone, respectively, which are important sex differentiation hormones. While many 17beta-HSDs are characterized in mammals, only few have been identified in non-mammalian species. In zebrafish, *Danio rerio*, only three 17beta-HSDs have been characterized so far, namely 17beta-HSD type 1, 2, and 3. In an attempt to identify new 17beta-HSDs in the genome of zebrafish, we performed phylogenetic analyses in unfinished zebrafish genome. By this approach we discovered a candidate gene in close relationship to 17beta-HSD type 3 and type 12. The aim of this study was a functional characterization of this candidate to elucidate whether this enzyme could be classified as a new zebrafish 17beta-HSD. The ORF was overexpressed in different human cell lines. In an initial screen for enzymatic activity (tritiated steroids and following analyses with HPLC) we observed that the candidate enzyme did not convert estrogens or androgens, despite its close relationship to 17beta-HSD type 3. However, we could demonstrate that the enzyme catalyzes the conversion of cortisone to an unknown product, which we identified to be 4-Pregnen-17alpha,20beta,21-triol-3,11-dione by LC-MS/MS. Therefore we named this new enzyme 20beta-HSD. The novel 20beta-HSD from zebrafish is localized in the endoplasmic reticulum as demonstrated by overexpression with appropriate tags facilitating immunofluorescence microscopy analyses. By RT-PCR and whole mount in situ hybridization we observed that the gene expression starts at very early stages and continues throughout the zebrafish development. In adult tissues, 20beta-HSD shows a ubiquitous expression pattern. In conclusion, we propose that the novel 20beta-HSD plays a role in the catabolism of the stress hormone cortisol in zebrafish.

Nothing to Disclose: JT, RM, JA

P3-28

6-Phosphogluconate Dehydrogenase - An NADPH-Generating Enzyme in the Lumen of the Endoplasmic Reticulum.

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6-Phosphogluconate dehydrogenase (6PGDH) is the third enzyme of the oxidative phase of the pentose phosphate pathway. The cytosolic form of this pathway is well characterised, but the details and significance of the endoplasmic reticulum (ER) version are only beginning to be understood. We have previously identified hexose-6-phosphate dehydrogenase (H6PDH), which catalyses the first two steps of this pathway within the lumen of the ER, as a pivotal regulator of ER reductases such as 11b-hydroxysteroid dehydrogenase type 1 (11b-HSD1). The cortisone-reductase activity of 11b-HSD1 is dependent on a high NADPH/NADP⁺ ratio, which in turn is dependent on the NADPH-generating activity of H6PDH. Activity of 6PGDH also generates NADPH, and hence its presence within the ER would be of potential significance. 6PGDH activity has previously been reported in microsomes, but neither the protein nor the corresponding gene has been characterised. Significant 6PGDH activity was observed in microsomes isolated from mouse liver. This activity was lower than the cytosolic activity (44.6±10.6 vs 300.1±15.4 mmol NADPH/min/mg protein, respectively), and was only measurable upon solubilisation - indicative of a true microsomal form. There was no detectable cytosolic contamination of the microsomes, as indicated by either Western immunodetection or activity measurements, using the known cytosolic marker, lactate dehydrogenase. On gel filtration and ion-exchange chromatography, the microsomal activity behaved identically to the cytosolic activity. FT-ICR mass spectrometry of trypsin digests of the chromatographically and electrophoretically-separated microsomal form, indicated a protein sequence identical to the cytosolic form. In addition, in Western blot analyses, antibody against the cytosolic form of 6PGDH cross-reacted with the active microsomal fractions. These data indicate that microsomal 6PGDH is likely to be derived from its cytosolic counterpart, and suggest the cytosolic 6PGDH can be shuttled into the ER where it can additionally contribute to the NADPH pool for luminal reductases such as 11b-HSD1.

Sources of Research Support: Wellcome Programme Grant (UK) awarded to PM Stewart and EA Walker.

Nothing to Disclose: IJB, JPR, PMS, EAW

P3-29

Identification and Functional Analysis of Novel Mutations in the Gene Encoding Hexose-6-Phosphate Dehydrogenase in Patients with Premature Pubarche.

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In peripheral target tissues, levels of active glucocorticoid are controlled by 11beta-hydroxysteroid dehydrogenase type 1 (11beta-HSD1) which catalyses the reduction of cortisone to cortisol within the endoplasmic reticulum. For functional 11-ketoreductase activity, 11beta-HSD1 requires the NADPH-generating enzyme hexose-6-phosphate dehydrogenase (H6PDH). Loss of 11-ketoreductase activity results in increased cortisol clearance and activation of the HPA axis with hyperandrogenism. Previously, we identified mutations in H6PDH as the cause of disease in adult cases – a condition termed Apparent Cortisone Reductase Deficiency. Here we present data from two young girls with premature pubarche. Case 1 was a 7yr old girl who presented with premature onset of pubic and axillary hair, and elevated serum and urinary androgen metabolite levels. Crucially, urinary steroid profiling showed a low cortisol to cortisone metabolite ratio (THF+allo THF/THE ratio) - reflecting 11beta-HSD1 activity - of 0.18 (normally >0.5). Case 2 was a 4yr old girl with premature pubarche, accelerated bone maturation and high serum and urinary androgen metabolite values. In addition, she exhibited low cortisol levels (90.2nmol/l) and an exaggerated response to Synacthen testing (cortisol 0 = 90.2nmol/l, cortisol 60mins = 1,336nmol/l). The THF+ allo THF/THE ratio in this case was markedly reduced at 0.056. Sequence analysis revealed 3 novel and 1 previously identified sequence variants in *H6PD*. *HSD11B1* was normal in both cases. Case 1 was compound heterozygous for a Q325X in exon 4, and Y446X in exon 5. Both mutations cause major truncation of the H6PDH protein and *in vitro* expression studies confirmed the resulting proteins to be inactive. Case 2 was compound heterozygous for a previously identified deleted C at position 325 in *H6PD*, resulting in a frame shift and generation of a stop codon (325delC), and a proline to leucine mutation at position 146 (P146L). The P146 residue is highly conserved within both H6PDH and G6PDH enzymes across species. Modelling this residue on the structure of G6PDH indicates that P146L disrupts the co-factor binding pocket. *In vitro* expression studies confirmed that the resulting protein was devoid of H6PDH activity. These data offer insight not only into the genetic basis of the premature pubarche seen in these individuals, but also indicates the importance of cofactor regulation in the control of glucocorticoid hormone action.

Sources of Research Support: Wellcome Trust 082809/Z/07/Z.

Nothing to Disclose: EAW, GGL, MS, SAW, MFH, JDS, WA, PMS

P3-30

Cortisol Metabolism and Hepatic Expression of 11 β -Hydroxysteroid Dehydrogenase Type 1 in Patients with Non Alcoholic Fatty Liver Disease.

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Non alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome. The role of glucocorticoids (GC) in its pathogenesis, is highlighted in patients with GC excess, Cushing's syndrome, who develop central adiposity, insulin resistance and in 20% of cases, NAFLD. Although circulating cortisol levels are normal in patients with NAFLD, hepatic cortisol availability is controlled by enzymes that regenerate cortisol from inactive cortisone (11 β -hydroxysteroid dehydrogenase type 1, 11 β -HSD1) or inactivate cortisol through A-ring metabolism (5 α - and 5 α -reductase, 5 α R and 5 β R).

We characterised hepatic cortisol metabolism in 16 patients with histologically proven NAFLD (8 steatosis, 8 steatohepatitis (NASH)) and 32 BMI-matched controls. We also analysed 11 β -HSD1 mRNA expression and protein expression by immunohistochemistry in liver from 5 NASH patients and 5 normal controls.

In patients with steatosis 5 α R activity was increased, (urine 5 α THF/THF ratio: controls 0.80 \pm 0.07, steatosis 1.31 \pm 0.21, p<0.05), paralleled by a decrease in hepatic 11 β -HSD1 activity (cortisol generation curve (CGC) after oral cortisone acetate 25mg AUC \pm SEM: controls 382 \pm 22, steatosis 304 \pm 27 mg/L.min, p<0.05, urine cortols/cortolones ratio: controls 0.43 \pm 0.02 vs. steatosis 0.33 \pm 0.02, p<0.05). Furthermore, total cortisol metabolites were increased consistent with increased GC production rate (12168 \pm 1028 vs 8690 \pm 786 mg/24h, p<0.01).

Patients with NASH had increased cortisol generation consistent with increased hepatic 11 β -HSD 1 activity compared with controls and with steatosis, (CGC AUC \pm SEM: controls 382 \pm 22, steatosis 304 \pm 27, NASH 496 \pm 22 mg/L.min; NASH vs controls, p<0.05, NASH vs steatosis, p<0.01).

Immunohistochemical analysis in patients with NASH showed markedly increased 11 β -HSD1 expression in peri-septal areas and within the inflammatory infiltrate. In addition 11 β -HSD1 mRNA expression was also increased when compared with controls, (dCT 9.65 \pm 0.29 vs 11.96 \pm 0.29, p<0.01).

Patients with hepatic steatosis have increased clearance and decreased regeneration of cortisol, a possible protective mechanism to decrease local GC availability to preserve hepatic metabolic phenotype. With progression to NASH, increased 11 β -HSD1 activity driven cortisol regeneration may serve to limit hepatic inflammation (Figure 1). These results provide a crucial insight into the pathophysiology of the NAFLD disease spectrum.

Nothing to Disclose: AA, TB, CB, PG, AW, PN, SGH, EE, DHA, JWT, PMS

P3-31

Is Increased 11 β -HSD1 Expression a Key Factor Underpinning Intrinsic and Extrinsic Skin Aging?.

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Glucocorticoids are highly detrimental to skin integrity and function both when used locally for anti-inflammatory treatments and during conditions of raised systemic concentrations such as Cushing's syndrome. Many of the adverse effects of glucocorticoids on skin are also symptoms associated with natural intrinsic aging and extrinsic photoaging. Locally, glucocorticoid availability is regulated independently of circulating levels by 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) which activates cortisol from cortisone. Surprisingly, studies investigating 11 β -HSD1 activity in a dermal context are limited.

We aimed to characterize the localization of 11 β -HSD1 in skin and analyze differential expression between old, young, photoprotected and photoexposed dermal fibroblasts.

Immunohistochemistry of full-thickness human skin (Hs) sections (obtained following local ethical approval, n=6) localized 11 β -HSD1 to epidermal keratinocytes and dermal fibroblasts. Mouse skin (Ms) sections (n=6) also revealed specific staining in these cell types and in outer root sheath cells. Negligible staining was observed in isotype control-treated sections or those obtained from 11 β -HSD1 knock-out (KO) mice (n=6).

Using radioactive substrate conversion assays, 11 β -HSD1 oxoreductase activity was identified in skin tissue explants from both species (mouse, 170 \pm 30 fmol/mg/h \pm SD, n=4; human, 9.6 \pm 3.6 fmol/mg/h \pm SD, n=15). Activity was undetectable when samples were co-incubated with an 11 β -HSD1 inhibitor or when tissue used was obtained from 11 β -HSD1 KO mice.

In Hs tissue, there was a positive correlation between 11 β -HSD1 oxoreductase activity and age (p<0.05). This was endorsed by real-time PCR data on primary Hs dermal fibroblasts. Fibroblasts derived from both photoexposed and photoprotected sites indicated that 11 β -HSD1 expression increased with age (photoprotected, r²=0.49, p<0.05, n=10; photoexposed, r²=0.78, p<0.01, n=10). Additionally, donor-matched photoexposed fibroblasts displayed 2- to 18-fold higher 11 β -HSD1 expression than photoprotected fibroblasts (mean Δ Ct 23.1 vs. 20.8 respectively, p<0.05, n=7). We conclude that age- and site-dependant increases in dermal 11 β -HSD1, through increasing local GC activation, may play an etiological role in the aging skin phenotype.

Nothing to Disclose: AT, AEM, RH, PMS, EAW

P3-32

Non-Invasive In-Vivo Monitoring of 11 β -HSD1 Activity Using ¹⁹F-Magnetic Resonance Spectroscopy.

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11 β -Hydroxysteroid Dehydrogenase 1 (11 β -HSD1) amplifies intracellular glucocorticoid levels in many tissues, including liver, adipose tissue and hippocampus. Selective inhibitors reduce tissue glucocorticoid action and are efficacious in pre-clinical models of obesity, diabetes, atherogenesis and cognitive dysfunction. Tissue-specific dysregulation of 11 β -HSD1 has been shown in obese patients using invasive tools (biopsy, microdialysis, arteriovenous sampling with stable isotope tracers), but non-invasive methods are needed to assess enzyme activity in inaccessible sites. We have studied the use of ¹⁹F-magnetic resonance spectroscopy (MRS) to detect conversion of fluorinated keto-substrate tracers to hydroxy-metabolites by 11 β -HSD1 *in-vivo*.

Fluorinated biomarkers were proposed based on known keto-substrates for 11 β -HSD1. *In-vitro* screening (HEK293 cells stably transfected with 11 β -HSD1) provided three candidate tracers containing trifluoromethylphenyl cores. The corresponding hydroxy-metabolites were chemically synthesised and the substrate-metabolite pairs analysed by ¹⁹F-nuclear magnetic resonance (Bruker NMR, 250 MHz and Varian 7T Small Animal Scanner). The fluorine signals of each pair differed ($\Delta\delta$ Fs) by 0.5-1.1ppm.

In biological matrixes signal width (1-2ppm) caused substantial overlapping of keto/hydroxy MRS peaks. However $\Delta\delta$ Fs \geq 0.7ppm allowed specific quantification in blood and liver. Our trifluorinated tracers also yielded a reasonable limit of detection (LOD) with short scan times (7-14min). One trifluorinated keto-candidate (Compound A, LOD: 0.16 μ moles in CHCl₃, 2.5 μ moles in blood), was studied further.

Compound A was infused *ex-vivo* via the portal vein (25mg in Krebs buffer, male Wistar rat, 420g). After 1 hour compound A and its hydroxy-metabolite were detected in liver and production of the metabolite continued for a further hour. Compound A was also administered by oral gavage to rats (n=3, 8-10mg/kg) and after 15 minutes MRS signals for substrate and metabolite were detected (7 min scan).

Our data shows that trifluorinated compounds, in combination with ¹⁹F-MRS, could be used as tracers to measure 11 β HSD1 activity non-invasively *in-vivo*. The technique could be useful in assessing pathophysiology of 11 β HSD1, and for tissue specific pharmacodynamic studies of inhibitors. Furthermore, as scanner sensitivity increases with increased amount of tracer, this method could be directly applied to humans using lower doses and/or shorter scanning times.

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Disclosures: MKH: Employee, Pfizer Global R&D. BRW: Consultant, Pfizer Global R&D.

Nothing to Disclose: GN-G, MAJ, GDM, OBS, RA

P3-33 Glucocorticoids Turn over Slowly in Human Adipose Tissue *In Vivo*.

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Background: Lipophilic plasma glucocorticoids are thought to gain rapid access to intra-cellular compartments in adipose tissue. However, in other organs transport can be regulated in a steroid- and tissue-specific manner. Moreover, 11 β HSD1 generates additional cortisol within adipose.

Aim: To measure the rate of exchange of cortisol between plasma and adipose for comparison with rates of intra-cellular cortisol generation by 11 β HSD1.

Methods: With ethical approval, otherwise healthy females (n=6) undergoing hysterectomy for benign indications were infused with tracer 9,11,12,12-²H₄-cortisol (d4-cortisol). Adipose biopsies and peripheral venous samples were obtained during surgery after 3.9-5.5 hours of infusion. Glucocorticoids were quantified using LC-MS/MS.

Results: In plasma, d4-cortisol and appearance rates of cortisol and d3-cortisol (reflecting 11 β HSD1 activity) did not change during surgery. In both omental and subcutaneous adipose, not only were cortisol concentrations substantially lower than in plasma, consistent with differences in corticosteroid binding globulin, but enrichment with d4-cortisol was low (subcutaneous 7.2 \pm 0.6% and omental 7.4 \pm 0.7% versus plasma 15.5 \pm 1.0%). The rate of accumulation of d4-cortisol in adipose depots was only 0.5 \pm 0.1 (subcutaneous) and 0.5 \pm 0.1 (omental) nmol/kg/h, and the proportion of intra-adipose cortisol replaced each hour was only 10.7 \pm 1.0% and 10.4 \pm 0.7%, respectively. The contribution of 11 β HSD1 to this turnover could not be quantified since very little substrate d3-cortisone accumulated in adipose.

Conclusion: Slow turnover of the adipose glucocorticoid pool suggests that rapid acute fluctuations in circulating cortisol are markedly damped, whereas 11 β HSD1 (previously estimated to generate 9nmol cortisol/kg/h in subcutaneous adipose) plays a relatively important role in modulating activation of glucocorticoid receptors.

Sources of Research Support: Award from the Translational Medicine Research collaboration.

Nothing to Disclose: KAH, RMR, HODC, RA, BRW

P3-34

Regulation of 11beta-Hydroxysteroid Dehydrogenase Type 1 (11beta-HSD1) Expression in the Rat Heart by Androgens.

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Introduction: 11beta-HSD1 converts inactive cortisone (or dehydrocorticosterone) to active cortisol (or corticosterone) and therefore is the key enzyme of intracellular glucocorticoid metabolism. Cortisol binds to the glucocorticoid and mineralocorticoid receptor in the heart. Cortisol excess as seen in Cushing's syndrome leads to left ventricular hypertrophy, but also androgens cause myocardial remodelling. The impact of testosterone on the cardiovascular system is controversial as androgens have beneficial and deleterious effects on cardiovascular disease factors. We investigated the effect of testosterone on 11beta-HSD1 in the rat heart.

Methods: Male Wistar rats aged 8-10 weeks were orchietomized and put on a low-salt (chow 0.03% NaCl + tap water) or high-salt diet (chow 4% NaCl + water 0.09% NaCl) for 5 weeks. In addition, they received either placebo, testosterone (1mg/animal) or 5alpha-dihydrotestosterone (1mg/animal) as daily s.c. injection for 16 days (each group n=6). In further experiments other rats were treated with the mineralocorticoid antagonist spironolactone (50mg/kg body weight per day), the androgen receptor antagonist flutamide (30mg/kg body weight per day), or amiloride (1mg/kg body weight per day) in addition to testosterone or 5alpha-dihydrotestosterone. After sacrifice cardiac 11beta-HSD1 mRNA expression was assessed by real-time PCR.

Results: Testosterone treatment led to a significant upregulation of 11beta-HSD1 mRNA expression in the heart of salt-depleted orchietomized rats. 5alpha-dihydrotestosterone showed the same trend, but did not reach significance. Pretreatment with spironolactone or flutamide completely abolished this testosterone effect, but not the pretreatment with amiloride. In addition, spironolactone significantly decreased cardiac 11beta-HSD1 expression in rats on a high-salt diet compared to those on a low-salt diet.

Conclusions: We suggest that testosterone increases intracardiac cortisol (corticosterone) concentrations by stimulation of 11beta-HSD1. This might contribute to left ventricular concentric remodelling as seen in Cushing's disease and in patients with androgen abuse.

Nothing to Disclose: TK, MM, PH, FG, MQ

P3-35

Decreased Abundance of Carbon 13 in Endogenous Ouabain from Bovine Adrenal Glands versus Plant Ouabain: Implications for the Biosynthesis of Cardiotonic Steroids by Mammals and Plants.

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Numerous studies link Endogenous Ouabain (EO) with blood pressure and vascular function in a large portion of patients with essential hypertension (EH), primary aldosteronism (PA), heart failure (HF) and in renal failure (RF). EO and digoxin-like materials (DLFs) have been repeatedly identified in the circulation, brain, adrenal glands and urine of mammals. Much evidence indicates that EO and DLFs are natural products of the adrenal cortex (1). However, some (2) have questioned the identity of the human EO isolated from plasma and its origin (3). During a large scale isolation intermediates in EO biosynthesis, we also isolated ~6 µg of pure EO from bovine adrenal glands. The isolation followed the bioactivity of EO through serial chromatography using a radioreceptor assay. The exact mass and carbon isotope ratios in the pure bovine EO were explicitly compared with commercially available plant sources of ouabain (Sigma Chemical Co. and Calbiochem) using high resolution mass spectrometry. For those molecules in which all carbon atoms had a mass of 12, the exact mass to charge ratios for protonated molecular ions of EO and the two plant ouabains were identical ($M+H^+ = 585.292$ m/z). However, the absolute abundance of carbon 13 containing EO molecules (m/z 586.292) was significantly lower (delta -53.7‰, $p < 0.001$) than in the plant ouabains (delta -22.4‰, -16.6‰). Tandem MS-MS experiments showed also that carbon 13 atoms were not randomly distributed in the product molecular ions confirming expectations. In conclusion, bovine adrenal EO and plant ouabain are structurally identical molecules. However, EO differs from plant ouabain at the subatomic level by virtue of reduced neutron abundance. Furthermore, the depletion of carbon 13 in bovine EO extends well beyond that known for terrestrial plants using C3 or C4 metabolism to fix atmospheric CO₂ (-10 to -30‰). Thus, the bovine EO is not due to contamination of the laboratory by plant ouabain and did not originate in terrestrial plants. The reduced abundance of carbon 13 indicates that the biosynthetic pathway of EO involves a mammalian enzyme(s) that preferentially uses substrates with adjacent carbon 12 atoms. In summary, the subatomic signature of bovine EO adds further evidence for EO biosynthesis by the mammalian adrenal cortex. Our results support the idea that the raised circulating levels of EO in patients with EH, PA, HF and RF are of adrenocortical origin.

(1) Manunta et al, J Hypertens 2009;27:9-18

(2) Nicholls et al, J Hypertens 2009;27:3-8

(3) Hamlyn et al, Proc Natl Acad Sci 1991;88:6259-63

Sources of Research Support: NIH HL75584.

Nothing to Disclose: GT, RR, VT, BPH, JMH

P3-36

Transgenic Mice Expressing Human Hydroxysteroid (17beta) Dehydrogenase 1 (HSD17B1) Provide a Novel Tool To Monitor Action of HSD17B1 Inhibitors *In Vivo*.

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Estrogens influence the pathological processes of hormone-dependent diseases in women, such as breast and endometrial cancers. Hydroxysteroid (17beta) dehydrogenase 1 (HSD17B1) catalyzes the conversion of biologically low-active estrone to a highly potent estrogen, estradiol, in various sex steroid target tissues. We have recently developed transgenic mice expressing human HSD17B1 (HSD17B1TG mice), and applied the mice also to study the efficacy of HSD17B1 inhibitors *in vivo*. Among other disturbances, the HSD17B1TG mice invariably develop endometrial hyperplasia at the age of 4 months, and the mice also fail to ovulate. Interestingly, similarly to that in human, the endometrial hyperplasia in HSD17B1TG mice could be treated by ovulation induction or by a progestin treatment. Moreover, treating the mice with a HSD17B1 inhibitor restored endometrial glandular morphology of epithelial cells, but less in the luminal epithelium. HSD17B1TG mice were also cross-bred with reporter mice carrying three estrogen responsive elements (EREs) in front of a luciferase reporter gene (ERELuc mice). The data revealed that the estrogen receptor activation in the intact bi-TG mice was significantly increased as compared with the ERELuc mice. This was observed by an increased luciferase activity in several tissues, including liver, kidney and mammary gland. The properties of HSD17B1 inhibitors were also studied in immature bi-TG mice by analyzing the uterus weight and luciferase activity in the uterus after treating the mice with estrone (0,3 µg/kg), and with or without HSD17B1 inhibitor (20 mg/kg), for five days. The estrone treatment increased the uterus weight significantly more in the bi-TG mice as compared with the ERELuc mice, and the HSD17B1 inhibitor significantly prevented estrone induced uterus weight increase. Identically, the luciferase activity measured *ex vivo* in the uterine tissues was lower in the in mice treated with the HSD17B1 inhibitors. As a conclusion, the HSD17B1TG mice and the bi-TG ERELuc-HSD17B1TG mice serve as preclinical models for testing the efficacy of HSD17B1 inhibitors *in vivo*.

Disclosures: PK: Co-author, Hormos Medical Ltd., Subsidiary of QuatRx Pharmaceuticals, Turku, Finland.

Nothing to Disclose: PJ, TS, PTvdS, HK, MP

P3-37

Measurement of Testosterone in Muscle Tissue Using Microdialysis: Preliminary Results.

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Introduction: For diagnostic purposes, total-testosterone levels are generally assessed in plasma. However, as the primary site of action of testosterone is in tissue, evaluation of the distribution of testosterone in tissue fluids could give enhanced insight into the (patho)physiological state. Clinical microdialysis allows for sampling at tissue level based on diffusion.

Objective: To compare the increase in testosterone levels in tissue and plasma after cutaneous administration of testosterone.

Methods: A CMA20 microdialysis probe was continuously perfused with Ringer's lactate containing 0.6nmol/L D5-testosterone as internal reference. A sample interval of 15 minutes was used, with a perfusion rate of 10 microliter per minute. Microdialysis was performed on the vastus lateralis muscle in two healthy male volunteers for 4h, preceded by a 1h run in period. Additionally, blood specimens were drawn in each interval. After one hour, 100 mg testosterone gel was applied to the skin of the back in an attempt to vary plasma and tissue testosterone levels. Testosterone was analyzed using a highly sensitive assay involving derivatization and ID-LC-MS/MS.

Results: Testosterone profiles in the collected microdialysis and plasma samples from both volunteers were comparable. Testosterone levels increased after cutaneous administration, in both microdialys fluid(70%) and plasma(60%).

Conclusion: The initial results of clinical microdialysis are promising. Low testosterone levels as well as increased concentrations after supplementation could be reliably detected. Further research is necessary to thoroughly evaluate the microdialysis procedure. Hopefully, in vivo experiments in the near future will enrich our knowledge about testosterone at the tissue level.

Nothing to Disclose: VLW, HNB, ACH, MAB, WdR

P3-38

Metformin Increases LKB1 Expression and Inhibits Aromatase in Primary Human Breast Adipose Stromal Cells.

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There is strong evidence that obesity is linked to an increased breast cancer risk. After menopause, it is the local expression of aromatase, responsible for converting androgens to estrogens within the breast adipose, that is believed to be responsible for the increased proliferation of breast cancer cells.

We have recently demonstrated that the LKB1/AMPK pathway is inhibitory of aromatase expression in primary human breast adipose stromal cells by inhibiting the nuclear entry of CRTC2, a CREB co-activator. Factors produced in obesity, such as leptin, and by tumors, such as PGE2, were shown to inhibit LKB1 expression and activity and cause the nuclear translocation of CRTC2, resulting in the increased expression of aromatase. Conversely, a factor produced in lean individuals, adiponectin, and the drug AICAR, known to activate AMPK, inhibited the PGE2-mediated expression of aromatase.

There is growing evidence that the action of metformin, a commonly used oral anti-diabetic drug, is mediated primarily by stimulation of AMPK in association with interaction with its upstream kinase LKB1. We have recently demonstrated that metformin increases the phosphorylation of AMPK and inhibits aromatase expression in breast adipose stromal cells. In the present study, we aimed to examine the effect of metformin on LKB1 expression and promoter activity. Primary human breast adipose stromal cells obtained from breast reduction surgeries were treated with 50 μ M 1,1-dimethylbiguanide hydrochloride (metformin) and LKB1 expression and promoter activity were quantified using real-time PCR, Western blotting and luciferase reporter assays. Interestingly, treatment with metformin resulted in a significant increase in LKB1 transcript and protein expression. Treatment with metformin also resulted in the significant increase in LKB1 promoter activity.

Phase III aromatase inhibitors are proving highly successful as endocrine therapy for breast cancer in postmenopausal women. However long-term contraindications may limit the use of these compounds in the prevention setting. This is the first study to demonstrate the increase in LKB1 transcript and protein expression as a mechanism of action of the drug metformin and suggests that using metformin, which increases insulin sensitivity, causes weight loss and decreases the local biosynthesis of estrogens, may provide a multi-faceted approach at preventing and treating obesity-related postmenopausal estrogen-dependent breast cancer.

Sources of Research Support: National Health and Medical Research Council of Australia Grant 494819; Victorian Breast Cancer Research Consortium; Terry Fox Foundation Fellowship awarded to KAB.

Nothing to Disclose: KAB, MD, ERS

P3-39

Characterization of the Anabolic and Androgenic Properties of the Unapproved Aromatase Inhibitor Androsta-1,4,6-Triene-3,17-Dione.

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Since a few years several steroids are available on the Internet as sport supplements, often sold by bodybuilding sites. Recently androsta-1,4,6-triene-3,17-dione (ATD) was also detected in products labeled as dietary supplements. It was reported that ATD effectively reduces estrogen biosynthesis by irreversible aromatase inhibition (1,2). Since 2010 the World-Anti-Doping Agency has explicitly listed ATD as prohibited substance in sports (3). Sanctions on an athlete strongly depend on the classification of the administered drug.

Thus, we have investigated the ability of ATD and its main metabolite 17OHAT to interact with the androgen receptor (AR). Its anabolic and androgenic properties were determined in vivo and metabolism was studied.

Using a yeast AR transactivation system ATD and its 17-OH metabolite 17OHAT could be identified to be moderate AR agonists with transactivation potencies ~10times lower than the natural AR ligand dihydrotestosterone. In addition 17OHAT was found to be a functional AR antagonist.

To investigate potential androgenic/anabolic effects of ATD in vivo a rodent Hershberger assay was performed: Castrated male Wistar rats were treated once a day s.c. with ATD (1 mg/kg BW/day), TP (1 mg/kg BW/day) or vehicle only for 12 days. After necropsy the wet weights of the prostate, seminal vesicle, and levator ani muscle, were determined. Neither anabolic nor androgenic properties of ATD were detected. Analogous results were already published for the administration of 17OHAT (4).

Additionally blood plasma was taken using heparin tubes during necropsy. The concentrations of testosterone (T) and the main ATD metabolites were determined using ELISA and GC-MS techniques and compared between the administration groups.

Following ATD administration in men (single oral dose of 50 mg) a significant decrease in the urinary steroid profile ratio of androsterone (AND)/T was observed together with increases in the ratio of T/epiT, 5 α -/5 β -androstane-3 α ,17 β -diol and AND/etiocholanolone. This indicates either the formation of T as metabolite or its accumulation due to the inhibition of the aromatase (5).

The results support the classification of ATD as aromatase inhibitor. However whether ATD and 17OHAT may also act as an antiandrogen, and how ATD effects testosterone levels has to be investigated in future studies.

(1) Schwarzel WC, Kruggel WG, Brodie HJ. *Endocrinology*. 1973;92:866-80

(2) Covey DF, Hood WF. *Endocrinology*. 1981;108:1597-9

(3) World Anti-Doping Agency. *The 2010 Prohibited List*: World Anti-Doping Agency; 2009

(4) Vida JA. Academic Press; New York and London: 1969

(5) Parr MK, Fußhöller G, Schlörer N, et al. *Rapid Commun Mass Spectrom*. 2009;23:207-218

Sources of Research Support: German Federal Ministry of the Interior, Berlin, Germany; Manfred Donike Institute for Doping Analyses e.V., Cologne, Germany.

Nothing to Disclose: MKP, PD, OZ, WS

P3-40

Analysis of Ketosteroids in Serum Using Derivatization Chemistry and LC/MS/MS.

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Introduction:

LC-MS/MS is becoming more widely used in the field of ketosteroid analysis due to its specificity and accuracy. However, the electrospray ionization efficiency for many neutral steroids may not be sufficient to reliably quantify low pg/mL levels in human plasma. Derivatization with ionizable moiety could significantly improve the ionization efficiency of neutral steroids.

Herein presented a novel derivatization chemistry for high sensitivity analysis of ketosteroids in serum samples.

Methods:

The ketosteroids (e.g. Testosterone, Pregnenolone, Progesterone, etc.) are enriched from 200uL of serum samples by solid phase extraction. The samples are dried and the derivatization reaction is performed in acidic conditions (MeOH+acetic acid) for 15min at room temperature. LC-MS/MS separation and analysis is performed using a Reverse Phase (RP) column, H₂O/organic solvent/Formic acid gradient and API 4000 (or 3200) MS/MS (AB Sciex) ESI source, operating in MRM mode. Quantitation of the derivatized ketosteroids is enabled by using an isotopically enriched reagent as an Internal Standard (IS). A known amount of IS is spiked into the sample before analysis and the unknown concentration is extrapolated from the analyte/IS peak area ratio of a known standard curve.

Results:

Product ion spectra of the derivatized ketosteroid (e.g. Testosterone, Progesterone, Pregnenolone, etc.) produced signature ions which are predominant and specific to the product. The resultant signal enhancement factor relatively to the underivatized ketosteroid is ~30-100 folds. The LODs for the derivatized ketosteroids in double charcoal stripped serum are 2.5-15pg/mL. The geometrical isomers of the ketosteroids which are formed upon derivatization can be chromatographically separated, or co-eluted as a single peak using a fast LC gradient. The ketosteroid derivatization method produced linear and reproducible data (<10%CV at LOQ). The Derivatization efficiency is >95% and the whole workflow (sample extraction plus derivatization) is amenable to automation.

Conclusions:

A novel derivatization reagent for ketosteroids significantly improves the limits of detection by ESI/MS/MS and enables the detection of trace amounts in serum samples.

Nothing to Disclose: MW, SD, BW, SP, BP

P3-41

LC-MSMS Method Development for Steroids Panel Analysis in Human Serum.

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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. Steroidogenesis is the process wherein desired forms of steroids are generated by transformation of other steroids.

A method for the quantitation of eleven steroid biomarkers representing different biosynthesis pathways with corresponding clinical relevant concentration ranges in human serum has been successfully developed utilizing APCI-LC/MS/MS. The steroid panel consists of Dehydroepiandrosterone Sulfate (DHEAS), Dehydroepiandrosterone (DHEA), Cortisol, Corticosterone, 11-Deoxycortisol, Androstenedione, Testosterone, 17-OH-Progesterone, Progesterone, Estradiol and 25-Hydroxyvitamin D3.

One major challenge of the assay is that the 11 steroids have a wide concentration of interest range from ug/mL to pg/mL. The method development also included the evaluation of the ionization pattern of steroids using ESI and APCI sources; internal standard evaluation; cross interference from high concentration steroid analytes to low concentration analytes and isomer separation and sample preparation. Simple in house developed protein precipitation using organic with excipient was used for sample preparation. The method of 11 steroids at corresponding concentration of interested range was evaluated and verified.

Nothing to Disclose: H-FL, JM, RH, AL, LS

P3-42

An LC-MSMS Analytical Method for Steroids and Mono-Hydroxy-Vitamin D3 Analysis in Dried Blood Spots.

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An over or under secretion of steroids can cause an imbalance resulting in various types of diseases, hence steroid analysis is important in medical diagnosis. However analytical challenges occur when analyzing steroids, due to low concentrations and/or the structural similarities between different steroids. Dried blood spot screening (DBS) is the concept that blood is obtained from a prick of the finger and blotted onto filter paper. DBS is an ideal method since the sample is easy to obtain and utilizes only a small amount of blood from the patient. The goal is to have a simple extraction method (underivatized) without sacrificing sensitivity nor specificity.

The following steroids: cortisol, 17-alpha hydroxy hydroxyl progesterone, progesterone, testosterone and mono-hydroxy metabolites of vitamin D3 are extracted from one whole blood spot using the liquid- liquid extraction method, prior to analysis with LC-MS/MS. The challenge of calibration curve generation for endogenous level analyte analysis will be examined, and will be a key factor in this experiment. The method development included: the evaluation of different dry blood spot sample preparation procedures, calibration standard curve construction for endogenous steroids, HPLC interferences and separation, and the reproducibility of the assay.

The sample preparation procedure was optimized for sample analysis. A whole spot (~60µL) is cut and soaked in water for around 60 min, followed by liquid-liquid extractions with a mixture containing hexane and ethyl acetate. The organic supernatant was transferred to glass tube and then evaporated to dryness with nitrogen gas and reconstituted with a solution of 50:50 methanol: water before loading into the LC-MS/MS system.

A sensitive and well separated LC-MS/MS method was developed on a triple quadrupole mass spectrometer. The detection limits of all steroids and mono-hydroxy metabolites of vitamin D3 achieved the requirement of endogenous steroids testing in general population screening. The low limit of quantitation for cortisol, 17-alpha hydroxyl progesterone, progesterone, testosterone, and mono hydroxy vitamin D₃ is as follows respectively: 5ng/mL, 100pg/mL, 100pg/mL, 100pg/mL, and 10 ng/mL. The reproducibility of the assay is under 15% accuracy ±100 in real patient sample set.

Nothing to Disclose: TJL, SL, LS, HFL

P3-43

Chemical Synthesis of 20S-Hydroxyvitamin D3, Which Shows Anti-Proliferative Activity.

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20S-hydroxyvitamin D3 (20S-(OH)D3), a new metabolite of vitamin D3 derived from the action of enzyme P450scc, was recently isolated and its structure defined [1-3]. This compound have shown capability to inhibit proliferation and to induce cell differentiation in epidermal keratinocytes [4, 5] and cancer cells including melanoma, leukemia and breast cancer, without inducing hypercalcemia. The enzymatic production of 20S-(OH)D3 is tedious, expensive, and cannot meet the demanding amount for extensive chemical and biological studies. Here we report for the first time *chemical synthesis* of 20S-(OH)D3, which retained biological activity characteristic for P450scc generated compound. Specifically, chemically synthesized 20S-(OH)D3 inhibited proliferation of human epidermal keratinocytes and stimulated expression of involucrin (marker of differentiation). It was 100 fold less effective in stimulation of CYP24 than 1,25(OH)2D3. Testing performed with hamster AbC1 melanoma demonstrated dose dependent inhibition of cell proliferation and colonies forming capabilities in vitro. Thus, we developed chemical method of the synthesis of biologically active 20S-(OH)D3 that can be used for anticancer studies using in vivo models and to prepare a series of its analogs for structure-activity relationship studies to further optimize this class of compounds.

1. Slominski, A., et al., The cytochrome P450scc system opens an alternate pathway of vitamin D3 metabolism. FEBS J, 2005. 272(16): p. 4080-90.
2. Guryev, O., et al., A pathway for the metabolism of vitamin D3: unique hydroxylated metabolites formed during catalysis with cytochrome P450scc (CYP11A1). Proc Natl Acad Sci U S A, 2003. 100(25): p. 14754-9.
3. Tuckey, R.C., et al., Pathways and products for the metabolism of vitamin D3 by cytochrome P450scc. FEBS J, 2008. 275(10): p. 2585-96.
4. Zbytek, B., et al., 20-Hydroxyvitamin D3, a product of vitamin D3 hydroxylation by cytochrome P450scc, stimulates keratinocyte differentiation. J Invest Dermatol, 2008. 128(9): p. 2271-80.
5. Janjetovic, Z., et al., 20-Hydroxycholecalciferol, product of vitamin D3 hydroxylation by P450scc, decreases NF-kappaB activity by increasing IkappaB alpha levels in human keratinocytes. PLoS One, 2009. 4(6): p. e5988.

Sources of Research Support: NIH grant R01AR052190.

Nothing to Disclose: WL, JC, ZJ, TTK, TTS, YL, JZ, RCT, DM, ATS

P3-44

Molecular Cloning of a Functional Zebrafish 25-Hydroxyvitamin D3 1 α -Hydroxylase.

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Activation of precursor 25-hydroxyvitamin D3 (25D3) to hormonal 1,25-dihydroxyvitamin D3 (1,25D3) is a pivotal step in vitamin D physiology. In mammals, the enzyme that catalyzes this reaction, vitamin D3-1 α -hydroxylase (CYP27B1) is expressed predominantly in the kidneys. However, CYP27B1 is also detectable in diverse extra-renal tissues where it mediates tissue-specific intracrine responses to 25D3, including stimulation of innate immune antibacterial activity. To establish new models for assessing immune activity of the CYP27B1-25D3 axis, we have characterized CYP27B1 expression and biological activity in *Danio rerio* (zebrafish), an in vivo model routinely used for studies of mycobacterium infection. Based on published sequences for zebrafish vitamin D target gene 24-hydroxylase (Cyp24A1-L), studies were carried out to compare responses to 25D3 or 1,25D3 in day 5 zebrafish larvae. Treatment of larvae with inactive 25D3 (5 nM) or active 1,25D3 (1 nM) for six hrs induced expression of Cyp24A1-L 16-fold relative to larvae treated with vehicle (0.1% ethanol), suggesting the presence of a CYP27B1 activity capable of converting 25D3 to 1,25D3. To assess the biological activity of zebrafish Cyp27B1 (z-Cyp27B1), we assembled a full-length cDNA using 5', 3' RACE and RT-PCR methods on day 5 zebrafish RNA. Sequencing of the resulting clone revealed an ORF encoding a protein of 505 amino acids with 53% identity and 72% similarity to human CYP27B1. The cDNA was then transferred to an expression vector and transfected into a human kidney proximal tubular epithelial cell line (HKC-8) known to support vitamin D-associated cytochrome P450 activity. Following overnight transient transfection, cells containing empty vector or varying levels of z-Cyp27b1 expression construct were incubated with vehicle or 25D3 for six hrs and RNA extracted for qPCR. Analysis of mRNA for the 1,25D3 target Cyp24A1 showed 3.3 fold \pm 0.52 SE greater induction of this gene by 25D3 in cells receiving 1 μ g z-Cyp27b1 compared to those transfected with empty vector. These data indicate that we have cloned a functional zebrafish equivalent of CYP27B1, representing the most phylogenetically ancient DNA sequence currently available for this key enzyme in vitamin D metabolism. Further analysis of CYP27B1 expression and activity in zebrafish may provide new perspectives on the biological importance of 25D3 metabolism and in particular its effects on immune function.

Sources of Research Support: NIH grant AR050626 to MH.

Nothing to Disclose: RFC, EB, SR, AS, JSA, MH

P3-45

The Glucagon-Like Peptide 1 (GLP-1) Receptor Agonist Exendin-4 Upregulates cAMP Levels, Activates AMP Kinase and Inhibits In Vitro and In Vivo Growth of Breast Cancer Cells.

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The hormone GLP-1 is secreted from intestinal endocrine L-cells in response to food intake. It activates a G-protein coupled receptor, GLP-1 receptor (GLP-1R) and promotes β -cell proliferation. Exendin-4 is a stable GLP-1R agonist, which is prescribed for the treatment of type 2 diabetes. As exendin-4 enhances proliferation of hepatic and islet cells, we aimed to study its effects on breast cancer cells.

We examined the effects of GLP-1 and exendin-4 on proliferation and signaling of MCF-7 and MDA-MB-231 breast cancer cells, non-cancerous human embryonic kidney (HEK)-293 cells and human primary liver (PL) cells. Viability was measured using MTT and colony formation assays, expression and phosphorylation of signaling proteins was tested using Western blotting and cAMP levels were measured using RIA. In vivo activity of exendin-4 was tested in athymic mice.

Short-term treatment with exendin-4 (5nM, 48 hrs) reduced viability of breast cancer cells by 50%, increased apoptosis and elevated p53 and p21 levels. Long-term treatment (5nM, 14d) inhibited colony formation of these cells. Importantly, exendin-4 did not inhibit growth of the non-cancerous cells.

We studied exendin-4 signaling. GLP-1R was not detected in breast cancer cells. Yet, exendin-4 or GLP-1 elevated cAMP levels in these cells. Surprisingly, exendin 9-39, an inhibitor of GLP-1R also induced cAMP accumulation, and induced CRE-luciferase accumulation, suggesting the existence of an alternative GLP-1R in breast cancer. Further studies revealed that either exendin-4 or GLP-1 induced CREB phosphorylation and activated AMP kinase (AMPK), but did not affect ERK1/2 or AKT.

Finally, MDA-MB-231 cells were injected into both flanks of athymic mice and mice were implanted with osmotic pumps delivering exendin-4 (500ng/d or 2ug/d) or a vehicle continuously for 28 days. Exendin-4 inhibited tumor formation in a dose dependent manner.

GLP-1 and exendin-4 are potent inhibitors of breast cancer cells growth. Their activity may be mediated by a novel signaling pathway which involves activation of an alternative GLP-1R. Further exploration of this pathway may foster understanding of breast cancer development and lead to the discovery of novel therapies. Further studies are needed to determine if exendin-4, an approved treatment for diabetes, can also be used against breast cancer.

Nothing to Disclose: TR, HL, AK, IW

P3-46

The Diabetic Environment Impacts the Metastatic Potential of the Murine Mammary Tumor Cell Line MVT1.

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There is evidence from several epidemiological studies that the metabolic disorders found in Type 2 Diabetes (T2D) may predispose women to greater risk of breast cancer although the precise mechanisms involved in the heightened progression of the disease in these patients still remain under investigation. Recently, mouse models for studying breast cancer development in a diabetic setting have proved extremely useful in demonstrating that the characteristic hyperinsulinemic conditions of T2D promote pre-cancerous mammary gland lesions as well as the progression of primary mammary tumors, primarily through increased activation of IR/IGFR and Akt signaling pathways. The most common cause of mortality resulting from breast cancer is the frequent emergence of aggressive metastatic cells which, after detachment from the primary tumor, typically form lesions in lung and bone tissue. To investigate how the TD2 setting affects this metastatic progression we used the well-characterized hyperinsulinemic female MKR mouse model. To study the effect of hyperinsulinemia on the formation of breast tumor-derived lung metastases, the murine mammary tumor cell line MVT1 was injected into the fourth mammary fat pad of 8-week old MKR and wild-type (WT) mice. Primary tumor formation was followed for 6 weeks and then both groups of mice were examined for formation of lung metastases. Compared to WT mice where only 3/9 mice demonstrated metastases, all the hyperinsulinemic MKR mice (10/10) developed lung metastases. In addition, upon gross examination of the lungs we observed a significant increase in the mean number of metastases present in these MKR mice (WT=1.66; MKR=7.5, p=0.01) suggesting that the TD2 setting may in some way facilitate the metastatic spread of breast cancer cells from the primary tumor site to distant organs of the body.

Nothing to Disclose: RDF, RN, YF, SY, DLR

P3-47

In Asian-American Women, Westernization Influences Estrogen Metabolism, but Not Total Estrogen Production.

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Background Historically, breast cancer incidence has been 4-7 times higher in the United States than in China and Japan. When Asian women migrate to the U.S., risk rises over several generations to almost equal that of U.S. Whites. Endogenous estrogen has been postulated to explain these international differences.

Methods In a population-based case-control study in Chinese, Japanese, and Filipino women living in California and Hawaii, we documented a 6-fold gradient in breast cancer risk with Westernization. Recently we investigated associations of estrogens/estrogen metabolites (EM) with Westernization using 12-h urines from 263 premenopausal luteal and 168 postmenopausal control subjects. Fifteen EM were measured by liquid chromatography-tandem mass spectrometry with high accuracy, precision, and sensitivity. Log-transformed EM measures were regressed on Westernization, which was measured by birthplace or migration history.

Results Total urinary EM (pmol/mg creatinine) was not associated with birthplace in premenopausal or postmenopausal women. When the three estrogen hydroxylation pathways were expressed as % total EM, %2-pathway was 15% lower ($p=0.009$); %4-pathway, 7% lower ($p=0.37$); and %16 pathway, 10% higher ($p=0.008$) among Asian-American women born in the West, compared to the East. Similar results were observed for individual EM in each pathway, premenopausal and postmenopausal women, and the three ethnicities. In both menopausal groups, 2-pathway concentration decreased steadily with increasing breast cancer risk across six Westernization categories.

Conclusions Total estrogen production may not explain the increased breast cancer risk in Western societies. However, estrogen metabolism patterns, especially decreased 2-hydroxylation, may contribute to the elevated risk, and underlying mechanisms merit further exploration.

Nothing to Disclose: RGZ, BJF, XX, MHG, LKK, TDV, RNH

P3-48

Insulin Glargin: Life Time Toxicology Studies in Rats, Mammary Tumor Incidence and Drug Exposure.

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Preclinical risk assessment is based on toxicology studies. Insulin glargin was tested in 6 and 12 months dose-finding studies and in lifetime toxicology for 24 months [1]. The maximum tolerated dose was 12.5 IU/kg qd s.c., the reference compound was human NPH insulin 5 U/kg qd. We report here updated statistical analysis for mammary tumor incidence and exposure data. In studies with [B10-Asp] insulin an increased incidence of mammary tumors was found after 12 months treatment. The lifetime studies with glargin targeted the incidence of spontaneously occurring tumors. Dose levels were 2 - 5 - 12.5 IU/kg and 5 IU/kg in the human NPH insulin group. Animals were housed individually, rats found dead during the study were autopsied on the same day. Serum concentrations of glargin and human insulin were measured in satellite groups, by specific RIA. Results: Survival rates in female rats were 74 - 72 - 60% by week 80, 56 - 60 - 36 % by week 90, and 36 - 32 - 14 % at terminal sacrifice (of 50 rats per group). Survival in the human NPH group was 66% by week 90 and 18% by study end. Mammary tumours observed in each glargin group were mainly fibroadenoma (26 - 22 - 15 vs. 26 in saline controls and 28 in the NPH insulin group) and mammary gland adenocarcinoma (glargin groups incidence 7 - 8 - 7 vs. 9 in saline controls and 7 in the NPH insulin group). Statistical analysis by two different methods (modified Peto lifetime adjusted analysis and a Bieler-Williams poly-3-test) did not reveal any significant differences between the glargin groups and the control groups regarding the incidence of these tumor types. Exposure data: In female rats after glargin injection AUC values (uIU x h/ml) on days 28, 189 and 371 of the study were 2802 - 4387 - 5782 at dose 5 U/kg, and 4233 - 6763 - 12148 at dose 12.5 U/kg. In the human NPH insulin group, AUC values were 1910 - 3585 - 5429 at 5 U/kg. The high dose of glargin induced Cmax and AUC values about 2-3fold higher than those induced by 5 U/kg human NPH insulin.

Conclusions: the design of the lifetime toxicology study was adequate to assess the effect of insulin glargin on mammary tumor incidence in female rats. There was no significant effect of glargin on mammary gland neoplastic lesions in female rats as assessed by two different statistical methods taking into account the survival rates per group. Exposure of glargin treated female rats was 2-3 fold higher than insulin exposure in the NPH insulin reference group.

[1] Stammberger et al (2002). *Int J Toxicol* 21: 171-179

Disclosures: JS: Planning Group Member, Sanofi-Aventis. IS: Employee, Sanofi-Aventis.

P3-49

Potential Role for Neuroendocrine-Induced Changes in Adipose Tissue Biology in the Promotion of Tumor Cell Proliferation.

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The physiological and molecular mechanisms that link chronic stressors (e.g. social isolation) to cancer biology have been long suspected but are poorly understood. We have previously observed that chronic social isolation is associated with a significantly higher mammary tumor burden in both the transgenic C3(1) SV40 Tag mouse and spontaneous Sprague-Dawley rat models of human breast cancer. In transgenic mice, social isolation was found to be associated with a significant upregulation of lipid synthesis and glycolytic pathway gene expression in the mammary gland of 15 week old virgin mice, prior to invasive tumor formation, suggesting that altered metabolism in either the epithelial or stromal tissues is associated with the greater tumor burden observed in mice subjected to the stress of chronic social isolation. To determine whether the stromal +/- the mammary epithelial cells account for increased metabolic gene expression in the isolated versus grouped mice, we separated stromal and epithelial compartments and investigated expression of the previously identified metabolic genes (Acaca, Hk2 and Acly) using Q-RT-PCR. Preliminary data suggest that the increased metabolic gene expression is primarily in the adipose compartment of the isolated mouse mammary gland. We also examined distal fat depots in mice from both social environments for evidence of secretion of tumor epithelial cell-promoting molecules. For these experiments we took gonadal fat pad explants from our animals and made conditioned media. Fat explant-conditioned media from isolated versus grouped animals indeed resulted in increased growth of cancer cells in vitro, suggesting that pro-proliferative factors secreted from the isolated mice depots are more tumorigenic than the grouped mice fat depots. Taken together, our preliminary data suggest that stress-associated neuroendocrine factors modulate gene expression within the adipose tissue depots, and result in changes in adipose tissue biology that promotes tumor cell proliferation.

Nothing to Disclose: PAV, ELW, SWK, MKM, SDC, MJB

P3-50

Signaling Crosstalk between Leptin and IGF-1 in Human PC-3 Prostate Cancer Cells.

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Obesity is considered as a risk factor for many cancers including prostate cancer, particularly aggressive tumors. Leptin, a product of the obese (*ob*) gene is a hormone that plays an essential role in appetite regulation and metabolism. Evidence also suggests that leptin can act as a growth factor to regulate cancer cell proliferation, invasion and metastasis. Insulin-like growth factor type I receptor (IGF-1R) is widely expressed in human epithelial cancers, and is emerging as an important therapeutic target. We hypothesized that leptin and IGF-1 signaling might collaborate to modulate cellular functions in prostate cancer cells. Here we utilized an androgen-insensitive human prostate cancer cell line, PC-3, as an advanced cancer cell model to investigate potential signaling crosstalk between leptin and IGF-1 and subsequent outcomes. We first demonstrated that both leptin receptor (Ob-R) and IGF-1R were expressed in PC-3 cells; however, the Ob-R level was relatively low. IGF-1 treatment caused phosphorylation of insulin receptor substrate-1 (IRS-1) and activation of Akt while leptin activated JAK2 (a tyrosine kinase downstream of Ob-R) and Akt in a time-dependent manner. Interestingly, when PC-3 cells were cotreated with IGF-1 and leptin (referred to as IGF-1/Leptin), the degree of both IRS-1 phosphorylation and Akt activation was significantly lower than that in IGF-1 single treatment ($P < 0.05$, IGF-1/Leptin vs. IGF-1). We next examined cell survival in PC-3. MTT assay (measuring cell survival) and Hoechst/propidium iodide (PI) double staining (measuring cell apoptosis) results indicated that IGF-1 treatment markedly prevented hyperthermia-induced apoptosis, suggesting an anti-apoptotic effect of IGF-1. Although leptin itself had no apparent effect on hyperthermia-induced apoptosis, it significantly antagonized the IGF-1's anti-apoptotic effect when cotreated with IGF-1. Finally, we performed wound healing assays to assess cell motility. IGF-1 but not leptin promoted PC-3 cell migration; however, this IGF-1 effect was significantly inhibited by leptin cotreatment. Taken together, our results suggest that leptin antagonizes IGF-1 signaling and IGF-1-mediated cellular processes such as anti-apoptosis and migration. We are currently investigating the mechanism how leptin interacts with IGF-1 signaling system to exert its inhibitory effects on IGF-1 actions. The study will provide new insights into signaling crosstalk between leptin and IGF-1 in cancer.

Sources of Research Support: St. Joseph's Foundation Startup Fund and partially by a Science Foundation in Arizona (SFAz) Award (to Y.H.).

Nothing to Disclose: KF, IB, JB, YH

P3-51

High Dose Isoflavones Do Not Improve Metabolic and Inflammatory Parameters in Androgen Deprived Men with Prostate Cancer.

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Background: The profound hypogonadism that results from the use of androgen-deprivation therapy (ADT) in men with prostate cancer (PCa) results in complications such as insulin resistance and metabolic syndrome. Since phytoestrogens have been shown to improve glycemic parameters, we evaluated their role in men undergoing ADT.

Methods: This was a randomized, double-blind, placebo-controlled, 12-week pilot study. Participants were randomly assigned to receive 20 g of soy protein containing 160 mg of total isoflavones vs taste-matched placebo (20 g whole milk protein). Fasting glucose, insulin and lipid profiles were measured at baseline, 6-weeks and 12-weeks. Adipokines and inflammatory cytokines were also measured at these time points. The study was conducted at a tertiary care center in the United States.

Results: Thirty-three men (isoflavones=17, placebo=16) undergoing ADT for PCa completed the study. The two groups were well-matched at baseline. Mean age of the subjects was 69 years and 80% were Caucasian. Mean duration of ADT in both groups was approximately 2 years. After 12-weeks of intervention, there was no significant difference in either metabolic or inflammatory parameters between the two groups.

Conclusion: High-dose isoflavones in androgen deprived men with PCa do not improve metabolic or inflammatory parameters.

1. Basaria S et al., Cancer 2006; 106: 581
2. Braga-Basaria M et al., J Clin Oncol 2006; 24: 3979
3. Jayagopal V et al., Diabetes Care; 25: 1709

Sources of Research Support: Physicians Pharmaceuticals, Inc, Kernersville, NC, provided the soy compound; GCRC, Johns Hopkins University School of Medicine.

Nothing to Disclose: JN, RGS, DM, OC, TGT, MM, JE, SB

P3-52

Separate and Combined Actions of Tamoxifen and Metformin in Hormone Resistant Breast Cancer Cells.

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Measures to increase tamoxifen (TAM) effectiveness or overcome resistance to this antiestrogen are critically needed in breast cancer patients. A series of prior studies have suggested that antidiabetic biguanide metformin (MF) might be useful to enhance effectiveness of a number of oncologic agents. For that reason, we examined the effects of TAM and MF in several MCF-7 breast cancer models. Methods: For quantitation of cell number, wild-type (wt) and TAM resistant (TAM-R) MCF-7 cells were grown in the absence or presence of 10⁻⁷ M TAM ± 2.5 mM MF for 5 days in IMEM medium containing 5% FBS or (in experiments with estradiol) dextran-coated charcoal-stripped serum (DCC). Long-term estrogen deprived (LTED) cells were grown in DCC medium with the same concentrations of ingredients. For immunoblot analysis and aromatase activity measurements, wt, TAM-R and LTED cells were grown for 48 hrs ± TAM (10⁻⁷ M) and ± MF (5.0 mM). Results: A. Cell counts. In wt MCF-7 and LTED cells, TAM and MF inhibited growth, although TAM-R cells were less sensitive to TAM than to MF. The highest partial additive effect of TAM and MF was observed in LTED cells which clinically correspond with the long-term postmenopausal condition or with long-term treatment with aromatase inhibitors. In addition, differences between the effects of TAM+MF vs isolated TAM action were highest in TAM-R cells. B. Western-blots. The analyses performed revealed that AMP-kinase is stimulated by MF approximately equally in MCF-7, TAM-R and LTED cells. Inhibition by MF of p-S6K as a target of mTOR was strongest in TAM-R cells which may be important clinically. Contrary to the findings in TAM-R cells, expression of the oncogene suppressor BRCA1 was increased by MF in wild-type MCF-7 and LTED cells. In contrast with cell number values, no difference was discovered in PCNA levels in cell lines treated with MF or TAM. Under the influence of MF, expression of ER-alpha was decreased in wt MCF-7 cells and a modest trend toward a decrease in PR expression was seen in response to the combined actions of MF and TAM in wt MCF-7 and TAM-R cells. C. Aromatase activity was decreased in response to co-cultivation with MF in TAM-R cells only whereas a change of this enzyme activity in other cell lines was variable. Conclusions: The cellular context (including loss of sensitivity to TAM and estrogen deprivation) is important in modulating the response of breast cancer cells to metformin and its combination with tamoxifen.

Sources of Research Support: UICC grant ICR 09-130.

Nothing to Disclose: LMB, WY, JPW, RJS

P3-53

JunB and JunD Mediate PGE₂ Activation of Breast Cancer-Associated Aromatase Promoters in Human Breast Adipose Fibroblasts.

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Aromatase is a key enzyme for estrogen biosynthesis. A distal promoter I.4 maintains baseline levels of aromatase expression in normal breast adipose tissue. In contrast, malignant breast epithelial cells secrete prostaglandin E₂ (PGE₂), which is thought to stimulate aromatase expression via its proximal breast cancer-associated promoter I.3/II in a cAMP/PKA and PKC-dependent manner in adjacent breast adipose fibroblasts, leading to strikingly high local concentrations of estrogen. In cultured human breast adipose fibroblasts (BAFs), PGE₂ stimulation of JNK1 is necessary for aromatase induction. To identify transcription factors downstream of JNK1 that regulate aromatase promoter I.3/II, we examined the roles of the Jun family transcription factors c-Jun, JunB, and JunD known to be phosphorylated and activated by JNK. PGE₂ treatment of BAFs resulted in biphasic c-Jun phosphorylation on Ser73 and marked induction of JunB and JunD protein prior to peak aromatase induction. Surprisingly, c-Jun siRNA knockdown enhanced PGE₂ induction of aromatase mRNA level and enzyme activity and did not change basal expression or activity. Aromatase promoter-specific RT-PCR revealed that c-Jun knockdown did not change basal or PGE₂-induced activity of promoter I.3/II, but it dramatically increased constitutive promoter I.4 activity. By contrast, JunB or JunD knockdown markedly reduced both basal and PGE₂-induced levels of total and promoter I.3/II specific aromatase mRNA and aromatase enzyme activities. In addition, JunB siRNA, with its target sequences sharing no homology with JunD mRNA, not only abrogated JunB protein expression, but also JunD mRNA and protein expression, suggesting that JunB regulates JunD gene transcription. Jun family transcription factors regulate gene expression by interacting with AP-1 and CRE sites in the promoter region as homo- or heterodimers. Using avidin-biotin conjugate DNA (ABCD) precipitation assay, chromatin immunoprecipitation (ChIP), and luciferase reporter assay, we determined that JunB and JunD, as well as c-Jun, bound to CRE(-211/-199) critical for aromatase promoter I.3/II activity. We conclude that JunD homodimer and its heterodimer with JunB or c-Jun are key transcription factors directly stimulating aromatase promoter I.3/II by binding to CRE(-211/-199) following PGE₂ treatment in BAFs, and that JunB also activates aromatase promoter I.3/II indirectly by maintaining JunD gene transcription.

Sources of Research Support: NIH grant CA67167 and the Penny Severns Breast, Cervical and Ovarian Cancer Research Fund from the Illinois Department of Public Health.

Nothing to Disclose: DC, SR, JC, SB

P3-54

Tumour Necrosis Factor α Induces Early Growth Response Genes in Breast Adipose Fibroblasts: A Novel Aromatase Regulatory Pathway.

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Breast cancer remains the leading cause of cancer-related death in Australian women. In post-menopausal cases, up to 70% of tumours are classed as estrogen receptor positive (ER+), dependent on estrogen for continued growth and proliferative advantage. Adjuvant anti-estrogen therapies are considered the cornerstone approach to the treatment of such tumours, and research is ongoing to maximize the effectiveness of drug treatments.

The major source of estrogens for growth of ER+ breast cancers is local conversion of androgen precursors by the enzyme P450 aromatase. Inflammatory factors such as TNF α stimulate transcription of the *CYP19A1* gene that encodes aromatase, via its adipose-specific promoter I.4 (PI.4). The pathways by which this is achieved are not fully understood. This study aims to identify the mechanisms underlying TNF α -dependent aromatase induction in the context of estrogen-dependent breast cancer.

Primary human breast adipose fibroblasts (BAFs) were treated with TNF α and potential regulatory factors of PI.4 were assessed for changes in mRNA expression through the use of a custom array. The family of Early Growth Response genes *Egr1*, *Egr2*, *Egr3* and *Egr4* were all found to be significantly upregulated in a time-dependent manner in response to TNF α , as validated by RT-PCR. Previous studies have indicated that the SP1 binding site within the PI.4 region is critical to transcriptional activation (1), and our studies reveal that this site is also an overlapping Egr consensus site. Such overlapping sites are known to exist on a number of human promoters, with Sp1 and Egr's co-operating to control gene transcription. No change in mRNA expression of *Sp1* was observed in BAFs in response to TNF α . This creates the potential for interplay of these transcription factors in a competitive manner to regulate PI.4 expression in response to TNF α , a hypothesis further being investigated through CHIP and overexpression studies.

Understanding TNF α signaling and the transcription factors it activates to turn on PI.4 *CYP19A1* expression in BAFs is vital to the understanding of breast cancer pathology. These insights may help in the development diagnostic tools or novel therapeutics in order to help tackle the growing instances of this disease.

(1) Zhao Y et al., Mol Endocrinol 1995; 9:340

Nothing to Disclose: SQT, KCK, ERS, CDC

P3-55

Species-Dependent Susceptibility of Inhibition of 17beta-Hydroxysteroid Dehydrogenase Type 1: Enzyme Inhibition and Molecular Docking Study.

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In search for new inhibitors of human 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD 1) for the treatment of endocrine-related diseases like breast cancer or endometriosis we validated novel substances based on 15-substituted estrone for their specificity to other 17beta-HSD types. The compounds were tested by in vitro activity assays on recombinant human 17beta-HSD types 1, 2, 4, 5 and 7. Most of the tested substances are specifically inhibiting 17beta-HSD1 except a 15alpha-substituted compound, which was effective on 17beta-HSD5 to the same amount. We also analyzed 17beta-HSDs type 1 of several other species including marmoset, pig, mouse, and rat and therewith present the quantification of inhibitor preferences between human and animal models used in the processes of pharmacophore screening. Profound differences in the susceptibility to inhibition of steroid conversion among the 17beta-HSDs type1 were observed. Especially the rodent 17beta-HSD types 1 were significantly less sensitive to inhibition compared to the human orthologue, while the most similar inhibition pattern to the human 17beta-HSD 1 was obtained with the marmoset enzyme. Two compounds were found to inhibit the 17beta-HSD types 1 of all species studied, but were not the most effective for the human enzyme. Therefore, activity screens in heterologous species systems must be evaluated with caution. We show that good candidate inhibitors for human 17beta-HSD 1 would out-select them during pre-clinical optimization step using rodent models. Further we performed molecular docking experiments to check the predictive value for homology build structures of 17beta-HSDs. With these analyses we were able to confirm the ranking of one of the universal inhibitors and to select the most specific human inhibitor. However, species-specific prediction of inhibitor performance by modeling was not possible.

Nothing to Disclose: GM, BH, DK, LH, DP, LR, JM, HT, JA

P3-56

Modulation of Oxidant and Antioxidant Enzymes in Cancer Cells by Progesterone.

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Progesterone (P4) has been implicated as a protective factor for epithelial ovarian and endometrial cancers, yet little is known about its mechanism of action. We previously reported that pregnancy-equivalent doses of P4 inhibited the growth of normal and malignant human ovarian surface epithelial (HOSE) and endometrial cells. Increased generation of reactive oxygen species (ROS) and an altered redox status have been observed in cancer cells. Here, we investigated if P4-induces cell death by modulating major antioxidant enzymes. The exposure of ovarian and endometrial cancer cell cultures to 10^{-6} M P4 induced time-dependent increases in early and late apoptotic cells and activation of caspase-3. A general caspase inhibitor Z-VAD effectively blocked the P4- induced cell death in a dose-dependent manner. Expression levels of glutathione peroxidase (GPX1), NAD(P)H:quinine oxidoreductase (NQO1) and copper and zinc SOD (CuZnSOD) were high in cancer cells. Treatment of cancer cells with progesterone at 10^{-8} M for a period of 72 h induced significant loss of GPX1, NQO1 and CuZnSOD protein expression. The inhibitory action of progesterone was blocked by the specific progesterone antagonist (mifepristone, 10^{-5} M), confirming P4 specificity. Further investigation on the regulation of antioxidant enzymes by P4 may provide new insights into P4's anticancer activities and facilitate the development of new strategies to use P4 as an anticancer agent.

Nothing to Disclose: HN, VS

P3-57

Neurotensin Promotes Breast Cancer Growth and Metastasis Emergence.

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Intratumoral heterogeneity is a critical phenomenon in the natural history of breast carcinomas. The clinical repercussions from these events include the formation of clones with metastatic potential, as well as the development of drug-resistant variants in primary cancers. Several cellular factors are suspected to interact in concert forming a signaling network capable to increase mitogenic or invasive signals. In this context, we investigated the potential contribution of the neuropeptide neurotensin (NTS), and its high affinity receptor (NTSR1) in human breast cancer progression.

NTSR1 is the target of wnt/beta catenin pathway resulting in early and high expression in breast tumors and its ligand-receptor complex is involved in the growth of several other solid tumors (1). NTSR1 is not expressed in normal breast epithelial cells but is expressed in invasive breast cancer. In contrast, NTS is expressed in normal breast cells, as well as cancer cells, and is upregulated by estrogens (2).

NTS and NTSR1 expression was studied in a series of patient with invasive ductal carcinomas. Most of patient expressed NTSR1 (over 10% of positive cells) with the majority (50 to 30%) exhibited a high proportion receptor-positive (50 to 100%), and 40% expressed NTS (3). We observed a correlation between the high expression of NSTR1 and the patient death, the size of tumor, the SBR grade, and a higher number of invaded lymph nodes. No correlation was observed with NTS and predictive clinical criteria. Simultaneous expression of NTS and NTSR1 in the tumors (20% of the patients) suggested the establishment of inappropriate and detrimental autocrine and/or paracrine NTS signaling loops. Using experimental tumors generated by the xenograph of MDA-MB 231 wild type and silenced for NTSR1, or MCF-7 cells wild type or stably expressing NTS, we demonstrated that NTS drastically induces tumor growth and metastasis emergence. In vitro studies showed the potential of NTS to accelerate and activate the cellular invasion, migration and anchorage independent growth.

Our findings support the contribution of the neurotensinergic system in human breast cancer progression and point out the interest to develop therapeutic molecules targeting the NTS-NTSR1 signaling cascade.

(1) Souaze F, Viardot-Foucault V, Rouillet N et al. *Carcinogenesis* 2006; 27(4):708-16.

(2) Dupouy S, Viardot-Foucault V, Alifano M et al.. *PLoS One* 2009; 4(1):e4223.

(3) Souaze F, Dupouy S, Viardot-Foucault V et al. *Cancer Res* 2006; 66(12):6243-9.

Sources of Research Support: INSERM and Grants from ARC: 3543 and 3905, "Ligue contre le Cancer" 07/75-85 and GEFLUC. Sandra Dupouy was supported by the "Ligue nationale contre le cancer".

Nothing to Disclose: SD, VKD, NM, ODW, AG, PF

P3-58

Activated Farnesoid X Receptor Inhibits Growth of Tamoxifen-Resistant MCF-7 Breast Cancer Cells, through Down-Regulation of HER2 Expression.

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Tamoxifen treatment is still the first-line endocrine therapy for ER-positive breast cancer patients. Unfortunately, not all breast cancers respond to tamoxifen, and many of the responsive tumors eventually relapse developing resistance to this drug. It has been demonstrated, using “in vivo” and “in vitro” models, that HER2/neu (erbB2) overexpression can trigger tamoxifen resistance in human breast cancer cells. HER2 overexpression results in activation of the Ras/MAPK signaling pathway that in turn can promote growth and progression in breast cancer cell lines and carcinomas. The nuclear receptor FXR (Farnesoid X Receptor), mainly expressed in enterohepatic district where regulates lipids and glucose homeostasis, is found to be expressed also in several non enterohepatic tissues including normal and tumoral mammary gland. Particularly, it has been reported that FXR induces apoptosis in breast cancer cells, but its mechanisms of action in these cells are not fully elucidated.

Here we evaluated the effects of FXR activation, by its natural ligand the chenodeoxycholic acid (CDCA), on growth of tamoxifen-resistant breast cancer cells (MCF-7TR), developed to mimic “in vitro” the occurrence of acquired Tam-resistance. Using MTT assay we found that CDCA as well as GW4064, a specific FXR agonist, reduced cell viability in MCF-7 and in a higher extent in MCF-7TR cells, but had no effects on growth of normal breast epithelial cells MCF-10. Furthermore, CDCA treatment induced a significant reduction in both anchorage-dependent and anchorage-independent tamoxifen-resistant growth. CDCA was also able to inhibit Epidermal Growth Factor (EGF) induced growth in MCF-7 TR cells. Interestingly, FXR activation determined a down-regulation of HER2 expression at both mRNA and protein levels, and concomitantly reversed EGF-mediated HER2 and p42/44 MAPK phosphorylation in MCF-7 TR cells. Preliminary transient transfection experiments, using a vector containing human HER2 promoter region, revealed that CDCA was able to reduce basal HER2 promoter activity. However, further studies are needed to elucidate the molecular mechanisms through which FXR regulates HER2 transcriptional activity in breast cancer cells. Collectively, these data suggest that FXR ligand-dependent activity, blocking HER2/MAPK signaling, may overcome antiestrogen resistance in human breast cancer cells and could represent a new therapeutic tool to treat breast cancer patients that develop resistance.

Nothing to Disclose: CG, DV, SP, IB, DB, SF, SC, SA

P3-59

Development of Stable Isotope Dilution Liquid-Chromatography/Mass Spectrometry (LC/MS) Methods for the Determination of the Androgen Metabolome.

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Although prostate cancer is initially responsive to androgen ablation therapy, it often recurs as castrate resistant prostate cancer (CRPC). The success of the CYP17A1 inhibitor abiraterone acetate in the treatment of CRPC indicates that this disease typically remains hormone dependent. Intratumoral levels of testosterone, but not 5 α -dihydrotestosterone, are elevated in CRPC compared to primary tumors. However, levels of other androgen precursors and metabolites (the androgen metabolome) have not been examined. Determination of the androgen metabolome would elucidate which reactions are likely to be rate limiting, and therefore most likely to respond to pharmacotherapy for CRPC. To this end, we have developed stable isotope dilution LC/MS methods for the determination of androgens in biospecimens. The basis of our method is to use deuterated internal standards for each analyte of interest so that the ratio of labeled and unlabeled analytes can be used for quantitation. Normal phase separation of the steroids coupled to atmospheric pressure chemical ionization-MS allows the androgen metabolome to be separated and quantified. In order to improve sensitivity, derivatization of ketosteroids as oximes (i.e. Girard derivatives) and hydroxysteroids as esters (i.e. picolinate derivatives) has been achieved. The derivatized steroids can be separated with reverse phase HPLC and quantified through comparison to deuterated standards using electrospray ionization-MS detection. With this method, we will be able to examine androgen metabolism by prostate cancer cells in vitro and androgen levels in biospecimens from prostate cancer patients undergoing different drug treatments.

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Nothing to Disclose: MCB, CM, IAB, TMP

P3-60

Altered Hormonal Regulation of Gene Expression in Anti-Hormone Drug-Resistant and Hormone-Deprived Breast and Prostate Cancer Cells.

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Background: Endocrine therapies targeting hormone receptor (HR) actions are widely used and effective treatment for estrogen receptor α (ER α)-positive breast and androgen receptor (AR)-positive prostate cancers, respectively. Many patients treated with endocrine therapies, however, will relapse with endocrine-resistant cancers. To understand molecular mechanisms underlying the resistance, we investigated gene expression signatures in endocrine-resistant cancer cell models derived from hormone-sensitive breast and prostate cancer cells.

Methods: Tamoxifen (1 μ M)-resistant (TamR) and long-term estrogen-deprived (LTED) breast cancer cells were generated from MCF7 cells, and bicalutamide (1 μ M)-resistant (BicR) and long-term androgen-deprived (LTAD) prostate cancer cells were generated from LNCaP cells. The culture duration from the parental cells was >12 weeks, >16 weeks, and >24 weeks for TamR, LTED, and BicR/LTAD cells, respectively.

Results: Quantitative RT-PCR showed that AR was upregulated at basal and at R1881 treatment in BicR/LTAD cells compared to parental LNCaP cells. AR levels were even slightly increased by Bic treatment in BicR/LTAD cells. In breast cancer cells, ER α expression was upregulated in LTED cells compared to parental MCF7 cells, but not in TamR cells at mRNA level. Glucocorticoid receptor (GR) was upregulated in TamR, LTED, and BicR cells. In BicR cells, GR expression was substantially induced by Bic rather than by R1881. Expression of some HR downstream genes including progesterone receptor and PSA was repressed in TamR cells (and some LTED clones) and BicR/LTAD cells, respectively. We also interested in some of forkhead box family members, because FOXA1 has been shown as a critical transcriptional factor associated with the AR and ER α signaling (1), and we have previously shown that FOXP1 is an androgen-responsive transcription factor that negatively regulates AR signaling (2). We found that the expression of FOXP1 and FOXA1 was increased in TamR/LTED cells. FOXP1 was also slightly increased in BicR/LTAD cells.

Conclusion: The present study suggests that endocrine therapies will alter the expression of nuclear receptors and its related genes, as well as the responsiveness to steroid hormones and its antagonists. Elucidation of molecular mechanisms underlying endocrine resistance will be useful to develop alternative therapeutic options for resistant disease.

(1) Lupien M et al., *Cell* 2008;132:958

(2) Takayama K et al., *Biochem Biophys Res Commun* 2008;374:388

Sources of Research Support: The Cell Innovation Program from the Ministry of Education, Culture, Sports, Science & Technology, Japan, awarded to KH, SI.

Nothing to Disclose: KH-I, KI, SI

P3-61

In Vitro Cultures of Prostaspheres from Aged Rats Are Enriched for Stem/Progenitor Cells Capable of Regenerating Prostate Glands In Vivo.

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In the aging Brown Norway rat, epithelial cell hyperplasia occurs in the dorsal and lateral lobes of the prostate. We initiated studies to determine whether hyperplasia of epithelial cells is due to age-related changes inherent in prostate stem/progenitor cells. We used both in vitro and in vivo approaches to isolate and characterize prostate epithelial stem/progenitor cells from the hyperplastic lateral lobe of 24-month old rats. Prostatic cells were completely dissociated by enzymatic digestion and enriched for epithelial cells by allowing stromal cells to attach to tissue culture plates by incubation overnight in DMEM/F12 medium containing FBS. Non-adherent epithelial cells were counted (500 cells/ml) and incubated in serum free DMEM/F12 medium supplemented with EGF and bFGF on ultra low attachment culture plates. A subpopulation of epithelial cells formed prostaspheres as evidenced by the increasing size of cellular spheres over time in culture. After 15 days of growth, 55-60 spheres were formed/plate. The floating spheres were composed of viable cells based on propidium iodide staining. Dissociation of spheres and serial passage increased the efficiency of sphere formation by 4-fold compared to parental cells. Prostaspheres were enriched for stem/progenitor cells as confirmed by RT-PCR for Sca-1, CD133, Bcl-2 and b-catenin mRNA expression. The prostate regenerative potential of these cells was tested in vivo in tissue recombination experiments. Prostaspheres were dissociated and the cells were mixed with E17 rat UGM, combined with collagen and grafted under the renal capsule of nude mice. After 4 weeks, prostate glandular structures were regenerated as revealed by H&E staining. Prostatic glands did not form in the absence of E17 UGM. The prostate glands were positive for p63 and AR by immunostaining that identified basal and luminal epithelial cells, respectively. The successful isolation and propagation of prostaspheres from the Brown Norway rat model of prostatic epithelial hyperplasia will enable us to study the effects of aging on the biology of prostate epithelial stem/progenitor cells.

Sources of Research Support: NIH Grant AG020999 awarded to TRB.

Nothing to Disclose: JO, TRB

P3-62

Effects of Prolactin (PRL) and the Selective PRL Receptor Modulator, S179D PRL, on the BRCA1-p21 Axis in Breast, Ovarian, and Prostate Cancer Cell Lines.

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The literature supports a role for prolactin (PRL) in the development of some cancers through PRL's promotion of the cell cycle and/or inhibition of apoptosis. S179D PRL, on the other hand, inhibits cancer cell proliferation and promotes differentiation and/or apoptosis. BRCA1 is a tumor suppressor in breast (B), ovarian (O), and prostate (P) cancer. The purpose of the current study was to determine the effect of the functionally distinct ligands for the PRL receptor (PRL and S179D PRL) on BRCA1 function. Contrary to expectation, both PRL and S179D PRL (at 500 ng/ml) elevated levels of BRCA1 protein (~3 fold in 24h and up to 10 fold in 48h) in all cancer types examined (B,O, &P). The promoter for the cell cycle inhibitory molecule, p21, has a BRCA1 binding site, but only S179D PRL increased levels of p21 protein as a result of BRCA1 elevation. Using deletion analysis of a p21 promoter-luciferase construct, we showed that the BRCA1 binding site (-84 to -129) was crucial to p21 expression in response to S179D PRL. An important question therefore was how elevated BRCA1 in response to each ligand had a different end result in terms of p21 expression. Both ligands promoted nuclear translocation of BRCA1. Moreover, both ligands similarly increased BRCA1 interaction with the important region of the endogenous promoter of the p21 gene (ChIP assay). A major difference in the signaling resulting from receptor engagement by PRL *versus* S179D PRL is that only PRL activates Stat5. With PRL, but not S179D PRL, BRCA1 formed a complex with phospho-Stat5 (IP with anti-BRCA1 and Western for phospho-Stat5) and this complex translocated to the nucleus. It appears therefore that formation of a complex between BRCA1 and phospho-Stat5 negates its ability to transactivate the p21 promoter. For already synthesized p21, additional studies demonstrated that stimulation of cells with PRL, but not S179D PRL, resulted in phosphorylation of p21, a posttranslational modification that inhibits nuclear translocation and therefore the ability of p21 to inhibit the cell cycle. This study has uncovered two ways in which p21 function is hampered by PRL and hence additional ways in which PRL promotes cell proliferation. At the same time, since S179D PRL inhibits Stat5 activation in response to PRL in addition to itself signaling to p21 elevation, this work also furthers our understanding of the anti-cancer activities of S179D PRL.

Nothing to Disclose: KEC, AMW

P3-63

Regulation of CYP3A4 and CYP3A5 Expression in Prostate Cells and Modulation of 'Intracrine' Metabolism of Androgens by Liganded Vitamin D Receptor (VDR).

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Androgen deprivation therapy (ADT) is a primary course of treatment for men with advanced prostate cancer. While such tumors typically exhibit a dramatic initial positive response to ADT, they inevitably relapse to an androgen-independent phenotype. It has become apparent that current ADT approaches are unable to completely suppress the presence of androgens at the tissue level which may contribute to adaptive changes allowing prostate cancer cell survival. The optimum application of ADT towards prostate cancer will require the suppression of both systemic and 'intracrine' contributions to the androgen microenvironment.

We and others have previously demonstrated that CYP3A4 gene expression, a pivotal factor in the metabolism of xeno/endobiotic compounds, can be induced by ligand-activated vitamin D receptor (VDR) in a number of colon-derived cell lines. As androgens such as testosterone, androstenediol, and DHEA, are also known to be oxidatively inactivated through CYP3A4 activity, we have investigated if the regulatory effects of VDR upon the CYP3A family could be extended to prostate cells and impact upon the prostatic metabolism of growth-promoting androgens. Our data reveal that the mRNA and protein levels of both CYP3A4 and CYP3A5 in LNCaP and RWPE-1 cells are significantly induced following exposure to 1,25(OH)₂D₃. Chromatin immuno-precipitation (ChIP) and promoter/response element-based reporter assays reveal that regulation of CYP3A4/A5 gene expression is achieved through direct binding of prostatic VDR to distinct regulatory motifs located within the 5' promoter region of both CYP3A genes. We observe that treatment of LNCaP cells with 1,25(OH)₂D₃ will enhance the cellular enzyme activities of CYP3A4 and CYP3A5 to levels comparable to that observed within caco-2 and hepG2 cells, and will lead to a significant turnover of cellular testosterone to its inactive 6β-OH metabolite. While we are continuing to probe through both genomic and metabolomic approaches how liganded VDR can impact upon androgen metabolism within the context of both prostate 'normal' and cancer cells, our present data point to the potential use of vitamin D-derived compounds used in combination with ADT as a means to limit the bioavailability of growth-promoting androgens with the tumour microenvironment.

Sources of Research Support: Prostate Research UK.

Nothing to Disclose: CP, OM, TS, SM, PM, AO, PT

P3-64

Long Range Chromatin Interactions of *AMACR* Gene in the Prostate.

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Alpha-methylacyl-CoA racemase (*AMACR*) is overexpressed in prostate cancer (PCa) and has been established as a diagnostic marker for PCa in needle biopsies. *AMACR* is responsible for the metabolism of branched-chain fatty acids and bile acid intermediates. siRNA knockdown of *AMACR* increased androgen receptor expression, decreased cell viability and impaired proliferation in PCa cells (1,2). We previously reported the deletion polymorphism at CG12-16 of a CpG island in the 5'-flanking region of the *AMACR* gene is a *cis*-element(s) controlling gene expression in prostate and colon cancer (3). Proximal promoter-reporter assays showed that the deletion of CG12-16 increased the gene activity by only 1-fold in PCa cells. In contrast, by comparing transcript expression in nine pairs of matched PCa and adjacent benign tissues, we observed an average of 31-fold increase of *AMACR* transcript overexpression in cancer tissues. We therefore hypothesize that changes in distal *cis*- and *trans*-regulatory factors play important role in *AMACR* expression. This study seeks to identify and characterize the long range regulatory sequences that regulate *AMACR* expression by employing the circular chromosome conformation capture (4C) technology to uncover long-distance chromosomal interactions (4). 4C technology cross-links cells to fix DNA-protein interactions, and studies *in vivo* physical interactions between chromosomes without a preconceived idea of the interacting partners. Using normal prostate epithelial cell line (NPrEC) with low *AMACR* expression and PCa cell lines LNCaP and its derivative C4-2 with high *AMACR* expression, our preliminary data identified inter-chromosomal interactions flanking *AMACR* gene transcriptional start site (chromosome 5p13) and foci at 6p22, 7q35 and 8q21.1, ranging from 113 to 600 bp with no known genes in these regions. Interestingly, putative repressors are identified in these sequences and their interaction with *AMACR* gene shown in 4C is higher in NPrEC than in LNCaP and C4-2 cells, suggesting distal repressors suppressed *AMACR* expression in NPrEC cells. Further identification and characterization at chromatin architecture, promoter, and *cis*- and *trans*-regulatory elements should uncover the mechanism of *AMACR* gene regulation in prostate carcinogenesis.

- (1) Zha S et al., Cancer Res 2003; 63:7365
- (2) Takahara K et al., Anticancer Res 2009; 29:2497
- (3) Zhang X et al., PLOS Genetics 2009; 5:e1000334
- (4) Zhao Z et al., Nat Genet 2006; 38:1341

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Nothing to Disclose: XZ, S-MH

P3-65

GEMS (Gene Expression MetaSignatures), a Web Resource for Querying Meta-Analysis of Expression Microarray Datasets: Dihydrotestosterone in LNCaP Cells.

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Several gene expression microarray resources are available to the scientific community, one of which is the Nuclear Receptor Signaling Atlas (NURSA) website, www.nursa.org, which archives datasets according to ligand, nuclear receptor or coregulator. Once datasets are accessed however, a present limitation is availability of a common software platform for evaluation of expression relationships across different hormonal or tissue variables. To this end, we have developed a simple meta-analysis algorithm, GEMS (Gene Expression Metasignatures) which has been incorporated into the NURSA website, and which allows for querying any given meta-analysis by gene name or symbol; future versions will provide for querying by cut-off in addition to other parameters such as Gene Ontology terms or disease. The tool is designed to be convenient for use by the average bench scientist with little or no bioinformatics or microarray expertise. Here we report use of the GEMS meta-analysis tool to establish a consensus across multiple independent datasets for genes regulated by the androgen dihydrotestosterone (DHT) in the human LNCaP prostate tumor cell line. The initial seed for the meta-analysis combines published and unpublished expression datasets utilizing Affymetrix Human Genome U133 arrays encompassing 19,622 Entrez Gene Identifiers. This meta-analysis provides, for each gene, a combined measure of significance for its transcriptional response after 24 h treatment with ≥ 10 nM DHT in LNCaP cells across all the datasets in the meta-analysis. Importantly, the meta-analysis method is platform independent and is amenable to the inclusion of any gene expression array platform. The method provides confidence limits for expression significance, and allows the end-user to identify reliable gene signatures for specific hormonal conditions. We anticipate that with the inclusion of additional future datasets, the DHT/LNCaP GEMS will develop into an important resource for modeling gene expression in this widely-used model system.

Nothing to Disclose: SAO, CMW, ESC, DLS, BR, IUA, NLW, WTS, NJM

P3-66

Oxytocin as a Potential Autocrine Regulator of Prostate Cancer Metastasis.

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Oxytocin (OXT) and vasopressin (AVP) are highly homologous peptide hormones that have diverse endocrine functions (1). To assess potential autocrine roles of OXT and AVP in normal and diseased prostate, we examined the expression of these hormones and their cognate receptors in normal and cancerous prostate epithelial cells. Because chemotaxis has been associated with activation of GPCRs that couple exclusively to G α i (2, 3), we hypothesized that OXT would induce migration of prostate cancer cells if endogenous OXTRs couple effectively to the Gi-dependent pathway. Reverse transcription and polymerase chain reaction techniques (RT-PCR) were used to examine the expression profile of OXT, AVP, OXT receptor (OXTR), AVP type 1a and type 2 receptors in normal prostate epithelial and stromal cell lines (PrEC, RWPE1, PrSC), k-ras transformed prostate epithelial cell line (RWPE2), and in four prostate cancer cell lines (LNCaP, DU145, PC3, PC3M). OXTR expression was also examined by quantitative PCR. Secreted and cell-associated OXT peptide was measured by an enzyme immunoassay. The cell migration assay was performed with transwell inserts. OXT and OXTR were expressed in all eight prostate cell lines. Cell-associated OXT peptide was found in all prostate epithelial cell lines except in DU145 cells. On the contrary, neither AVP nor its cognate receptors were expressed in any prostate cell line examined. These data point to the OXTR as the primary target of OXT and AVP and suggest that OXT may be an autocrine/paracrine regulator in human prostate. OXT was found to induce migration of PC3 and PC3M, but not DU145 prostate cancer cells. The effect of OXT (100 nM) is distinct from the epidermal growth factor (EGF, 3ng/ml)-induced migration of prostate cancer cells, in which extracellular signal-regulated kinase 1/2 and EGF receptor kinase activities were required. When cells were pretreated overnight with pertussis toxin (100 ng/ml), the effect of OXT, but not EGF, on cell migration was abolished. Pretreatment with the cell permeable cAMP analogue, 8-Br-cAMP (0.3 mM), did not affect the OXT-induced cell migration, which eliminated the non-specific effect of pertussis toxin. Collectively, these data point to a Gi-dependent mechanism underlying the OXTR-mediated migration of prostate cancer cells, and indicate the potential contribution of OXTR to prostate cancer invasion and metastasis.

(1) Gimpl G, Fahrenholz F. *Physiol Rev* 2001; 81:629

(2) Neptune ER, Bourne HR. *PNAS* 1997; 94:14489

(3) Arai H, et al., *PNAS* 1997; 94:14495

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Nothing to Disclose: MZ, MLB, ACM, SAK

P3-67

Caveolin-1 Expression Regulates the Proliferation and Apoptosis of Malignant but not Benign Human Prostate Cells.

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Within the human prostate a balance between proliferation and apoptosis exists. Disruption of this leads to development of malignancy and prostate cancer is the second most common cause of cancer related death worldwide. Levels of caveolin-1 (cav-1), a membrane protein associated with caveolae, are correlated with tumour grade and progression to androgen-independence labelling this protein a 'tumor promoter'. Whilst cav-1 localisation to malignant cells is well reported, we have localised this protein to basal epithelial cells in the benign prostate suggesting a role for this protein in normal cell function. To investigate this we have used siRNA technology to downregulate cav-1 expression in both malignant (PC-3) and benign (PrEC) prostate epithelial cells. In both cell lines siRNA transfection reduced cav-1 levels by >80%. In PrEC's a loss of cav-1 had no significant effect on the rate of proliferation or apoptosis. In contrast, cav-1 down-regulation in PC-3 cells resulted in a significant reduction in proliferation (cell counts of 568 ± 25.2 in negative control vs. 503 ± 28.9 in cav-1 knockdown, $p < 0.01$) and a significant increase in apoptosis ($9.8 \pm 1.48\%$ in negative control vs. $14 \pm 2.64\%$ in cav-1 knockdown, $p < 0.05$). Following loss of cav-1 expression, PC-3 cells were not more susceptible to the apoptotic challenge of C2 ceramide treatment. To investigate possible pathways of action for cav-1 in proliferation and apoptosis we used Western blotting to determine levels of total and phosphorylated Akt and Erk. Levels of total Akt were significantly reduced (by $40 \pm 7.3\%$, $p < 0.01$) following cav-1 siRNA treatment. However, these cells were still able to respond to EGF stimulation and the resultant level of phosphorylated Akt was not significantly affected by a loss of cav-1. In contrast, EGF stimulation following cav-1 siRNA treatment lead to a significant increase in the level of Erk phosphorylation ($53 \pm 13.2\%$, $p < 0.01$) when compared to cells with unaltered levels of cav-1. This work confirms the tumor promoter role of cav-1 in prostate cancer cells and suggests that in benign prostate cells cav-1 loss does not disrupt proliferation or apoptosis indicating a change in the role of this protein with cancer development. The signalling pathways of both Akt and Erk are likely to be involved in mediating cav-1's effects on proliferation and apoptosis.

Nothing to Disclose: EHD, BMC, HB, KW

P3-68

Molecular Mechanism of Androgen-Specific Down Regulation of *CYP24* in Human Prostate Cancer (PCA) Cell Lines.

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Prostate cancer (PCa) is androgen dependent during the early stages of the disease. However, Vitamin D₃ (1,25(OH)₂D₃), (VD₃) as well as other hormones regulates PCa growth. Hydroxyvitamin D₃ 24 hydroxylase (*CYP24*) gene is one of VD₃ target genes. *CYP24* is a multi-catalytic cytochrome P450 enzyme that initiates inactivation of VD₃. Similar to the androgen-independence that develops; our studies show over-expression of *CYP24* in advanced PCa leads to VD₃ resistance inhibiting the antiproliferative actions of VD₃. The study explores how androgens regulate the expression of *CYP24*, to delineate potential therapeutic targets for treating PCa. Cells were treated with vehicle or 10nM VD₃ alone and in combination with 10nM or 100nM R1881. Cell growth was measured using a cell proliferation assay using WST-1. Cell lysates were immunoprecipitated with anti *CYP24* and probed with AR and *CYP24* antibodies. *CYP24* and other VD₃ target genes expression levels were estimated by real-time RT PCR. Interactions between VD₃ and R1881 groups in AR dependent cells show statistical significance. R1881, at low doses has a growth stimulatory effect and at 100nM has an inhibitory effect on AR dependent cells. The combined effect of R1881 and VD₃ were more pronounced, showing 70% growth inhibition. The synergistic effect of the androgens and VD₃ is more potent, exhibiting a strong antiproliferative effect in AR dependent cells in contrast to AR independent PC3 cells. Western blot analysis demonstrated a robust expression of AR in LNCaP and PC3AR cells with little or no expression in PC3 and C42 cells. AR was stabilized and showed a graded response with induction of 10nM VD₃ and 10nM R1881 in AR dependent cells. Induction of VD₃ enhances *CYP24* expression in PCa cells regardless of androgen dependency. In contrast, pre-incubation with 10nM/100nM R1881 together with 10nM VD₃ significantly suppressed the expression of *CYP24* (7-10fold in AR dependent cell lines), indicating that R1881 at physiological concentration protects VD₃ from catabolism. The effect of androgen on *CYP24* is very specific compared to other Vitamin D₃ targets. Androgen selectively upregulates cyclin dependent kinases *CDKN1A*, E-cadherin *CDH* and growth factor *IGFBP3* in a dose dependent manner. Thus, the inhibitory action of androgens on *CYP24* is unique and distinct from other Vitamin D₃ targets. These studies suggest an important role of androgens together with Vitamin D₃ in the growth inhibition of PCa.

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Nothing to Disclose: SM, AS, JA, DJL

P3-69

Functional Dissection of the Methylation Centers in Estrogen Receptor- β Promoter in the Prostate.

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Estrogen receptor (ER) β is a predominant ER subtype and plays important roles in the prostate. Studies of prostate cancer (PCa) progression revealed dynamic regulation of ER β expression via methylation of a CpG island (CGI) in ER β ON promoter. We previously reported that three CpG methylation clusters or Centers were identified in three Regions of the CGI (1). *In vitro* promoter-luciferase analysis indicated the upstream Center 1 with a methylation-sensitive AP-2 *cis*-element in it, but not exonic Center 2 and 3, is crucial in controlling the gene expression (2). However, further mechanism of epigenetic-mediated ER β gene regulation in relation to the function of the CpG Centers is unclear. Here, using bisulfite sequencing analysis, methyltransferase accessibility assay, stable transfection of region-specific methylated ON promoter-luciferase fragments (ON-Luc) combined with long term observation of dynamic changes of ON promoter methylation status in PCa cells, our data suggest the function of the downstream Center 3 but not Center 1 and 2 is to serve as a “docking site” for DNA methylation “seeding”. Methylation seeded CpG cluster triggers methylation “spreading” upstream to Center 2 and then Center 1 to repress the gene expression. In addition, the methylation status is tightly correlated to chromatin accessibility in the promoter. Stable transfection of ON-Luc with ON promoter heavily methylated or unmethylated indicated the promoter maintains relatively stable methylation status for over three months. In contrast, Center 1 and 3-specific methylated ON-Luc revealed that methylation seeding is a crucial step for methylation spreading at both directions. However, no significant correlation between methylation statuses and the promoter activities or chromatin accessibility was observed in the stable-transfected colonies, suggesting the location of the promoter in chromosome is important for proper gene regulation. These findings revealed important epigenetic functions of methylation Centers in gene regulation.

(1) Zhu X et al., Am J Pathol 2004; 164:2003

(2) Zhang X et al., Oncogene 2007; 26:7346

Sources of Research Support: NIH Grants CA015776, CA112532, ES06096 and OH008432; US Army Prostate Cancer Program DAMD-W81XWH-04-1-0165 SMH and W81XWH-06-1-0376.

Nothing to Disclose: XZ, EC, S-MH

P3-70

Estrogen-Driven Prolactin-Mediated Gene Expression Networks in Hormone-Induced Prostatic Intraepithelial Neoplasia.

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Testosterone (T) plus 17 β -estradiol (E2) co-treatment is an established regimen to induce prostate intraepithelial neoplasia (PIN) and prostate cancer in rodent models. Previously, we used the pure antiestrogen ICI 182,780 (ICI) and bromocriptine (Br), a dopamine receptor agonist, to inhibit PIN induction and systemic hyperprolactinemia in Noble rats. We found that the carcinogenic action of T+E2 is mediated directly by the E2 effects on the gland and/or indirectly via E2-induced hyperprolactinemia. In this study, we aimed at delineating the unique action(s) of E2 and prolactin (PRL) in early prostate carcinogenesis by global transcription profiling. We identified 2,504 differentially expressed genes in the T+E2-treated lateral prostate. The expression changes of a subset of 1,990 genes (~80%) were blocked upon co-treatment of ICI and Br, respectively, whereas those of 262 genes (~10%) were blocked by ICI only. These data suggest that E2-induced hyperprolactinemia primarily mediates the prostatic transcriptional response to the altered hormone milieu. In silico analyses identified hormone-responsive gene networks involved in immune responses, oxidative/nitrosative stress, stromal tissue remodeling, and the Erk-signaling pathway. In particular, our data suggests that IL-1 β , a potent cytokine, may play an important role in this hormone-induced prostate carcinogenesis model. Taken together these data highlight the importance of pituitary PRL and inflammatory cytokines in estrogen-induced prostate tumorigenesis. The identification of distinct E2- and pituitary PRL-responsive gene signatures provides a new resource for future investigations on the complex mechanisms underlying hormone-induced prostate carcinogenesis in vivo.

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Nothing to Disclose: NNCT, CYYS, JMF, ANF, MM, SMH

P3-71

Comparison of the Differential Estrogen Receptor Beta Versus Estrogen Receptor alpha Binding Affinities of 3 Natural Isoflavone Compounds (Spinacetin, Patuletin, and Luteolin) and 2 Endogenous Hydroxylated Cholesterol Derivatives (25- and 27-Hydroxycholesterol): Implications for Hedgehog Signaling in Prostate Cancer.

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Introduction: Prostate cancer (PCa) risk increases with age and is influenced by dietary factors. It has been proposed that ER α (Estrogen Receptor alpha) and ER β (Estrogen Receptor beta) act in opposition in PCa. These findings suggest ER β serves a protective role while ER α may promote PCa. On this basis, compounds which stimulate ER β or inhibit ER α are desired for possible PCa suppressive therapy. Interestingly, the natural soy isoflavone genistein has been shown to have agonist properties and it binds ER β with approximately 20 fold higher affinity than ER α . It is also possible that an ER β -specific antagonist may increase PCa incidence. Micromolar concentrations of 25- and 27-hydroxycholesterol (25- and 27-OHC) have recently been reported to be SERMs, which displayed a 3 fold preference for ER β over ER α in transcriptional activities. This is important because 27-OHC is the most prevalent form of hydroxylated cholesterol (oxysterol) found in circulating serum and is known to increase in concentration with age.

Hypotheses: Spinach-derived isoflavones, spinacetin and patuletin, as well as the isoflavone, luteolin, which all have a structural similarity to genistein, will bind selectively to ER β . 25- and 27-OHC will also bind to ER β with a higher affinity than to ER α .

Results/Conclusions: Using a ³H-estradiol dextran coated charcoal competitive binding assay, we found that luteolin bound to ER β with ~15nM Kd, while it had a worse binding affinity to ER α (Kd > 1 μ M). However, spinacetin and patuletin displayed only poor affinity binding to both ER α and ER β (Kds > 6 μ M). In our system 25- and 27-OHC bound to ER β (~25nM Kd) 200 times better than to ER α (~5 μ M Kd). In conclusion, luteolin is an ER β -specific ligand and its agonist/antagonist functional activity on ERs is currently being tested in our lab. Additionally, 25- and 27-OHC are endogenous ER β -specific compounds, which have the potential to explain some of the increased prevalence of PCa in the aging population. This is presumably due to oxysterols recently described ability to stimulate hedgehog signaling via LXR. In exciting new data, we found that the ER β -specific agonist DPN will inhibit hedgehog signaling but the ER α -specific agonist PPT will not. Thus, if luteolin is an ER β -specific agonist, then a diet rich in isoflavones, like luteolin and genistein, and low in oxysterols, such as 25- and 27-OHC, could lead to a marked decrease in prostate cancer risk via an ER-Hedgehog interaction mechanism.

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Nothing to Disclose: NJES, SKD, DP, AS, GER, DBL

P3-72

Urinary Steroid Profiling as a High-Throughput Screening Tool for the Detection of Malignancy in Patients with Adrenal Tumors.

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Adrenal tumors have an incidence of 2-3 % in the general population. Differentiating adrenocortical adenoma (ACA) from adrenocortical carcinoma (ACC) represents a continuous challenge, with unfavorable sensitivities and specificities provided by tumor size, imaging and even histology. Here we aimed to develop a reliable screening tool for the detection of adrenal malignancy. We performed urinary steroid profiling by gas chromatography/mass spectrometry (GC/MS) in scanning and selected-ion-recording mode, quantifying 35 distinct steroids. We cross-sectionally collected 24-h urine samples from 487 adrenal tumor patients assessed in centers participating in the European Network for the Study of Adrenal Tumors (www.ensat.org). Only samples from treatment-naïve patients with radiological evidence of tumor at time of urine collection were included (n=147) comprising 102 ACA (38m, 64 f, 19-83 yrs) and 45 ACC (24m, 21f, 20-80 yrs). Underlying diagnosis was confirmed by evidence of metastasis in ACC; mean duration of follow-up in ACA was 54 (range 18-193) months without evidence of metastasis. Total steroid excretion was independent of tumor size (ACA 26 (9-78) mm; ACC 90 (14-230) mm). Steroid data from ACA, ACC and healthy controls (n=88) were subjected to distance-based machine learning techniques, employing a prototype-based relevance learning approach to identify the most discriminative steroids. Matrix Relevance Learning Vector Quantization, carried out over 1000 random splits into training (90%) and test (10%) data sets, identified a subset of nine steroids that performed best in differentiating ACA from ACC. Receiver-operated characteristics analysis revealed sensitivity=specificity=91% (AUC 0.97) employing all 32 steroids and sens=spec=89% (AUC 0.96) when using only the 9 most differentiating markers. While GC/MS was invaluable in documenting the distinct ACC metabolome, it would be unsuited to use in widespread screening because of staff effort involved and sample preparation time. Therefore we have transferred the methodology to a uPLC/tandem mass spectrometry platform. All nine targeted steroid markers can be separated and quantified within 5 minutes using positive ion mode. These results suggest that urinary steroid profiling is a highly sensitive and specific biomarker tool for differentiating benign from malignant adrenal tumors. Prospective studies are required to establish its role in the routine diagnostic work-up of adrenal incidentalomas.

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Nothing to Disclose: AET, MB, SH, RL, BAH, HS, PS, DJS, NK, EP, GO, JB, FM, BA, MT, PJN, CHLS, XB, MF, PMS, WA

P3-73

WNT/Beta-Catenin Pathway Dysregulation in Childhood Adrenocortical Tumors Harboring the R337H p53 Mutation.

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Introduction: The prevalence of childhood adrenocortical tumors (ACTs) is 10-15 times higher in Brazil. The R337H p53 germline mutation is found in most of these Brazilians patients. Beta-catenin (CTNNB1) mutations are found in some ACT and very recently a direct and functional connection between p53 and the Wnt signaling pathway was shown *in vitro*. **Objective:** To analyze the mRNA expression of p53 and *Wnt/beta-catenin* pathway genes (CTNNB1, Axin, DKK3 e WISP2) in ACT and verify if differential expression patterns are associated with the R337H p53 mutation, tumor stage and mortality. **Patients and Methods:** We study 25 pediatric patients with ACT (median age: 2.6 yrs [0.4 to 15.4]; 17F/8M) and 8 normal adrenals (controls). All patients presented hormone excess (8 virilization, 16 virilization/Cushing's syndrome, 1 Cushing's syndrome). 12 patients presented tumoral stage I, 2/II, 4/III and 7/IV (Sandrini classification). At a median follow-up of 3.9 yrs (0.1 to 13.1), 7/25 patients (28%) had died and 6 patients lost to follow up. Mortality rate was 16% in stages I and II and 28% in stages III and IV. Relative gene expression was measured by real-time PCR and calculated by $2^{-\Delta\Delta C_t}$ method. Statistics: one way ANOVA and Mann-Whitney test; $P < 0.05$. **Results:** The R337H p53 mutation was found in 20 out of 25 (80%) patients. Compared to controls, ACTs presented hyperexpression of CTNNB1 (2.3 fold; $p = 0.005$), a Wnt signaling activator and hypoexpression of DKK3 (-12.2 fold; $p = 0.0001$), a Wnt signaling inhibitor. Low expression of MYC (-2.9 fold; $p = 0.02$), a target gene of this pathway, was also found. No different expression of Axin and WISP2 was observed. ACTs stage III/IV presented significant reduction of p53 expression compared to ACT stage I/II and controls (-2.5 fold; $p = 0.004$). ACTs stage I/II showed lower expression of DKK3 compared to controls (-9.4 fold; $p = 0.0004$) and ACTs stage III/IV also showed lower expression of DKK3 compared to controls (-13.3 fold; $p = 0.0004$). Compared to survivors, patients who died presented significant hypoexpression of p53 (-2.9 fold; $p = 0.02$). **Conclusion:** Wnt/beta-catenin pathway is dysregulated in childhood ACTs harboring the R337H p53 mutation. These *in vivo* findings reinforce the link between Wnt signaling and p53 in adrenocortical tumorigenesis.

(1) Gajoux Set al. J Clin Endocrinol Metab. 2008 Oct;93(10):4135-40.

(2) Bourdeau I et al. Clin Endocrinol (Oxf). 2008 Feb;68(2):264-70.

(3) Lee K-W et al. Proc Natl Acad Sci U S A. 2010 Jan 5;107(1):69-74.

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Nothing to Disclose: LFL, LMM, CEM, ACM, LGT, CAS, MC, SRA

P3-74

Therapeutic Approaches in a Family of Neurofibromatosis Type 1 Accompanied by Pheochromocytoma Using Antisense Morpholino Oligonucleotides (AMOs).

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Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant inherited disorder affecting the nervous system and is associated with mutations in the *NF1* gene, which is located on chromosome sub-band 17q11.2 and contains 58 exons spanning approximately 300 kb of genomic DNA. NF1 is caused by the loss of function mutation of the *NF1* gene resulting in inactivation of neurofibromin, as a tumor suppressor, which encodes a GTPase activating protein (GAP) involving in the negative regulation of Ras activity. GAP related domain, which is encoded by exons 20-27a, and cysteine-serine rich domain are well known to be the most important functional domains in neurofibromin. In spite of many genetic analysis of NF1 related pheochromocytoma, there still remains the question of whether pheochromocytoma is a true component of NF1. We found a novel germline mutation of *NF-1* gene (c.7907+1G>A) in a 31-year-old female patient with NF-1 accompanied by pheochromocytoma and genetic analyses of her family members showed that this mutation led to skipping of exon 54 during *NF1* mRNA splicing. As this novel germline mutation falls into neither GAP related domain nor cysteine-serine rich domain but into C-terminal region of *NF1* gene, which is relatively not well known for its function. This suggests that there must be an essential function in the C-terminal region of *NF1* gene, especially in the development of NF-1 related pheochromocytoma. In this family, skipping exon 54 frameshifts downstream sequence, resulting into premature termination of translation of *NF1*, in other words truncated neurofibromin. Structurally and functionally defected neurofibromin is the causal provider of this disease. From the therapeutic points of view, we used antisense morpholino oligonucleotides (AMOs) to skip exon 54 as well as adjacent exon 55 and 56 (101 bases + 143 bases + 47 bases = 291 bases, which is divisible by 3; $291 / 3 = 97$) and restored the reading frame of *NF1* in a primary cultured fibroblast from the patient's skin lesion. To assess the restored neurofibromin function, we conducted an indirect analysis by measuring Ras-GTP levels and showed decreased Ras-GTP levels, suggesting the restoration of the neurofibromin activity.

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Nothing to Disclose: SL, HSY, JML, SH, SHK, YSE, YSK, IBP

P3-75

Correlations between the Expression of BUBB1, DLG7 and PINK1 Genes and Outcomes in a Brazilian Cohort of Adrenocortical Tumors of Adult and Pediatric Patients.

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Background: Adrenocortical carcinomas (ACC) are rare tumors with a dismal prognosis with a 5-year survival rate below 30%. Moreover, histopathology is sometimes insufficient to establish the diagnosis of malignancy and is a poor indicator of prognosis. Recently, the combined expressions of *BUBB1*, related to mitosis and chromosomal segregation; *DLG7* and *PINK1*, both implicated with cell cycle regulation were identified as strong predictors of overall survival and disease-free survival in adult ACC. **Objective:** To validate these findings in adult adrenocortical tumors (ACTs) and to evaluate if these predictors applies to pediatric ACTs. **Methods:** Thirty-five ACTs from 19 adults and 16 children (1 to 64 yrs) were studied. Of these, 29 were hormone-secreting. Nine adult ACTs were carcinomas (Weiss score ≥ 3), 5 of them had metastasis at diagnosis and had a fatal outcome. Among pediatric ACTs, 5 developed metastasis during the follow-up and 3 died from progressive disease. *BUBB1*, *PINK1* and *DLG7* genes expression were evaluated by qRT-PCR TaqMan assays. *GUS* was selected as the endogen gene. The differences of DCts of *BUBB1* and *PINK1* (*BUBB1-PINK1*) and *DLG7* and *PINK1* (*DLG7-PINK1*) were used in statistical analyses. **Results:** In all patients, *BUBB1-PINK1* and *DLG7-PINK1* were different between metastatic (M) (n=12) and non-metastatic (NM) (n=23) ACTs. (*BUB-PINK1*) median for M and NM tumors = -1.3642 and 4.3 respectively, $p=5 \times 10^{-4}$; (*DLG7-PINK1*) medians = 0.80 and 7.45 respectively ($p=3 \times 10^{-4}$). In pediatric ACTs, these differences remain significant for *DLG7-PINK1* and marginally significant for *BUBB1-PINK1* ($p=2 \times 10^{-2}$ and $p=5,1 \times 10^{-2}$ respectively). A significant correlation between both *BUBB1-PINK1* and *DLG7-PINK1* and Weiss score was found in children ACTs ($p=1 \times 10^{-3}$ and $p=1 \times 10^{-2}$; $r=-0.76$ and 0.62 for *BUBB1-PINK1* and *DLG7-PINK1* respectively). In adults, a correlation between tumor size and *DLG7-PINK1* and *BUBB1-PINK1* was observed ($p=7 \times 10^{-3}$ and $p=4 \times 10^{-2}$ $r= -0.59$ and -0.48 , respectively). For all 35 patients the cox-proportional hazards regression survival analysis identified *DLG7-PINK1* and *BUBB1-PINK1* as predictors of disease-free survival ($p=1 \times 10^{-3}$ and $p=4 \times 10^{-3}$) and overall survival ($p=2 \times 10^{-2}$ and $p=4 \times 10^{-2}$). **Conclusion:** The differences of *BUBB1-PINK1* and *DLG7-PINK1* expressions correlate with ACT aggressiveness and were predictors of disease-free survival and overall survival in both adult and pediatric patients, suggesting that it might be useful prognostic markers.

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Nothing to Disclose: MCBVF, TLM, LPB, BGM, IB, TCG, ACL, BBM, AL, AML

P3-76

Polymorphisms of the Glucocorticoid Receptor Gene Associated with Hormonally Inactive Adrenal Adenoma.

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Introduction Little is known about the pathogenesis of sporadic adrenal adenomas, which are relatively common incidental findings due to modern imaging techniques. Altered sensitivity against glucocorticoids is influenced by polymorphisms (SNP) of the glucocorticoid receptor gene (GR). Earlier, our group found that the carrier frequency of the N363S variant of the GR was higher among patients with bilateral incidentalomas than in healthy population. The aim of the present study was to explore whether other SNPs or a specific haplotype of the GR could be associated with tumor formation or hormonal activity.

Methods. The study included 134 patients with adrenal adenoma (102 with hormonally inactive adrenal adenoma: HI; and 32 with cortisol producing: CP tumor) and 129 controls. Hormonal evaluation of the hypothalamo-pituitary-adrenal (HPA) axis, measurement of metabolic parameters (body mass index: BMI, blood pressure, blood glucose and lipid concentrations) was carried out in patients, and genetic analysis in all subjects. Polymorphisms of GR were detected by allele-specific PCR methods for N363S and BclI polymorphism, with RFLP for ER22/23EK and with Taqman allele discrimination assay for A3669G.

Results. As previously observed the carrier frequency of N363S was higher in HI than in CP or healthy population, while the prevalence of A3669G was lower in patients with HI than in CP or controls (N363S: 7.4% vs 2.7% $p < 0.05$; A3669G: 14.7% vs. 22.1% $p < 0.05$). These associations were even stronger in patients with bilateral HI tumors: N363S: 10.5% vs. 2.7% $p < 0.05$; A3669G: 10.5% vs. 22.1% $p < 0.05$). The prevalence of haplotypes containing N363S was significantly higher in patients with HI especial in bilateral HI than in controls or patients with CP (0.23 in bilateral HI vs. 0.05 in healthy or 0.03 in CP, $p < 0.05$), while the prevalence of haplotypes containing A3669G was lower in HI than in controls or patients with CP (0.15 vs. 0.34 or 0.34, $p < 0.05$). The ER22/23EK was identified both in patients and controls together with A3669G. In CP group no associations between GR SNPs were observed

Conclusions: The increased prevalence of N363S and the decreased prevalence of A3669G variants of the GR by increased sensitivity against endogenous glucocorticoids can play a role in the pathogenesis of hormonally inactive adrenal incidentalomas especial in bilateral cases.

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Nothing to Disclose: AP, BA, AS, KF, JM, PI, MT, KR

P3-77**Plasma Concentration of o,p'DDD (Mitotane), o,p'DDA and o,p'DDE as Predictors of Tumor Response in Adrenocortical Carcinoma: Results of a Retrospective European Network for the Study of Adrenal Tumors (ENS@T) Multicentre Study.**

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Introduction Lysodren (Mitotane) is the drug of choice for patients with adrenocortical carcinoma (ACC). Monitoring o,p'DDD plasma level has been proposed as predictor of tumor response. Measurement of o,p'DDD metabolites involved in the active pathway may provide an improved prediction of o,p'DDD activity. The objective of our study was to (1) confirm the currently used threshold o,p'DDD plasma level of 14mg/l for achieving a tumor response and (2) evaluate the value of o,p'DDD, o,p'DDA, or o,p'DDE levels in predicting tumor response. **Methods** Retrospectively o,p'DDD and its metabolites were measured in available samples from 91 patients with advanced ACC in 5 ENS@T centers. Samples within 3 months of best response were used for analyses. ROC curves were used to define cut-off values. **Results** Mitotane was given as monotherapy (30%) or in combination with chemotherapy (70%). Tumor response was observed in 17 patients (19%). Metabolites o,p'DDE and o,p'DDA showed significant correlation with o,p'DDD ($r=0.61, <0.001$ resp. $r =0.67, p< 0.001$). Univariate analysis revealed significantly higher o,p'DDD plasma levels in patients with partial and complete response ($p=0.03$). Subgroup analysis in patients treated with mitotane monotherapy revealed significant higher o,p'DDA levels ($p=0.03$). Using o,p'DDD cut-off value of 14mg/L, 11 out of 36 patients (31%) reaching o,p'DDD levels ≥ 14 mg/L were responders compared to only 11% responders in patients with levels <14 mg/L ($p=0.02$). Using a cut-off value of 92mg/L for o,p'DDA, 6 out of 16 patients (38%) were responders. Percentage of responders was increased using combined o,p'DDD ≥ 14 mg/l and o,p'DDA ≥ 92 mg/l measurements (5 out of 11, 45% of the patients). **Conclusion** Our data confirm that plasma concentrations of o,p'DDD, including the 14mg/l threshold, are correlated with tumor responses. Higher o,p'DDD threshold and or additional o,p'DDA measurement may help to improve the prediction of response. A prospective study is warranted to confirm these results and look for additional predictors of response.

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Nothing to Disclose: IGCH, MF, MT, SH, JdH, SL, FD, BA, AB, RC, HRH, EB

P3-78

Survival of Patients with Adrenocortical Carcinoma (ACC) after Radical Resection Revisited.

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In patients with stage II ACC who underwent curative surgery tumor recurrence rates of up to 85 % have been reported. However, many patients with ACC are referred to specialized centers only when disease recurrence occurs. Thus, we hypothesized that recurrence rate and outcome of patients with ACC are strongly affected by patient selection.

All patients in the German ACC registry, in whom ACC was diagnosed after 1989 were included (n=489). Patients with a follow-up time <12 months (n=39) and children <16 years were excluded (n=36). All 149 patients presenting with stage II disease were grouped according to the registration date: prospective group (PG) (n=30) comprised patients with registration \leq 4 months after resection of the primary tumor; retrospective group (RG) included patients who registered >4 months after primary surgery (n=119).

There were no statistically significant differences between both groups concerning age, tumor size, mitotic count, Weiss score, and adjuvant tumor bed irradiation. PG included more females (80% vs. 61%, $p = 0.02$) and only 16% of the patients in the RG received adjuvant mitotane compared to 53% in the PG ($p < 0.001$). All patients of the PG were disease-free when they enrolled and 10 patients (33%) developed tumor relapse during follow-up. In contrast, the recurrence rate in the RG amounted to 71% and most patients (63%) had experienced recurrence at the time of registration. The 5-year survival of all patients with stage II disease was 58%. However, the PG had a much better survival (96% vs. 55%, $p=0.02$). A similar trend was found when only patients without mitotane treatment were included ($p=0.15$). Patients in the PG had a 83% lower risk of death compared to the patients of the RG (HR 0.17, 95 % CI 0.023-1.27). Patients who took mitotane displayed a significantly better 5- year survival than did the patients without mitotane treatment (87% vs. 53%, $p=0.03$). On multivariate analysis, adjuvant mitotane treatment led to a risk reduction of 55% (HR 0.45, 95% CI 0.2-1.3).

Our study strongly suggests that current survival data in patients with localized ACC are severely biased by patient selection and that patients who are followed prospectively have a greatly improved survival. In addition, our findings support a beneficial role of adjuvant mitotane.

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Nothing to Disclose: MF, SJ, SH, MQ, FB, HSW, MK, BA

P3-79

High Expression of DAX-1 Protein in Adult and Pediatric Adrenocortical Tumors.

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DAX-1 (dosage-sensitive sex reversal-adrenal hypoplasia congenital critical region on X chromosome gene 1) is an orphan member of the nuclear receptor superfamily. This transcription factor is a key determinant of human adrenogonadal development and function, acting as a repressor of SF-1 target genes in steroidogenesis. Recently, it was demonstrated that Dax-1 is expressed at high levels in murine embryonic stem cells and its disruption induced loss of pluripotency and differentiation of these cells. Furthermore, DAX-1 transcription is up-regulated by Wnt signaling, which is well known to be activated in adrenocortical tumors. Therefore, we investigated the involvement of DAX-1 expression in human adrenocortical tumorigenesis. Tissue microarrays of paraffin-embedded tissue samples were performed in 102 adrenocortical tumors (70 adenomas and 32 carcinomas) from 36 children and 66 adults. Endocrine syndromes were diagnosed in all children (isolated Cushing syndrome 8.3%, virilization 58.3%, and mixed syndrome 33.3%) and in 75% of adults with adrenocortical tumors (isolated Cushing syndrome 48%, virilization 9%, mixed syndrome 18%, and nonfunctioning 25%). Nuclear DAX-1 staining was semi-quantitated using H score by two independent observers (inter-observer agreement of 0.83). Negative sample was considered if H score = 0 and positive if H score > 0; the positive samples were also categorized as weak (H score from 0.1 to 0.49) or strong (H score ≥ 0.5). DAX-1 staining was positive in 78% and 65% of the pediatric and adult adrenocortical tumors, respectively. The frequency of adrenocortical tumors with a strong nuclear DAX-1 immunoreactivity was higher in children than in adults (67% vs. 41%; p = 0.03). A positive staining for DAX-1 was detected in 76% (53 out of 70) of the adrenocortical adenomas and in 56% (18 out of 32) of the adrenocortical carcinomas (p = 0.07). DAX-1 expression did not correlate with the functional status of adrenocortical tumors. Interestingly, an unexpected positive correlation was observed between DAX-1 and SF-1 protein expression in adrenocortical tumors (p < 0.0001, r = 0.61). In conclusion, DAX-1 protein was highly expressed in both pediatric and adult adrenocortical tumors, suggesting an important role of this transcription factor in human adrenocortical tumorigenesis. Additionally, the positive correlation between DAX-1 and SF1 suggests that these transcription factors might cooperate in the adrenocortical tumor development.

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Nothing to Disclose: MQA, ICS, MCBVF, VBD, AW, RAR, MYN, AML, VAFA, BBM, ACL

P3-80

Analysis of *FGFR4* Locus Amplification in Pediatric and Adult Sporadic Adrenocortical Tumors.

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Background: Adrenocortical tumors are heterogeneous neoplasms with incompletely understood pathogenesis. Fibroblast growth factors (FGFs) are master regulators of a spectrum of cellular and developmental processes, including, apoptosis, proliferation, migration and angiogenesis. Deregulation of FGF/FGFR signaling by activating mutations or ligand/receptor overexpression could allow this system to become hyperactive, leading to cancer development. *FGFR4* overexpression has been demonstrated in adrenocortical tumors by microarray analyses. Recently, our group also showed *FGFR4* overexpression in both pediatric and adult adrenocortical tumors by real time PCR.

Objective: To investigate the presence of *FGFR4* gene amplification in human sporadic adrenocortical tumors.

Patients: Eighteen adrenocortical tumors (9 adenomas and 9 carcinomas) diagnosed in 5 children (3 girls) and 13 adults (12 women) were studied. Seventeen of them are steroid-hormone producing tumors and caused endocrine syndromes: 6 isolated Cushing's syndrome (C), 5 virilization (V), 5 mixed syndrome (C+V) and 1 feminization. Eight normal adrenal gland samples were obtained during renal surgery or autopsy and used as controls. *FGFR4* overexpression was previously determined in 8 (5 adults and 3 children) of these cases by real time PCR.

Methods: Genomic DNA was extracted from adrenocortical tumors and normal adrenal tissues. *FGFR4* copy number was determined by multiplex ligation-dependent probe amplification (SALSA MLPA kit P026-C1 Sotos, MRC-Holland, Amsterdam). Two probes for *FGFR4*, 24 probes for *NSD1* and 14 control probes were used in this assay. Both *NSD1* and *FGFR4* genes are mapped to 5q35 chromosome.

Results: Amplification of *FGFR4* locus was detected in 3 adrenocortical carcinomas (16.7%) diagnosed in adult women. Interestingly, all 3 cases had mixed syndrome. Two of them were studied for *FGFR4* gene expression and both exhibited higher mRNA levels in comparison to normal adrenal samples. No *FGFR4* locus amplification was demonstrated in any of the pediatric tumors.

Conclusion: *FGFR4* gene amplification and, consequently, its overexpression were demonstrated only in adrenocortical carcinomas diagnosed in female adults with mixed hormonal secretion pattern. These findings suggest that the *FGFR4* could be implicated with malignant behavior of a subset of adrenocortical tumors and potentially make part of a distinct molecular signature.

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Nothing to Disclose: TCR, LPB, AML, BMPM, AAJ, MCF, BBM, ACL

P3-81

Parathyroid Carcinoma: A 43 Year Institutional Review.

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Background: Parathyroid carcinoma is a rare but ominous cause of primary hyperparathyroidism. In this article, we review outcomes of parathyroid cancer patients seen at our institution over a 43 year period and evaluate factors associated with mortality.

Methods: A retrospective review was performed on 37 patients with parathyroid cancer who were treated at a university tertiary care center between the years of 1966 and 2009.

Results: The average age at cancer diagnosis was 53 years (23-75), and 62% (23/37) of patients were men. 21.6 % (8/37) of patients had an associated benign parathyroid adenoma before (2/8, 25%), concurrent with (2/8, 25%), or after (4/8, 50%) their parathyroid cancer operation. A cystic component was present in 7/37 cancers (18.9%) on initial exploration. The average number of neck dissections done for cancer was 3 (1-11). 48.6% (18/37) of patients recurred after their initial cancer operation. 22 of 37 patients (60%) eventually developed complications as a result of their cancer treatment. Unilateral (11) or bilateral (3) vocal cord paralysis occurred in 38% of patients. Lymph node or distant metastases eventually developed in 30% (11/37) of patients. Median survival for patients was 14.3 years from the date of diagnosis (10.5-25.7). Factors associated with increased mortality included lymph node metastases, distant metastases, higher calcium level at recurrence, and a high number of calcium-lowering medications. Factors that were not associated with mortality included gender, age, tumor size, number of recurrences, and time to first recurrence. Extent of operation (parathyroidectomy alone vs. parathyroidectomy with partial or total thyroidectomy) was also not associated with mortality.

Conclusion: Parathyroid cancer patients have a long survival. However, these patients have a high rate of surgical complications, including a high proportion of recurrent laryngeal nerve injuries. They may also require multiple operations for recurrence. Lymph node and distant metastases were both associated with increased mortality. Extent of initial cancer operation (with or without thyroidectomy) was not associated with mortality.

Nothing to Disclose: AH, AW, GF-R, JH, IS, EM, RHG, WS, JG, QYD, OC

P3-82

PTHrP Expression and Lung Cancer Cell Growth Inhibition by Novel Bis-Cyclic Thioureas.

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Background: PTHrP (parathyroid hormone related protein) is abnormally expressed in a substantial majority of lung cancers, especially non-small cell lung cancers. This oncoprotein plays a key role in tumor progression for many cancers including lung. Since PTHrP is a marker of both the tumor and its invasiveness, PTHrP could be a reasonable target for treating patients with lung cancer. However, PTHrP-antibody studies notwithstanding, modalities involving PTHrP have received little attention as treatments for this malignancy. We have conducted in vitro studies that posit a novel role for bis-cyclic thioureas in tumor management.

Methods: We screen combinatorial libraries of small molecule heterocyclic amines for inhibitory effects on PTHrP expression and cell proliferation, as measured by immunoassays, qPCR and MTT assays. The studies were conducted in cultures of a panel of human derived PTHrP-positive lung cancer cells.

Results: After the initial screens of the combinatorial libraries, we identified six lead bis-cyclic thiourea compounds that demonstrated > 60% inhibition on PTHrP expression as measured by immunoassays from BEN and NCI-H727 lung cancer cells compared to vehicle control treatment. Another biomarker, calcitonin, was reduced by the bis-cyclic thioureas to a much smaller degree. The compounds decreased PTHrP mRNA levels, particularly promoter P3-specific transcripts, but had no effect on message stability in cells treated with actinomycin D. The lead bis-cyclic thioureas were next assayed for the effects on lung cancer growth in a 6 day time course study. 5/6 and 6/6 compounds significantly inhibited ($P < 0.01$) BEN and NCI-H727 cell proliferation, respectively, at Days 2, 4 and 6.

Conclusions: Contemporary combinatorial chemistry procedures identified PTHrP inhibitory bis-cyclic molecules that inhibited the PTHrP expression and the growth in vitro of PTHrP-producing lung cancers. These inhibitory effects may act on PTHrP transcription. This family of compounds has the potential for innovative treatments of lung cancer.

Nothing to Disclose: LJD, AN, DWB, ST, RQ, RHH

P3-83

The Utility of Multiphasic Computed Tomography as a Localization Procedure for Primary Hyperparathyroidism.

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Introduction: Four-dimensional or multiphasic computed tomography (M-CT) has been found to provide greater sensitivity and specificity in the localization of hyperfunctioning parathyroid glands compared with ultrasonography and sestamibi imaging. This novel imaging modality has been shown to be useful in the reoperative setting and allowed more successful focused neck explorations. The aim of this study was to further evaluate the clinical utility of this procedure in patients with primary hyperparathyroidism (PHPT).

Methods: A retrospective review of patients with PHPT was performed at Mayo Clinic from December 2008 to December 2009 who underwent preoperative M-CT and surgery. Clinical, radiologic, and pathologic data was analyzed and the sensitivity and specificity of the various imaging procedures were assessed.

Results: Nineteen patients met the predefined inclusion criteria: 9 had persistent PHPT and 1 had recurrent PHPT.

Eighteen patients were cured after parathyroid surgery. Only 1 of 8 ultrasounds and 5 of 10 sestamibi scans performed at outside institutions aided in preoperative localization. Of those who had negative outside imaging with either ultrasonography or sestamibi, 2 of 4 ultrasounds and 3 of 5 sestamibi scans performed at our institution accurately identified the hyperfunctioning parathyroid gland. Of the 18 patients who were cured, M-CT provided correct localization in 16 cases. In 3 of 4 cases, M-CT was able to provide accurate localization when ultrasonography and sestamibi were discordant. M-CT localized the correct single adenoma when sestamibi showed 2 abnormal foci in 4 of 5 patients. M-CT did not provide additional information in 5 of 6 patients with concordant ultrasonography and sestamibi scans. The sensitivities of M-CT (89%) and sestamibi scan (89%) were similar and were superior to ultrasonography (46%). The specificities of all three imaging modalities were comparable (96%, 91%, and 94%, respectively).

Conclusions: The overall sensitivity and specificity of M-CT as a localization tool in PHPT are similar to that of sestamibi imaging, likely due to the better sensitivity of sestamibi at our institution. Re-imaging with sestamibi may be beneficial when outside imaging results are negative. M-CT may be useful in cases of discordant ultrasonography and sestamibi results as well as finding of multiple abnormal foci on sestamibi, but offers limited additional information when both ultrasonography and sestamibi are unequivocal.

Nothing to Disclose: RPE, DRD, GBT, AEK, RAW

P3-84

Expression and Somatic Mutations of SDHFA2 (SDH5), a Novel Endocrine Tumor Suppressor Gene, in Parathyroid Tumors of Primary Hyperparathyroidism.

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Background: Loss of heterozygosity at chromosome 11q13 is the most common chromosomal aberration in parathyroid tumors occurring in about 40% of sporadic tumors, whereas only 15-19% displays somatic mutations in the MEN1 gene. Thus, this chromosomal region may harbour additional genes of importance in parathyroid tumor development. The SDHFA2 gene is a recently identified tumor suppressor gene within the region of frequent allelic loss in parathyroid tumors. SDHFA2 is involved in the electron transport chain and inherited mutations have been linked to familial paraganglioma. The expression in parathyroid tissue of SDHFA2 and the role of mutational aberrations in parathyroid tumor development was examined.

Methods: Total RNA (10 µg) was isolated from snap frozen parathyroid tissue. cDNA was synthesized using iScript cDNA synthesis kit (Biorad Hercules, CA). RT-PCR used specific primary primers and analyzed on a 1% agarose gel. Genomic DNA was isolated from matched whole blood and parathyroid tumor tissue from 80 patients with sporadic primary hyperparathyroidism (pHPT) due to a single parathyroid adenoma. The coding exons of the SDHFA2 gene were amplified and analyzed using automated sequencing.

Results: Gene expression of SDHFA2 was present in normal and parathyroid tumor tissue using RT-PCR. Sequencing of all coding regions of the SDHFA2 gene identified an A to G substitution in intron 2 in 9/80 parathyroid tumors. However, this variant was also present in the germline DNA from the patients, suggesting a non-pathogenic silent single nucleotide polymorphism. No tumor-specific somatic mutational aberrations, such as nonsense, frameshift, or other inactivating mutations were identified.

Conclusions: SDHFA2 is expressed in parathyroid tissue. Somatic mutations of the SDHFA2 gene are unlikely to frequently contribute to parathyroid tumor development in sporadic pHPT.

Nothing to Disclose: LFS, AD-V, RU, PB, TC

P3-85

Early-Onset, Progressive, Frequent, Extensive and Severe Bone Mineral and Urolithiasis-Related Renal Complications in Multiple Endocrine Neoplasia Type 1-Related Primary Hyperparathyroidism.

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Context: Differences in the bone mineral density (BMD) patterns have been recently reported between multiple endocrine neoplasia type 1-related primary hyperparathyroidism (HPT/MEN1) and sporadic primary HPT, however studies on early/late outcome of bone/renal complications in HPT/MEN1 are lacking.

Objectives: To study the bone and renal outcome in HPT/MEN1.

Design and Setting: Cross-sectional study performed in a tertiary academic hospital.

Patients: Thirty-six cases with uncontrolled HPT from eight unrelated MEN1 families.

Main outcome measure: BMD analysis was performed by DXA in the proximal one third distal radius (1/3DR), femoral neck, total hip and lumbar spine (LS).

Results: Patients' age was 38.9 ± 14.5 yrs. PTH/calcium values were mildly elevated, despite an overall high percentage of bone demineralization (77.8%). In the younger group (< 50 yrs), demineralization was more frequent, early-onset and severe at the 1/3DR (40%; Z, -1.81 ± 0.26). The older group (> 50 yrs) had higher frequencies of bone demineralization in all sites ($p < 0.005$); larger number of affected bone sites ($p < 0.0001$); and BMD was more severely compromised in the 1/3DR ($p = 0.007$) and LS ($p = 0.002$). BMD values were lower in symptomatic (88.9%) than in asymptomatic HPT cases ($p < 0.006$). Cases with long-standing HPT (> 10 yrs) and gastrinoma/HPT presented significantly lower 1/3DR BMD values. Urolithiasis occurred early (<30yrs), frequently (75%), associated with related renal co-morbidities (50%) and renal insufficiency in the older group (33%).

Conclusions: The outcome of the bone mineral and urolithiasis-related renal complications in HPT/MEN1 is early-onset, frequent, extensive, severe and progressive. These data should be considered in the individualized clinical/surgical management of patients with MEN1-associated HPT.

Nothing to Disclose: DML, FLC, RAT, FLMM, JEMC-D, SPAT

P3-86

Accuracy of Selective Intra-Arterial Calcium Injection in the Localization of Insulinomas.

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Insulinomas, the most frequent functioning pancreatic endocrine tumors, are usually small, benign and sporadic lesions. Successful surgical treatment depends mainly on the adequate identification of the source of insulin excess. Preoperative localization of the tumor improves the chance of surgical success and becomes important when a laparoscopic approach is programmed. Despite advances in imaging techniques, tumors smaller than 2 cm are still difficult to localize by conventional methods. Selective intra-arterial calcium injection of the major pancreatic arteries (CaStim) with hepatic venous sampling is useful to regionalize an insulin secreting tumor in a high percentage of patients when other studies have failed to detect a pancreatic lesion. We wish to report our experience with CaStim in the preoperative localization of insulinomas in adult patients. Accuracy of CaStim against computed tomography (CT) and/ or magnetic resonance imaging (MRI) was compared in 22 consecutive patients (14 females, 8 males) aged 25 to 80 years, with documented endogenous hyperinsulinemic hypoglycemia. The diagnosis of insulinoma was confirmed by pathology in 19 of 22 cases, 1 patient presented a nesidioblastosis and in 2 cases no tumor was found. Regionalization test was considered positive if it was consistent with surgical findings. CaStim's test allowed to accurately localizing the source of insulin in 18 out of 22 (81.8%) insulinomas; the test was falsely positive in 2 patients, one of them had a nesidioblastosis and in the other, no tumor was found. In one case in which the test failed to show a gradient, an insulinoma in the uncinete process of the pancreas was found (false negative). The remaining patient showed no gradient and no tumor was found during surgery. CaStim was the only test to show the correct localization of insulinomas in 12 patients (54.5%), and confirmed the findings of CT or MRI in 6 additional cases (27.3%). All positive tests showed a more than 1.56 fold rise in serum insulin levels within 60 to 90 seconds after calcium injection. CaStim confirmed previous data from other centers and was vastly superior to CT or MRI as a method of preoperative localization, especially when the last were negative. Less than the classical twofold rise gradient in insulin concentration after calcium injection could be enough to identify the insulin hypersecretion source.

Nothing to Disclose: AGD, RMG, AF, FJSA, SL, APdL, MCM, ODB

P3-87

Endocrinological Disturbances in Germ Cell Tumour Patients - Comparison of Hormone Levels and Kinetics in Cubital and Testicular Vein Blood.

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Objectives: Men with testicular cancer are often infertile. Impairments can be found in spermiogram and sex hormone levels. We investigated hormone levels following tumour ablation.

Patients and methods: We investigated the hormone levels in 249 patients with testicular germ cell cancer. At the time of the primary surgery we harvested testicular vein blood by puncture or venae sectio after preparation of the spermatic cord and peripheral blood was drawn from the cubital vein. The measurements of cubital vein blood were performed 24 hours and 4 to 7 days after surgery in a subset of patients. All samples had been analysed for the tumour markers Alpha-Fetoproteine (AFP), human chorionic gonadotropin (hCG), lactate dehydrogenase (LDH) and placental alkaline phosphatase (PLAP), the gonadotropins LH and FSH, and the steroids testosterone and estradiol. 134 patients had seminoma, 111 had nonseminoma. Half of the patients had stage I UICC (Union internationale contre le cancer), the other half was in different metastasized stages.

Results: In all patients we found distinctly increased levels of estradiol in the testicular vein blood. It is about 30-fold higher than in the cubital vein blood, showing that estradiol is produced in the testes of these patients. Patients with increased estradiol levels in cubital vein blood had a strong correlation to their testosterone levels. In patients with high estradiol and testosterone levels in peripheral blood the levels of hCG were increased and LH was suppressed to very low levels. After tumour ablation, high estradiol and testosterone levels returned to normal ranges parallel to the decrease of hCG. In 37 patients with a long follow-up the normalization of the disturbances took place in an average time of 17 days. LH increased to normal levels in the same interval. In patients with hCG producing germ cell tumours of the testis the analogous alpha-chain leads to the same effect on Leydig cells as LH-stimulation. This explains the high production of testosterone and the simultaneous aromatization to estradiol with high levels in peripheral blood. The negative gonadal-pituitary feedback leads to the LH-suppression.

Conclusion: Our findings show the reversibility of endocrinological disturbances after testicular tumours ablation with decreasing hCG.

Disclosures: AAY: Speaker, Bayer Schering Pharma, Berlin, Germany, Pfizer Germany, Ferring Pharmaceuticals. FS: Employee, Bayer Schering Pharma.

Nothing to Disclose: TP, TB

P3-88

cAMP and KIT-KITLG Signaling Interact in Testicular Tissue and Possibly Cooperate in Predisposition to Testicular Germ Cell Tumors: A Novel Function of PDE11A in Testis.

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Testicular germ cell tumors (TGCTs) account for 95% of the cases of testicular cancer, the most common solid malignancy affecting young males. Somatic activating mutations in the proto-oncogene *KIT* are found in patients with TGCTs, whereas common variations in the *KITLG* gene have been associated with these tumors. Phosphodiesterase (PDE) 11A (PDE11A) is expressed in several tissues, but is most highly expressed in testis (and prostate). PDE11A's enzymatic activity, thus, appears to be an important regulator of cAMP signaling in testicular tissue. Recently, we reported *PDE11A* mutations (and functional polymorphisms) as predisposing factors for the development of TGCTs (1). In this study, we investigated whether the two pathways of cAMP and KIT/KITLG signaling interact in testicular tissue. A TGCT cell line (NTERA-2) was transiently transfected with plasmid DNA expressing either the wild type (WT) or the mutated forms of PDE11A described in patients with TGCTs (R52T, F258Y, G291R, Y727C, R804H, V820M, R867G and M878V); we then determined cAMP levels, PDE and PKA activity, and RNA and protein levels for KIT, KIT/KITLG, and related molecules. cAMP levels were significantly higher in the NTERA-2 cells transfected with all the PDE11A mutations and relative PDE activity was lower. KITLG expression was consistently increased in the presence of *PDE11A* mutations. The investigation of co-segregation of *PDE11A* and *KITLG* TGCT-linked alleles in families with TGCT is now ongoing, along with immunostaining for the respective proteins. We conclude that KITLG expression consistently increases in the presence of *PDE11A*-inactivating defects, both at the RNA and protein levels, in TGCTs. This is the first demonstration, in any tissue, of a functional link between the cAMP and the KIT signaling pathways in predisposition to cancer.

Horvath A et al. Cancer research 2009; 69(13):5301-5306.

Nothing to Disclose: MFA, ERB, AH, CK, MN, MG, CAS

P3-89

Regulation of Protein Kinase C δ Expression and Sensitivity to Chemotherapy in Endometrial Cancers.

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Endometrial cancer is the most common gynecological malignancy in the US, however, its etiology is poorly understood and few prognostic indicators have been identified. We have recently shown that Protein Kinase C delta (PKC δ) is a critical regulator of endometrial cancer cell survival and apoptosis, modulating sensitivity to chemotherapy and radiation. Moreover, immunohistochemical analysis of a series of endometrial cancers, revealed PKC δ protein expression is progressively lost in tumors of increasing grade. Consistent with these observations, Hec-50 endometrial cancer cells, derived from a poorly differentiated tumor, exhibited reduced PKC δ protein, mRNA and increased resistance to chemotherapeutic agents, relative to the well-differentiated Ishikawa cell line. Thus, loss of PKC δ is characteristic of advanced disease and may underlie the observed chemoresistance of higher-grade tumors. Based on these observations we proposed that PKC δ functions as a tumor suppressor in endometrial cancers and that its expression may be subject to epigenetic regulation.

We have identified CpG islands within conserved regulatory elements of the human PKC δ promoter, which satisfy the criteria for predicted methylation sites. Genomic DNA derived from endometrial tumors and cell lines is being analyzed to determine their methylation status using bisulfite sequencing and methylation arrays. Consistent with epigenetic regulation of the promoter, treatment of Ishikawa and Hec-50 cells with the DNA methyltransferase (DNMT) inhibitor, 5-aza-2-deoxycytidine, markedly increased PKC δ protein levels. To examine the effect on chemosensitivity, cells were treated with DNMT inhibitor or paclitaxel alone or a combination of both agents and cell viability determined. Co-treatment with 5-aza-2-deoxycytidine and paclitaxel resulted in synergistic killing of Hec-50 cells and an additive cytotoxic response in Ishikawa cells.

These results suggest that analysis of PKC δ levels may be used to identify the subset of patients likely to respond to combination therapy with paclitaxel and currently approved 5-aza-2'-deoxycytidine derivatives. This provides a much-needed rational basis for treatment with methylation inhibitors as adjunct therapy in chemoresistant tumors. Thus, PKC δ has the potential to provide novel therapeutic options to target the most lethal forms of endometrial cancer.

Sources of Research Support: NCI CA104875.

Nothing to Disclose: AMT, APB

P3-90

Spontaneous and Programmed Transformation of Ovarian Surface Epithelium Using a Three Dimensional Organ Culture System.

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Ovarian cancer is the fifth leading cause of cancer death among women in the United States, and is the most lethal of the gynecologic cancers due in part to a lack of early detection methods. Over 95% of ovarian cancers are believed to arise from the ovarian surface epithelium (OSE), a single layer of flat-to-cuboidal epithelial cells covering the surface of the ovary. The "tear-and-repair" hypothesis proposes that repeated ovulations during a woman's lifetime, during which the OSE must rupture to allow release of an oocyte, encourages spontaneous DNA damage in OSE cells as they proliferate to cover the wound caused by ovulation. These spontaneous changes accumulate, leading to hyperproliferation of the OSE and potentially malignant changes in the ovarian surface, such as papillae, invaginations, the formation of inclusion cysts, and metastasis to the peritoneal cavity. To study changes in the OSE induced by aspects of ovulation, our lab has developed a three-dimensional organ culture system in which mouse ovaries are wounded with a scalpel and embedded in an alginate hydrogel matrix. Using this system, the OSE mimic ovulation-induced wound repair by proliferating and encapsulating the ovary. In order to study early transformative events in the OSE, the ovary organoids were cultured with the chemical carcinogens n-methylnitrosourea (MNU) or dimethylbenzanthracene (DMBA), or hydrogen peroxide (H₂O₂) to induce spontaneous DNA damage, as would be observed after repeated ovulation. Culturing organoids with H₂O₂, which recapitulates the oxidative stress that occurs during ovulation, causes an increase in OSE proliferation. As an additional indication of transformation, OSE enzymatically removed from organoids cultured in the presence of H₂O₂, MNU, or DMBA form colonies in a soft agar contact inhibition assay. As an alternative model of early events in the development of ovarian cancer, we have transfected organoids to express SV40 T antigen or combinations of shRNA against p53, PTEN, BRCA1, and BRCA2 to evaluate transformation of OSE after targeted genetic disruption. This transformation strategy reflects current mouse models of histotype-specific ovarian cancer. OSE isolated from organoids transformed by spontaneous mutagenesis or targeted genetic disruption can then be analyzed by transcriptional array for changes in genes involved in cancer initiation and progression to identify new targets for the treatment and prevention of ovarian cancer.

Sources of Research Support: NIH BIRCWH grant K12HD055892 awarded to JEB; NIH grant C06RR15482 awarded to JEB; UIC Cancer Center Pilot Grant to awarded JEB.

Nothing to Disclose: SMK, JEB

P3-91

Follicle-Stimulating Hormone and Luteinizing Hormone Induce Expression of Oncogenes in a 3D Model of Normal Ovarian Surface Epithelium.

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Ovarian cancer is the most lethal gynecological malignancy in the U.S. Epithelial ovarian carcinomas (EOC) accounts for 90% of all ovarian cancers and are believed to originate from the ovarian surface epithelium (OSE), a single layer of epithelial cells surrounding the ovary. Early signs or symptoms of ovarian cancer are often subtle and nonspecific, which are frequently ignored. The etiology of ovarian cancer is poorly understood and understanding the events leading to the initiation and progression are critical for detecting early stages of ovarian cancer. Ovarian surface proliferation, associated with ovulation, and the gonadotropins that regulate ovulation, are factors in ovarian surface transformation and cancer progression. Based on the gonadotropin theory, we hypothesized that follicle-stimulating hormone (FSH) and luteinizing hormone (LH), the key inducers of ovulation, play a role in the pathogenesis of EOC. This theory states the excessive levels of FSH and LH related to the surge during ovulation, and the loss of gonadal negative feedback from menopause and premature ovarian failure may play a role in the development and progression of EOC. A three-dimensional alginate-hydrogel organ culture system, developed in our lab, supported the growth of normal OSE and responds to the gonadotropins. Cultures treated with FSH and LH proliferated more on day 8 when compared to control tissues, while control tissues encapsulated more damaged surface by day 8. Using this culture system, the OSE can be removed with collagenase and studied further by isolating RNA to determine pathways regulated by FSH and LH using transcriptional array analysis. FSH transcriptionally up-regulates Mdm2 (double minute 2) and down-regulates Caspase 8, which can increase proliferation. LH transcriptionally up-regulates c-Met and c-Myc which can increase proliferation. FSH and LH both transcriptionally up-regulates CHK2 (checkpoint 2) and PI3K (Phosphatidylinositol-3-kinase) pathways that contribute to increased proliferation. The role of these pathways can be validated by using knockdown and overexpression analysis and quantified by IHC, qPCR and western blot analysis. A novel 3D ovarian organ culture identified key cancer pathways regulated by gonadotropins and can help uncover how these hormones contribute to OSE tumorigenesis.

Sources of Research Support: UIC Cancer Center Pilot Grant; K12HD055892; CO6RR15482.

Nothing to Disclose: TSH, JEB

P3-92 Papillary Thyroid Cancer with Undetectable Thyroglobulin and Metastases to Pituitary Gland and Spleen.

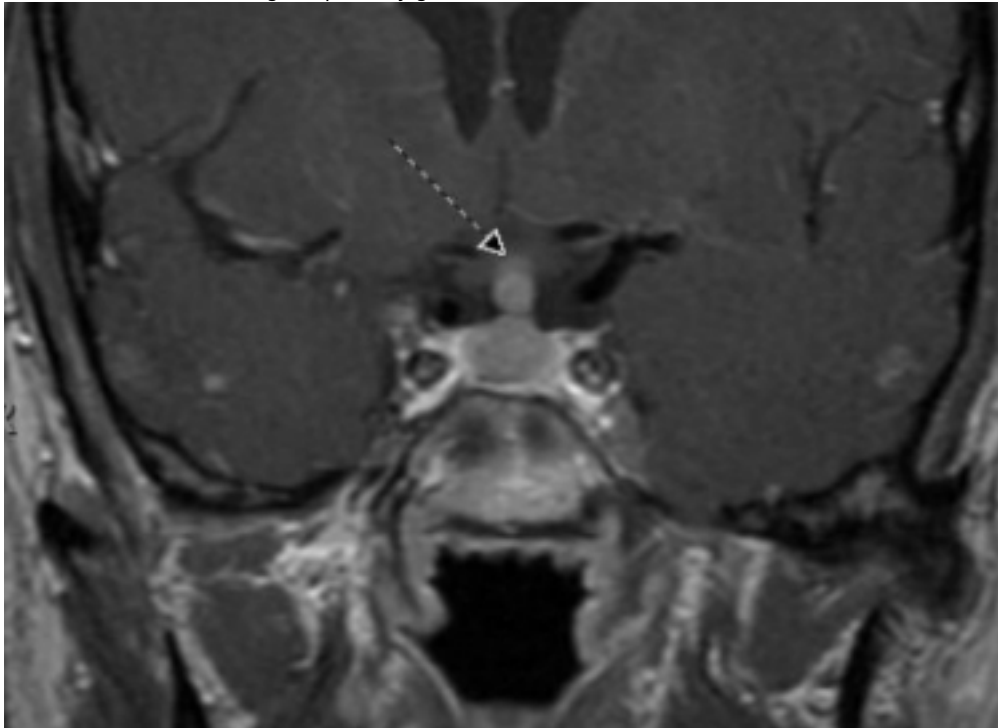
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Background: Papillary thyroid cancer (PTC) is thought of as a relatively non-aggressive malignancy, but in select cases it can present with widespread metastases. Metastases to the pituitary gland, brain and spleen are rare, and are more likely in the setting of other malignancies. We present a patient with history of PTC and prostate cancer, who presented with pituitary insufficiency and metastasis to pituitary, brain, bone and spleen.

Clinical Case: A 61 year old man with history of Gleason 6 prostate cancer and PTC presented with diabetes insipidus, hypogonadism, adrenal insufficiency and headaches. Ten years previously, he had a thyroidectomy and RAI remnant ablation for T4aN1M0 PTC. He has since had three surgeries for non-iodine avid local recurrences and external beam radiation therapy. His prostate cancer was diagnosed 2 years ago and was treated surgically.

He had normal visual fields. Labs showed a serum thyroglobulin (Tg) of 0.7ng/dL with no interfering antibodies, and suppressed TSH. Labs revealed secondary hypogonadism, adrenal insufficiency, and growth hormone deficiency. Prolactin was normal and prostate-specific antigen was undetectable. Imaging revealed multiple brain metastases and a 0.9 cm metastasis involving the pituitary gland.



There was also metastatic disease involving the lungs, spleen, and multiple bony sites. Known paratracheal disease was stable. Diagnostic transsphenoidal resection of the pituitary mass was performed, and pathology revealed PTC.

Discussion: This case provides several instructive points. It demonstrates the need for histological confirmation of disease, especially in the setting of a history of another malignancy or unusual clinical features, such as pituitary and spleen metastases in a thyroid cancer patient. It also is a reminder that aggressive cases of metastatic PTC can present with low or undetectable Tg levels. It also demonstrates an unusual presentation of apparently explosive, hematogenous showering of metastases to many different organs, which grew rapidly. This does not fit with the prevailing model of occult foci of metastatic disease that grow very slowly over time.

Disclosures: SIS: Researcher, Genzyme Corporation, AstraZeneca, Amgen, Eisai, V Foundation for Cancer Research, National Cancer Institute; Consultant, Bayer, Inc., Genzyme Corporation, Lilly USA, LLC, Oxigene, Celgene; Speaker, Genzyme Corporation, Exelixis, Plexxikon, Semafore, Veracyte; Speaker, Exelixis.

Nothing to Disclose: CLM, IEM

P3-93

Response to Sorafenib in a Pediatric Patient with Papillary Thyroid Carcinoma with Diffuse Nodular Pulmonary Disease Requiring Mechanical Ventilation.

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Background: Papillary thyroid cancer (PTC) presents with local and distant metastases more frequently in children¹. However, hypoxemia secondary to pulmonary metastases is not reported in the literature. Sorafenib is a small molecule multikinase inhibitor used in I-131 refractive papillary thyroid carcinoma.

Clinical case: An 8-year old boy presented with fevers and hypoxemia and was diagnosed with H1N1 virus and pneumonia requiring mechanical ventilation, anti-viral, antibiotic, and glucocorticoid treatment. After the initial resolution, he was readmitted 1 month later for hypoxemia. A high-resolution CT of the lungs showed multiple nodules throughout the lungs (1-4 mm) and multiple necrotic lymph nodes throughout the right aspect of the neck. A left lung wedge resection biopsy and right deep cervical node (2X1 cm) biopsy was consistent with PTC. He underwent total thyroidectomy with central neck node dissection and post-operatively required prolonged mechanical ventilation. Thus, treatment with sorafenib 200 mg daily (10 mg/kg/day, 250 mg/m²) was initiated. Eight days post initiation of therapy, ventilation support was sufficiently weaned for extubation. He failed extubation secondary to supraglottic edema and 7 days later tracheostomy was performed. The dose was eventually increased to 200 mg twice daily (20 mg/kg/day, 500 mg/m²). After 52 days of therapy, a repeat CT scan showed a reduction in the lung nodules (1-2 mm). He underwent I-131 therapy 87 days after sorafenib was started. Post treatment scan showed extensive uptake throughout out the lungs and bed of the thyroid, supraclavicular nodes and cervical nodes.

Conclusion: This is the first case described of severe hypoxemia and diffuse nodular respiratory insufficiency related to metastatic papillary carcinoma requiring mechanical ventilation. Sorafenib should be considered for gap therapy while the patient recovers pulmonary function. Treatment with this multi-kinase inhibitor does not seem to adversely affect the uptake of I-131 in radiation naive patients.

(1) Zimerman D, Curr Opin Pediatr 1997; 9:413

Nothing to Disclose: PI

P3-94

An Unusual Case of Syncope: Burkitt's Lymphoma Arising from the Thyroid.

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Burkitt's lymphoma is a disease that involves the lymphatic system and can affect a wide variety of organs. Unusual cases of Burkitt's lymphoma can present with a head or neck mass. In this case, a neck mass resulted in syncope. Our patient was a 31 year old Filipino female from jail, with a history of diabetes mellitus type 2 and history of crystal methamphetamine use. She initially presented with recent syncopal episodes. Within the past two months she had three prior syncopal episodes. In one of these instances, she noticed a left neck pain prior to syncope. She complained of headache, left neck pain, hoarseness, dysphagia, and history of night sweats. Two weeks prior to admission, the patient was seen by otolaryngology for an enlarged neck mass arising from the left thyroid lobe. Otolaryngology then performed an FNA biopsy which showed a large malignant high grade lymphoma, which was deemed Burkitt's lymphoma. In the emergency department, EKG showed sinus bradycardia and subsequent tachycardia. Physical exam revealed a hard neck mass anteriorly, 4cm in the vertical aspect and 10cm in the anterior aspect. Cardiac exam revealed tachycardia, no murmurs, and no carotid bruits. TSH and free T4 were within normal limits. Upon further workup, a computed tomography of the neck showed a large 7.4cm x5.7cm x9.4cm mass arising from the left thyroid lobe with displacement of the trachea to the right and left internal jugular vein compressed and displaced laterally. Hyper-CVAD chemotherapy treatment was started along with intrathecal chemotherapy with methotrexate and Ara-C. During her hospital stay, the patient reported that her neck mass had significantly reduced in size and was now soft. She reported no further episodes of syncope or lightheadedness. After chemotherapy was started the patient's bradycardia resolved. Furthermore after the patient was discharged, our patient has denied any syncopal episodes after a two year follow up.

Syncope involving the carotid body can result from a variety of causes, which can include carotid body compression, such as in our case, and physiological causes such as carotid body hypersensitivity. With carotid hypersensitivity, patients have been known to have syncope after wearing tight collars or turning their head while shaving. Carotid sinus stimulation can then result in bradycardia, hypotension, and syncope. In this unusual case, the patient's syncope was the result of Burkitt's lymphoma causing carotid body stimulation.

Nothing to Disclose: JKT, HL, SK, GZ

P3-95

Infants with Hereditary MEN 2B Should Undergo Prenatal Surgical Referral and Prophylactic Thyroidectomy within the First Month of Life.

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Background: Patients with Multiple Endocrine Neoplasia type 2 (MEN 2) are at high risk of developing aggressive medullary thyroid carcinoma (MTC) at a young age, with those harboring M918T RET proto-oncogene mutation (> 95% of MEN 2B) having the highest risk for early disease. Microscopic MTC with metastases has been reported within the first year of life and as young as 3 months of age. Therefore, current guidelines state that prophylactic thyroidectomy should

be performed by 6-12 months of life for MEN 2B, preferably in the first month.^{1,2}

Clinical Case: We describe the youngest patient in the literature (to our knowledge) with proven MTC due to hereditary MEN 2B. A full term male infant was born to a mother with known MEN 2B due to M918T RET proto-oncogene mutation. His mother was diagnosed at age 13 years with metastatic MTC, and has persistent hypercalcitoninemia despite total thyroidectomy, regional lymph node dissection, and a second exploratory surgery that identified and resected hepatic metastases. Our patient was confirmed as having an M918T RET proto-oncogene mutation by 4 weeks of age. Serum calcitonin drawn at 4 weeks was 81 pg/mL (normal reported range below 6 months of age < 41 pg/ml) and he underwent total thyroidectomy at age 9 weeks. Pathology showed a focal calcitonin positive nodule (2.5 mm) consistent with microscopic MTC. His calcitonin level dropped to 8 pg/ml a week post-operatively, and he recovered without complication.

Conclusion: This case underscores the importance of early prophylactic thyroidectomy in hereditary MEN 2B with M918T RET proto-oncogene mutation. Although current guidelines give room for surgery up to 12 months of age, MTC may occur very early, as evidenced by our patient. In order to achieve timely neonatal thyroidectomy in those at risk, we recommend prenatal genetic counseling and surgical referral (with scheduling of anticipated surgery), with gene testing soon after birth. Once the molecular diagnosis is established, prophylactic thyroidectomy should be undertaken at a center with expertise in neonatal thyroid surgery within the first month of life.

(1) Brandi ML et al., JCEM 2001; 86:5658

(2) Kloos RT et al., Thyroid 2009; 19:565

Nothing to Disclose: RKS, MJR, SDC, MMR

P3-96

Report of Two Patients with Neurofibromatosis Type 1 Presenting Features of Multiple Endocrine Neoplasia Type 2A.

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Background: Neurofibromatosis type 1 (NF1) is an autosomal disorder associated with pheochromocytomas. The association of NF1 with other endocrine tumors is rarely recognized. Few reports have described medullary thyroid carcinoma (MTC) or thyroid C cell hyperplasia (CCH) in NF1, and only two cases have been reported with the full spectrum of multiple endocrine neoplasia type 2A (MEN2A). We describe two new cases of NF1 presenting clinical features of MEN2A.

Clinical case (1): A 70-year-old woman diagnosed with NF1 since the age of 35 was referred for the incidental discovery of a right para-renal mass. The mass was not of an adrenal origin, and urinary catecholamine and metabolites were normal. During the investigation, a multinodular goiter was found, with one dominant nodule measuring 8 mm in the right thyroid lobe. Basal calcitonin levels were increased at 66 ng/L (N<20), and a calcium stimulation test led to levels up to 188 ng/L. Total thyroidectomy was performed, and MTC with focal areas of CCH was found at histology. Despite normal PTH and calcium levels, a parathyroid adenoma was found in the resected material.

Clinical case (2): A 35-year-old male with familial NF1 underwent an abdominal CT scan for intra-abdominal neurofibromas. Incidentally, a 2.2 cm left adrenal lesion with a Hounsfield Unit of 76 was found. Urinary catecholamines, plasma metanephrine and normetanephrine levels were increased and a MIBG scan suggested a diagnosis of pheochromocytoma, which was confirmed at pathology after adrenalectomy.

Investigating for the possible co-existence of other pheochromocytoma-related syndrome, we found serum calcium and PTH levels that may be compatible with primary hyperparathyroidism as well as high basal calcitonin levels (28 ng/L (N<10)). Two calcium stimulation tests increased calcitonin from 16 to 207 ng/L and 19 to 304 ng/L, thereby raising the possibility of MTC or CCH. Thyroid sonography showed multiple nodules (<1 cm), and parathyroid MIBI suggested increased retention posterior to the upper right thyroid lobe. The patient has thus far declined thyroidectomy. These findings prompted us to consider the diagnosis of MEN2A syndrome, but testing was negative for RET gene mutation.

Conclusions: These two cases support a possible co-existence of NF1 and MEN2A as described previously. Calcium and calcitonin levels should be measured in patients with NF1 to exclude rare MTC/CCH and primary hyperparathyroidism association.

Nothing to Disclose: JA, JMB, IB

P3-97

Pituitary Carcinoma in a 54 Year-Old Male.

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Background: Pituitary carcinomas typically arise from the transformation of originally benign, large adenomas with an average latency period of 7 years and are defined only by development of craniospinal and/or systemic metastases.

Clinical Case: A 54-year-old male presented for evaluation of a recurrent pituitary tumor. A pituitary macroadenoma was discovered in 2000 after he complained of weight gain, fatigue, and visual changes. He was diagnosed with Cushing's disease and underwent transsphenoidal resection and radiation therapy. Pathology confirmed an ACTH-positive adenoma. Subsequently, he went into remission. In 2007, he developed a 2.8cm invasive macroadenoma (ACTH-positive) for which he had a transsphenoidal hypophysectomy (TSH). After 3 months, he required repeat TSH and stereotactic radiotherapy for a recurrent 3.8cm invasive mass with optic chiasmal compression. He was lost to follow-up until the summer of 2009 when MRI revealed a stable residual mass in the sella, plus a new retropharyngeal mass. PET scan demonstrated hypermetabolic foci in a skull base mass, left retropharyngeal, bilateral cervical, and abdominal aortocaval lymphadenopathy. Lymph node excisional biopsy revealed metastatic pituitary carcinoma, which was ACTH-positive and histologically similar to his previously resected pituitary adenoma. An Octreoscan showed increased uptake in the retropharyngeal mass and aortocaval lymphadenopathy. On recent evaluation, he complained of fatigue, weakness, and easy bruising. Physical exam revealed mild central adiposity, skin atrophy, and proximal muscle weakness, but no other Cushingoid features. AM cortisol was 29.1 ug/dL (4.3-22.4 ug/dL) and ACTH 70 pg/mL (7-50 pg/mL). He is currently undergoing treatment with monthly Sandostatin LAR.

Conclusion: Pituitary carcinomas and adenomas cannot be distinguished by clinical, radiological, biochemical, or standard histological criteria. The diagnosis of carcinoma is only made once there is evidence of craniospinal and/or systemic metastases which have structural or biochemical features similar to those of the pituitary tumor. The majority of carcinomas are hormonally active, with ACTH and prolactin-secreting being most common. Once metastases occur, the mean survival is less than four years; corticotroph carcinomas exhibit the worst survival. Treatment, which is mainly palliative includes surgery, external beam radiation, and adjuvant medical therapy (mainly Sandostatin LAR).

(1) Kaltsas GA et al., J Clin Endocrinol Metab 2005; 90:3089

Nothing to Disclose: MKM, SJ

P3-98

Effectiveness of Etomidate in a Patient with Ectopic ACTH Syndrome Presenting with Disseminated Nocardiosis.

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Background: Severe hypercortisolism in ectopic ACTH syndrome (EAS), together with opportunistic infection, often portends a poor outcome. We report a case of a patient with fulminant hypercortisolemia and disseminated Nocardiosis successfully treated with etomidate pre-operatively with rapid normalization of serum cortisol levels and an uncomplicated adrenalectomy.

Clinical case: A 62-yo man presented with a 3 month history of rapidly progressive proximal muscle weakness, back pain due to multiple, vertebral compression fractures, hypokalemia and new onset DM and HTN. Exam revealed mild plethora, truncal fat redistribution and pronounced proximal muscle weakness. Labs showed elevated 24-hr urine free cortisol- 4440 ug/24 hr, random cortisol 40-70 ug/dL, lack of suppression to low dose dexamethasone (cortisol- 66 to 64 ug/dL) and ACTH 205 pg/mL (10-60). CT imaging demonstrated a right adrenal mass 5.7 x 3.4 cm and multiple pulmonary masses. MRI of brain revealed several enhancing lesions, and a normal pituitary gland. The patient underwent right adrenalectomy and biopsy of the right lung. Histology revealed *Nocardia farcinica* without malignancy, for which the patient received antibiotics. Postoperatively, cortisol dropped to 20-30's ug/dL. High dose dex test did not suppress (cortisol 35 to 28 ug/dl), consistent with an EAS. Extensive evaluation for tumor localization was negative. Because of progressive weakness, anorexia and new spinal fractures, the patient was given Ketoconazole, which was poorly tolerated. In preparation for adrenalectomy, he was given an Etomidate drip 2.5 mg/hr which rapidly normalized the cortisol level to < 15 ud/dl (8h), as per a previously described protocol.¹ Once cortisol levels reached 15 ug/dl, the etomidate infusion was lowered to 1.3 mg/hr (36h) and a Dexamethasone drip was initiated to a target cortisol level of 10-15 ug/dl. Patient underwent an uneventful laparoscopic left adrenalectomy. The underlying etiology for his EAS remains unknown.

Discussion: Systemic, opportunistic infections are a well-described and potentially fatal complication of Cushing's syndrome, and mandate prompt control of hypercortisolemia. Etomidate use, although rarely reported, should be strongly considered in patient with fulminant hypercortisolemia and life-threatening complications because, unlike the other available medical therapies for Cushing's syndrome, it results in a profound and rapid inhibition of 11-B hydroxylase.

1. Drake W. et al. Emergency and prolonged use of intravenous etomidate to control hypercortisolemia in a patient with Cushing's syndrome and peritonitis. *J Clin Endocrinol Metab.* 1998 Oct;83(10):3542-4.

Sources of Research Support: VA Merit Review (MEW).

Nothing to Disclose: KK-V, DH, JK, MEW

P3-100

CT Scan-Negative Ectopic ACTH Syndrome: Is There a Role for Tumor Localization with ¹¹¹Indium-Octreotide Scintigraphy?.

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Background: In patients with ectopic ACTH syndrome (EAS) and negative high-resolution CT scanning, the role of ¹¹¹indium-octreotide scintigraphy in identifying tumors is not clear. It has been considered by some investigators to be not useful.

Clinical case: A 36-year old woman presented with typical symptoms of Cushing's syndrome (CS) that progressed rapidly over the course of one year. Her biochemical and imaging studies were as follows: morning ACTH 102 pg/mL (8-42) with a simultaneous cortisol 24.1mcg/dL, cortisol during 1-mg and 8-mg dexamethasone suppression tests of 38 and 14 mcg/dL, respectively, normal appearance of pituitary MRI along with negative CT scan of chest, abdomen, and pelvis. She underwent inferior petrosal sinus sampling, which supported an ectopic source of ACTH. Repeat CT scan of chest, abdomen, and pelvis using high resolution technique was negative. Subsequently, ¹¹¹indium-octreotide scintigraphy was performed. It revealed an indium-avid left upper lobe pulmonary nodule (1.3 cm) suspicious for a neuroendocrine tumor. While awaiting surgery, she was found to have a serum cortisol of 2.8 mcg/dL when symptomatic with fatigue suggesting an underlying cyclic CS, which improved after treatment with hydrocortisone. Subsequently, the patient underwent left-upper lobectomy and surgical pathology revealed an ACTH-producing carcinoid tumor that stained positive for ACTH. Serum cortisol on post-operative day 1 (drawn at 12:55 AM) was 2.4 mcg/dL, indicating cure/remission of CS. At 3 months post-surgery, she has lost 36 lbs, stopped all her antihypertensive and anti-diabetic medications, and her Cushing features have resolved.

Conclusion: This case demonstrates the utility of ¹¹¹indium-octreotide scintigraphy for localizing the ACTH-secreting tumor in EAS when high-resolution CT scanning is negative.

Nothing to Disclose: GBM, AHH, MH

P3-101

Cushing's Disease Due to an ACTH-Producing Carcinoid Tumor of the Thymus Gland: A Case Report.

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Background: Cushing's disease in young patients is most commonly caused by a pituitary adenoma. Ectopic sources of ACTH are rare, and carcinoid tumor of the thymus is an exceedingly rare cause, with poor clinical prognosis¹. We herein report a patient with classical signs and symptoms of Cushing's syndrome due to carcinoid of the thymus.

Patient report: Our patient is a 17 year old male who presented with a six month history of muscle weakness, profound decrease in muscle mass, weight gain followed by 25 lb weight loss, difficulty sleeping, facial puffiness, centripetal fullness, acne, skin infections, poor skin healing, hair thinning on the scalp but overall hypertrichosis, violaceous striae, and decreased libido. He had decreased physical stamina for over a year. On physical exam he appeared grossly Cushingoid. His afternoon cortisol level and 24 hour free urinary cortisol were significantly elevated at 24.4 ng/mL (4-11) and 777 ug (0-50) respectively. ACTH level was elevated at 139 pg/mL (0-46). A CT of abdomen and pelvis showed no adrenal mass. An MRI demonstrated no lesion or lateral displacement of the pituitary. Bilateral petrosal venous sinus sampling demonstrated equally elevated ACTH levels from both petrosal veins and peripheral blood, indicating an ectopic source of ACTH. A chest CT demonstrated a 6.4x2.4x6.3 cm mass in superior anterior mediastinum, arising from the thymus. A PET scan demonstrated a large, hypermetabolic heterogeneous soft tissue mass with scattered hypodense areas occupying the anterior superior mediastinum with hypermetabolic prevascular and right hilar adenopathy, representing metastatic nodal disease. A biopsy was consistent with a neuroendocrine tumor that stained positive for ACTH. The patient had a resection of the tumor and local lymph nodes, with clean margins. Octreotide treatment was initiated. Glucocorticoid replacement was started post-operatively. Two and six weeks post operatively the ACTH levels were 20 ng/dL and <10 ng/dL, respectively. Cortisol level was undetectable six weeks post operatively (after omitting hydrocortisone dose). There is no defined consensus on treating our patient post-operatively because of the rarity of the disease. Radioactive octreotide and radiation treatment are proposed options.

Conclusion: Practitioners must always consider an ectopic source of ACTH in a patient with Cushing's disease. Much can be learned from this case about work-up and treatment of thymic carcinoid tumor.

(1) Tiffet O et al., "A Clinicopathologic Study of 12 Neuroendocrine Tumors Arising in the Thymus." Chest 2003; 124; 141-146

Nothing to Disclose: OL, MIN

P3-102

Carcinoid Heart Disease Secondary to a Primary Ovarian Carcinoid Tumor.

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Introduction: We present a rare case of primary ovarian carcinoid tumor.

Case presentation: A 71-year-old female presented with dyspnea, flushing and edema. A 4/6 holosystolic murmur, flushed face and bilateral lower extremity edema were noted on physical exam. An echocardiogram showed severe tricuspid regurgitation with thickened immobile tricuspid valve. A carcinoid heart disease was suspected. The diagnosis was confirmed by marked elevation of chromogranin A (2240 ng/ml), serotonin (2326 ng/ml) and 24 hour urine 5-hydroxyindoleacetic acid (5-HIAA) (117 mg/24hrs). An octreotide scan demonstrated uptake in the area of the terminal ileum and cecum. A CT scan and ultrasound revealed a right pelvic mass. Ovarian carcinoid tumor was suspected. The patient was started on sandostatin LAR and underwent total hysterectomy/bilateral salpingo-oophorectomy. A right ovarian mass measuring 11cm was resected. Histological examination confirmed classic ovarian carcinoid tumor (pure insular form) without evidence of metastasis. Postoperatively, chromogranin A, serotonin, 5-HIAA levels normalized, and cardiac symptoms markedly improved, but the patient still experienced occasional flushing.

Discussion: Primary ovarian carcinoid tumors represent 0.5 % of all carcinoid cases (1). Histologically, they have been classified into three groups: insular; trabecular and strumal. Most insular carcinoid tumors are benign and arise within teratomas: 23% occur in pure form (2). Five year survival rate is >90%. Treatment is surgical (2). Recurrence is rare. Carcinoid syndrome is associated with insular type only and is determined by tumor size (2). Ovarian carcinoids release serotonin and other vasoactive metabolites directly into systemic circulation, causing carcinoid syndrome without metastasis to the liver. Carcinoid heart disease may regress after removal of the primary tumor, however, treatment usually involves tricuspid valve replacement. Our patient remains on octreotide, which has been shown to lengthen the time of tumor progression in metastatic carcinoids (4) in addition to symptom control (3).

Conclusion: Right heart failure is one of the classic presentations in a patient with carcinoid syndrome. Diagnosis may be delayed by 2-3 years as symptoms are vague (5). Carcinoid heart disease has to be considered in all patients presenting with restrictive cardiomyopathy and right heart failure symptoms.

(1) Modlin IM, An analysis of 8305 cases of carcinoid tumours. *Cancer* 1997;79:813-829

(2) Robboy SJ Insular carcinoid primary in ovary. *Cancer* 1975;36: 404-408

(3) Norheim I Malignant carcinoid tumors: an analysis of 103 patients with regard to tumor localization, hormone production, and survival. *Ann Surg* 1987; 206:215

(4) Rinke A, Placebo-controlled, double-blind, prospective, randomized study on the effect of octreotide LAR in the control of tumor growth in patients with metastatic neuroendocrine midgut tumors: A report from the PROMID study group 2009 *Journal of clinical oncology*, 27: nov 28.oct 1: 4656-4663

(5) Bax N, octreotide therapy in carcinoid disease, *Anti-cancer Drugs* 1996; 7(1): 17-22

Nothing to Disclose: TA, PMJ, NVP, AG

P3-103

Parathyroid Carcinoma (PC): Report of Two Cases.

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Background- PC is a rare endocrine malignancy accounting for about 1% of patients with primary hyperparathyroidism (PHP) (1). In the cases reported in literature, the tumor size ranges from 1 to 12 cm, with a mean of 3.1 cm (1). Associated kidney, bone, gastrointestinal and psychiatric symptoms are much more frequent than in parathyroid adenomas, due to the markedly increased calcemia (up to 24 mg/dL) (1).

Clinical cases- The first patient was a 62-year-old woman with a two-year history of asthenia, hypertension and mental disorders. Serum levels of intact parathyroid hormone (iPTH) and calcium were 104.7 pg/mL (normal range: 12.0–72.0 pg/mL) and 10.21 mg/dL (8.2–10.4 mg/dL), respectively. No bone pain, abdominal symptoms or renal abnormalities were noted. ^{99m}Tc-MIBI scintigraphy demonstrated an abnormal focal uptake of the right inferior parathyroid gland. Minimally invasive surgery was performed; the resected node measured 35 mm. Histopathological examination permitted a diagnosis of PC. Serum iPTH and calcium decreased postoperatively (28.4 pg/mL and 8.6 mg/dL). The second patient was a 54-year-old woman who underwent total thyroidectomy two years earlier. Biochemical follow-up disclosed hypercalcemia and hypercalciuria (10.7 mg/dL and 540 mg/24 h), which prompted iPTH assay (320 pg/mL). No bone pain, abdominal symptoms or renal lithiasis were noted. A 21-mm hypoechoic nodule with indistinct margins was detected by echography in a position equivalent to the lower pole of the right thyroid. In this position, ^{99m}Tc-MIBI scintigraphy demonstrated an abnormal uptake. After minimally invasive surgery and histopathological examination, a diagnosis of PC was made. Serum iPTH and calcium dropped postoperatively (< 5 pg/mL and 8.4 mg/dL). No recurrent disease was observed 12 months after surgery in both patients.

Conclusion- Diagnosis of PC is difficult preoperatively; PC-associated PHP may be symptomatic or asymptomatic. Minimally invasive parathyroidectomy ensures a good outlook at least in the short term.

(1) Fernandez-Ranvier GG et al., *Cancer* 2007; 110:255-264.

Nothing to Disclose: AC, RMR, AS, AI, GT, EC, LC, SB, SB

P3-104

Do DNA Variants in the Calcium-Sensing Receptor Play a Role in the Development and Progression of Cystosarcomata Phylloides of the Breast?.

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Background: Approx. 0.2% of primary breast carcinomas (BC) occur before age 20 y. Cystosarcomata phylloides (CP) represent less than 1% of BC, are characterized by proliferation of mesenchymal and epithelial cells, and metastasize in about 9% of adults (1,2). The CaSR plays a role in the development and progression of cancers (3).

Case Report: A now 45-yo woman presented with hypercalcemia (S-Ca 10.7 mg), hyperparathyroidism (intact PTH 78 pg, nl: 15-65), 25-OH vitD 108 ng, TSH 1.9, no h/o of renal stones or bony fx but of bilateral CP at age 11 y. She had undergone several resections, chemotx, and reconstructive surg with saline implants. Mammograms to date were neg. Of her 2 daughters, the 25-yo had 3 breast bx of lumps, the results of which were negative. Mammograms were also negative. Her s-Ca was 9.3, PTH 53. The 18-yo daughter had no mammograms or issues, s-ca 8.9 mg, PTH 25. Family history is neg for cancer except for her maternal grandfather (colon cancer). Repeat lab: s-Ca 10.3, PTH 87, 25-OHD 36, calcitonin undetect, fract excret calcium 0.0052. We considered FHH vs. pHPT. BMD DEXA showed a T-score of -0.6 at left forearm, -0.7 at femur, -1.3 at spine. Sestamibi negative. CaSR mutation screen: heterozygous SNP in exon 7 (DNA change c2968A>G, AA change p.R990G). We initiated biphos tx for osteopenia. The pt was also taking premarin 1.25 mg, nexium 40 mg, L-thyroxine 125 mcg, and paxil. She had a complete hysterectomy in 2004 and primary hypothyroidism since 2001. Her brother had renal stones but nobody else had any features of pHPT or MEN-1.

Discussion: Bilateral, early-onset breast tumors may suggest a hereditary tumor condition which we could not confirm in our case of CP. Increased CaSR levels are found in highly metastatic primary BC cells, whereas reduced or absent CaSR expression is seen in colon and parathyroid cancers with a decline in cell proliferation upon activation of CaSR (3). Loss of function of CaSR by heterozygous inactivating mutations occurs in FHH. This may reduce growth of BC and CP cells, thereby explain the longterm remission/cure (34 y) of our now 45-yo pt. On the contrary, activating mutations of CaSR, seen in pts with hypocalcemia, may trigger development of neoplasms (4). We speculate that the prevalence of breast cancer and CP is lower in individuals with inactivating mutations/sequence variants of the CaSR and higher in those with activating mutations of CaSR compared to individuals without abnormal DNA variants in CaSR.

1. Confavreux C, Lurkin A, Mitton N, et al. Sarcomas and malignant phylloides tumours of the breast-a retrospective study. *Eur J Cancer* 2006;42:2715-21
 2. Stamatakos M, Tsakanaki S, Kontzoglou K, et al. Phylloides tumor of the breast: a rare neoplasm, though not too innocent. *Int Semin Surg Oncol* 2009;6:6
 3. Saidak Z, Mentaverrri R, Brown EM. The role of the calcium-sensing receptor in the development and progression of cancer. *Endocr Rev* 2009;30:178-195
 4. Hoff AO, Cote GJ, Fritsche HA, Qiu H, Schultz PN, Gagel RF. Calcium-induced activation of a mutant G-protein coupled receptor causes in vitro transformation of NIH/3T3 cells. *Neoplasia* 1999;1:485-491
- Liu G, Hu X, Chakrabarty S. Calcium sensing receptor down-regulates malignant cell behavior and promotes chemosensitivity in human breast cancer cells. *Cell Calcium* 2009;45:216-225

Disclosures: CAK: Consultant, Novo Nordisk.

Nothing to Disclose: SAB, GIU, LM

P3-105

Levels of Cardiac Corticotropin-Releasing Factor Type 1 Receptor Gene Expression Are Up-Regulated in Heart Failure and Altered in Association with a Large Inversion Polymorphism That Spans the Gene.

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Corticotropin-releasing factor (CRF) and related peptides urocortin (Ucn) 1, Ucn2 and Ucn3 signal through receptors CRFR1 and CRFR2 to restore homeostasis in response to stress. The Ucn3s exert potent cardioprotective effects via CRFR2 in the heart and vasculature and have considerable therapeutic potential in the treatment of heart failure (HF). We hypothesized that genetic variation within the genes encoding the CRF family of ligands and receptors may alter their expression within the heart. To test this hypothesis we investigated associations between levels of cardiac gene expression of the CRF family members (measured by RT-qPCR) and genotype in left ventricle tissue from 110 patients with heart failure and 108 unmatched donors with no previous history of heart disease. Subjects were genotyped for putative functional or tagging polymorphisms within the genes encoding *CRFR1* (17q21.31 H1/H2 inversion haplotype), *CRFR2* (rs733453, rs3779250) and *Ucn3* (rs10904481) using PCR and Taqman assays. We detected expression of genes encoding *CRFR1*, *CRFR2* (predominantly *CRFR2α*), *CRF*, *Ucn1*, *Ucn2* and *Ucn3* within the myocardium of donors and HF patients. Expression of *CRFR1*, *CRF* and *Ucn3* was markedly up-regulated ($p < 0.001$) and *CRFR2* was down-regulated ($p = 0.001$) in HF patients compared with donors. Also, in a subset of donors and HF patients, mRNA corresponding to alternatively spliced forms of *CRFR1* (α , C, E, F) was detected, as well as a novel splice variant in which exons 4 and 6 of the *CRFR1* gene had been spliced out. Levels of expression of *CRFR1* were markedly lower in individuals homozygous for the chromosome 17q21.31 H1 haplotype (direct orientation) compared with other individuals (donors: -2.9-fold, $p < 0.001$; HF patients: -3.1-fold, $p < 0.001$). The H1H1 genotype occurred more frequently in HF patients compared with donors (75% vs 52%, $p = 0.001$), suggesting that the H1 haplotype, and/or lower levels of *CRFR1* expression, may be associated with increased cardiovascular risk. There were no associations between levels of gene expression of *CRFR2* and rs733453 or rs3779250, or between levels of expression of *Ucn3* and rs10904481. Our findings suggest that levels of cardiac *CRFR1*, *CRF* and *Ucn3* gene expression are up-regulated in human HF. In HF patients and heart donors *CRFR1* gene expression was altered in association with the chromosome 17q21.31 inversion polymorphism that encompasses the *CRFR1* gene. *CRFR1* may also be regulated by alternative splicing in the heart.

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Nothing to Disclose: APP, VAC, KAL, MHP, WES, RWT, WHT, MOH, CSM, WWV

P3-106

Identification and Characterization of Mouse Endothelial Cell-Specific Molecule 2 (ECSM2) Isoforms: Implications in Angiogenesis.

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Angiogenesis is not only essential for normal growth and development but also a determinant for many pathological conditions. Endothelial cells (ECs) that line the lumen of blood vessels are important players in blood vessel formation, and directed cell migration of ECs is a key component of angiogenic process. Thus, identification of genes that are specifically expressed in vascular ECs and in-depth understanding of their biological functions may lead to discovery of new therapeutic targets. We have previously reported molecular characterization of human endothelial cell-specific molecule 2 (ECSM2), a candidate gene potentially involved in angiogenesis (1). In the present study, we cloned two full-length cDNAs from mouse by RT-PCR, which encode two putative ECSM2 proteins with considerable homology to the human ECSM2. Nucleotide sequence and exon-intron junction analyses suggested that they are alternative splicing variants (ECSM2 isoform-1 and -2), differing from each other in the first exon. Quantitative PCR results revealed that isoform-2 is the predominant form both *in vivo* and *in vitro*. For *in vivo*, isoform-2 was expressed highly in heart, lung, and muscle, and moderately in uterus and testis. In contrast, isoform-1 seemed enriched in testis. As for *in vitro*, both isoforms were specifically expressed in mouse endothelial cells (MS1) but not other tested cell lines. To explore their potential cellular functions, we expressed GFP- and FLAG-tagged ECSM2 isoforms, respectively, in an ECSM2 deficient cell line (HEK293). Interestingly, the actual sizes of either GFP- or FLAG-fusion proteins detected by immunoblotting are much larger than their predicted sizes, suggesting that both isoforms are glycosylated proteins. Fluorescent microscopy revealed that both GFP-ECSM2 and FLAG-ECSM2 are localized at cell-surface, which is consistent with the structural prediction. We finally performed transwell migration assays using MS1 transfectants transiently expressing GFP-isoform-1 and GFP-isoform-2, respectively. Both isoforms substantially inhibited vascular epidermal growth factor (VEGF)-induced cell migration. Taken together, we have demonstrated the presence of two ECSM2 isoforms in mouse. They are preferentially expressed in vascular ECs, and are likely involved in EC migration. We are currently investigating the mechanism(s) of how the endogenous ECSM2 isoforms modulate cell motility and thereby angiogenesis.

(1) Ma F et al., Genes to Cells. 2009; 14: 281-93

Sources of Research Support: St. Joseph's Foundation Startup Fund and partially by a CHW SEED Award (to Y.H.). W.W. is the recipient of a China Scholarship Council (CSC) Studentship.

Nothing to Disclose: WW, CS, FM, HH, JB, YH

P3-107

Cross-Talk between Inflammation and Stress Reaction in Toll-Like Receptor 4 (TLR4)-Mediated Growth of Endometriosis.

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Background: The pathogenesis of endometriosis is still unclear. Hormonal factors and inflammation are commonly involved in the regulation of endometriosis. However, information on the combined role of inflammation and stress reaction is scanty. We investigated mutual role of inflammation and stress reaction and their involvement in toll-like receptor 4 (TLR4)-mediated growth of endometriosis.

Material and Methods: Biopsy specimens were collected from the eutopic and ectopic endometria of 30 women with endometriosis and 20 control women. We measured endotoxin (LPS) levels in the menstrual fluid (MF) and peritoneal fluid (PF) derived from these women. Gene and protein expression of TLR4 in PF-derived macrophages and endometrial cells were examined by RT-PCR and immunohistochemistry. Tissue expression of human heat shock protein-70 (Hsp70) in eutopic and ectopic endometria was examined. The effect of LPS on TLR4-mediated production of macromolecules by macrophages and Hsp70 expression by endometrial cells and on cell proliferation was examined. Conversely, effect of Hsp70 on TLR4-mediated cytokine production by macrophages and cell proliferation was also examined.

Results: Endotoxin levels in MF and PF were significantly higher in women with endometriosis than that in non-endometriosis. Gene and protein expression of TLR4 were detected in macrophages and endometrial cells. Hsp70 was expressed in both gland cells and stromal cells derived from eutopic and ectopic endometria. LPS was able to significantly stimulate TLR4-mediated production of a number of macromolecules by macrophages, induced expression of Hsp70 by endometrial cells and promote cell proliferation. This effect of LPS was significantly higher in women with endometriosis than that in control women. Again, exogenous treatment of macrophages and endometrial cells with Hsp70 significantly induced TLR4-mediated cytokine production and cell proliferation. While polymyxin B, a potent LPS antagonist, was unable to suppress combined LPS+Hsp70-promoted cell proliferation, pretreatment of cells with anti-TLR4 antibody was able to abrogate their dual effect on cell proliferation.

Conclusions: A prominent inflammatory response and endogenous stress reaction was observed in women with endometriosis than that in non-endometriosis. Both endotoxin and Hsp70 were mutually involved in inducing stress reaction and inflammation in pelvic environment and may regulate TLR4-mediated growth of endometriosis.

Nothing to Disclose: KNK, MK, KH, AF, HM

P3-108

Epigenetic Regulation and Hormonal Control of the Mouse Prolyl Oligopeptidase Gene in the Ovary.

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Prolyl oligopeptidase (POP) is a housekeeping gene encoding serine endopeptidase which hydrolyzes the peptide bond at carboxyl side of internal proline residue. POP gene is highly conserved from bacteria to mammals and expressed in every tissue. Although much effort has been focused on understanding its physiological role, the regulatory mechanism of POP expression is poorly understood. This time we have found that the mouse ovary expressed a higher level of POP mRNA than other tissues. To understand the POP regulation in the ovary, we investigated the epigenetic status and hormonal control of the mouse POP gene. By in situ hybridization and qRT-PCR analysis, POP mRNA was detected exclusively in the ovarian granulosa cell and the expression level in the granulosa cell was 18 times as high as that in the liver. We examined DNA methylation and histone modification of two CpG islands at the POP locus. Bisulfite sequencing analysis showed that the CpG island at the promoter region (CGI-1) was almost completely demethylated in both the granulosa cell and liver. In contrast, the methylation rate of a CpG island in the gene body (CGI-2) was lower in the granulosa cell. An in vitro reporter gene analysis indicated that CGI-2 enhanced POP promoter activity and its effect was significantly reduced by DNA methylation. We also performed chromatin immunoprecipitation assay to evaluate histone H3 acetylation and H3K4 methylation in the two tissues because the two epigenetic marks are consistently associated with gene activation. As a result, CGI-1 including the promoter was highly modified in both tissues whereas CGI-2 was enriched in histone H3 acetylation and H3K4 methylation only in the granulosa cell. These findings suggest that CGI-2 is a novel regulator for POP expression. We then investigated the effect of reproductive hormones on POP expression. We treated primary granulosa cells with progesterone (P4), 17beta-estradiol (E2), follicle stimulating hormone, and luteinizing hormone. Our preliminary results indicated that POP might be down-regulated by P4 in the granulosa cell. This implies that POP expression is tightly controlled in the ovary during the estrous cycle.

Nothing to Disclose: SM, APK

P3-109

Ovarian Gene Expression after Melatonin Supplementation on the Adult Female Rats.

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Objective: the aim of this study was to evaluate the ovarian gene expression after melatonin supplementation on the adult female rats. **Design:** Thirty female rats (*Rattus norvegicus albinus*) with normal estrous cycle were divided into two treatment groups: G1- control that received vehicle (n=15); GII - experimental that received melatonin supplementation (10 µg/animal), during consecutive 60 days. After that, all animal were sacrificed under anesthesia and the ovaries were removed and prepared for RNA extraction and the samples were submitted to cDNA microarray procedure using the Kit GeneChip® Rat Genome 230 2.0 Array of Affymetrix, following the manufacture instructions. The procedures were repeated three times. The results were normalized and confirmed by GeneChip® Operating software (Affymetrix Inc., Santa Clara, CA, USA) and NA-Chip Analyzer (dChip) software (www.dchip.org). We considered as positive or negative, when the data of experimental group were three times different than control one. **Results:** 80 and 12 genes of the experimental were up and down regulated, compared to control group, respectively. In relation to steroidogenesis, the pregnancy-zone protein (PZP) were down regulated and dual specificity phosphatase 1 (DUSP1), luteinizing hormone/choriogonadotropin receptor (LHCGR), gonadotropin releasing hormone receptor (GNRHR) were up regulated. **Conclusions:** Our results suggested that melatonin supplementation interfered with ovarian gene expression of normal rats and may influence the expression of hormonal receptors on the ovarian tissue, mainly the LH and GnRH receptors.

Nothing to Disclose: CCM, MAH, GARM, SAYH, CSF, RSS, ECB, JMS

P3-110

Characterization of Two DNase I Hypersensitive Sites at the Mouse *Amhr2*/MISIIR Gene Locus.

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Anti-Mullerian hormone/Mullerian inhibiting substance (AMH/MIS), which belongs to the transforming growth factor β (TGF- β) superfamily, is responsible for regeneration of the Mullerian ducts during male sex determination. It is also synthesized in females and involved in the regulation of early follicular development. AMH/MIS signals via two receptors. While the type I receptor could be shared with another member of TGF- β family, the type II receptor (*Amhr2*/MISIIR) is specific for AMH/MIS. In adults, the *Amhr2*/MISIIR is exclusively expressed in testicular Sertoli and Leydig cells and ovarian granulosa cells. This specific expression has been reported to be regulated by some transcription factors such as SF-1 and WT1 binding to the proximal promoter. However, several lines of evidences suggest the existence of other control elements distal to the promoter. To identify novel regulatory sequences for the *Amhr2*/MISIIR gene, I searched for DNase I hypersensitive sites (HS) at the mouse *Amhr2*/MISIIR locus. This is based on the observation that many cis-elements crucial for gene expression coincide with the presence of DNase I HS. I examined the chromatin from granulosa and hepatic cells by Southern blot analysis and found two DNase I HS at the *Amhr2*/MISIIR locus. One (HSII) is located 3kb upstream to the gene and the other (HSI) within the intron 6. HSI and HSII are present in both the ovary and liver and the HSII sequence is conserved between mouse and human genomes. Interestingly, our in vitro reporter analysis demonstrated that HSII could enhance the *Amhr2*/MISIIR promoter activity only in the granulosa cell and HSI could repress it in fibroblast and hepatic cells. Epigenetic patterns also implied the importance of HSII in the ovary and HSI in other tissues. Chromatin immunoprecipitation assay showed that histones at the HSII region were highly acetylated in the granulosa cell but not in the liver while HSI was acetylated in the liver but not in the ovarian cell. These results support the model that HSII works as an enhancer for the *Amhr2*/MISIIR gene in the granulosa cell and HSI suppresses the promoter activity in the tissue not expressing the gene.

Nothing to Disclose: APK

P3-111

Glucocorticoids Upregulate Brain Aromatase Utilizing Promoter I.f.

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Estradiol (E₂) biosynthesis is dependent on aromatase, which catalyzes conversion of C₁₉ steroids to estrogens. Within the neuroendocrine system, aromatase and E₂ are crucial for regulation of gonadotropin secretion and libido in adults. The regulation of aromatase expression in the brain is not well understood. The Cyp11a1 (aromatase) gene is selectively expressed in distinct neurons of the hypothalamus through a distal brain-specific promoter I.f located ~40 kb upstream of the coding region. Here, we investigated the effect of glucocorticoids on aromatase mRNA expression in embryonic hypothalamic neuronal lines that express aromatase via promoter I.f. We found that dexamethasone induced aromatase mRNA expression by as much as 10-fold compared to vehicle in a dose-dependent manner. This glucocorticoid induction of aromatase mRNA was time dependent, increasing as early as 3 hrs, peaking at 8-12 hrs, and continuing for up to 48 hrs. Glucocorticoid antagonist RU486 inhibited this induction. Furthermore, knockdown of glucocorticoid receptor (GR) by siRNA abolished glucocorticoid-induced aromatase mRNA induction at all times. These results indicated that glucocorticoids regulate aromatase expression in hypothalamic neurons through GR. We created a series of deletion mutants of aromatase promoter I.f in a luciferase reporter system. We identified a 300-bp region (-500/-200) that is required for glucocorticoid-induced promoter activity in mouse hypothalamic neurons. We confirmed increased GR binding to this region of promoter I.f following glucocorticoid treatment using chromatin immunoprecipitation (ChIP). Taken together, glucocorticoids induce aromatase expression via GR and the distal promoter I.f region of the Cyp11a1 gene in a murine hypothalamic cell line.

Nothing to Disclose: DCB, BY, HZ, SEB

P3-112

Regulation of Translocator Protein 18-kDa (Tspo) Gene Expression through a Protein Kinase C ϵ (PKC ϵ)-MAPK Signal Transduction Pathway Targeting STAT3 and c-Jun.

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Translocator protein, TSPO, is an 18-kDa high affinity cholesterol- and drug-binding mitochondrial protein implicated in numerous cell functions. TSPO is highly expressed in secretory and glandular tissues, especially in steroid synthesizing cells where it has been shown to play a determining role in the transport of cholesterol into mitochondria, the rate-limiting step in steroidogenesis. The factors regulating Tspo expression in the rich-in-TSPO steroidogenic cells are unknown. We analyzed Tspo transcriptional responses to the tumor promoter, phorbol-12-myristate 13-acetate (PMA), in cells with varying TSPO levels. PMA, through a PKC dependent pathway, induced Tspo promoter activity, TSPO mRNA and protein levels in poor-in-TSPO non-steroidogenic NIH-3T3 fibroblasts and COS-7 kidney cells, but not in rich-in-TSPO steroidogenic MA-10 Leydig cells with high basal Tspo transcriptional activity. We showed that high levels of TSPO in steroidogenic cells might be due to high constitutive expression of protein kinase C ϵ (PKC ϵ) whereas PMA activation of PKC ϵ drives TSPO expression in non-steroidogenic cells, likely through activator protein 1 (AP1). The MEK1/2 specific inhibitor U0126, but not NF κ B inhibitors, reduced basal Tspo promoter activity in MA-10 Leydig cells, as well as basal and PMA-induced Tspo promoter levels in NIH-3T3 fibroblasts identifying the mitogen-activated protein kinase (MAPK) ERK1/2 as the signal transduction pathway through which PKC ϵ regulates Tspo gene expression. AP1 and signal transducer and activation of transcription 3 (STAT3) have binding sites in the Tspo promoter and are downstream targets of PKC ϵ and MAPK (Raf-ERK1/2) pathways. PKC ϵ overexpression induced STAT3 phosphorylation in NIH-3T3 cells, while PKC ϵ knockdown reduced STAT3 phosphorylation in Leydig cells. MEK1/2, ERK2, c-Jun, and STAT3 knockdown reduced Tspo mRNA and protein levels in Leydig cells. MEK1/2, c-Jun, and STAT3 knockdown also reduced basal as well as PMA-induced Tspo mRNA levels in NIH-3T3 cells. Taken together these results demonstrate that PKC ϵ regulates Tspo gene expression through a MAPK (Raf-1-MEK1/2-ERK1/2) signal transduction pathway, acting at least in part through c-Jun and STAT3 transcription factors.

Sources of Research Support: ES07747.

Nothing to Disclose: AB, VP

P3-113

Circadian Patterns of Peripheral Blood Gene Expression.

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OBJECTIVE: Circadian rhythms are central to many physiologic functions. The mechanisms underlying rhythmicity have been elucidated mostly in rodents, in which the suprachiasmatic nucleus appears partly to orchestrate these patterns, while some are intrinsic. In humans, the circadian pattern of cortisol secretion suggests another possible synchronization mechanism. While administration of glucocorticoids has been shown to alter CLOCK genes in peripheral blood mononuclear cells, relatively little is known about circadian rhythms in non-stimulated cells.

METHODS: After IRB approval and informed consent, blood was drawn from 7 healthy inpatient volunteers, using PAXgene™ Blood RNA tubes, every 4 hours over 24 hours, from 0600h to 0200h. Samples were inverted ten times and incubated at RT for 2 hours, before storage at -80 °C and subsequent RNA extraction (PreAnalytix) and storage at -80C. After hemoglobin mRNA reduction, 1 mcg samples were interrogated by the Human Genome U133 Plus 2.0 Array (Affymetrix Inc., Santa Clara, CA, USA). Results were analyzed with a program to determine a sine wave amplitude in levels over the day, using a 1.58-fold difference as a cut-off value, followed by GeneGo analysis.

RESULTS: 474 gene probe sets had a sine wave periodicity pattern with 10% FDR; 226 gene probe sets peaked at 0200h (fold change to 2.13), and 49 peaked at 1000h (fold change to 1.9). GeneGo analysis of the 1000h group showed genes involved in cytoskeleton (HB-EGF, TALIN, CTTN, CLDN5, MYL9, ITGA2B), cell shape and matrix synthesis (SPARC, CTBP2), apoptosis/endocytosis (REPS2, CLU) and metabolism (ALDOB). The 0200h peak group included genes involved in Rab protein trafficking (CHM, CHML, RABGGTB), protein translation (RPL17, RPL9, RPS6KJB1, EIF1A), nuclear transport (KPNA5, NUP54), Ras activation (RASGRP1, N-RAS), cellular metabolism (ACSL6, PRKACB), antigen presentation (HLA-DMB, HLA-DQB, HLA-DRA) and T cell responses (TRAT1, ICOS, IL6ST, JAK2). Compared to the 0200h values, the CLOCK gene *Per1* was 1.2 fold increased at 0600h, *CRY1* was 1.5 fold decreased at 1400h. A glucocorticoid response element is present in the promoter region of all genes except the *CHEM*, *EIF1A*, *PPM2C*, *CTBP2*, and *ALDOB*.

CONCLUSIONS: The circadian pattern of gene expression in PBMC seen here at 0200h and 1000h is compatible with the hypothesis that changes in endogenous cortisol synchronizes these rhythms.

Nothing to Disclose: QW, MS, NR, XX, PM, LN

P3-114

Regulation of MUC1 Expression by Activated EGFR and PPAR γ in Pancreatic Cancer Cell Lines.

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Pancreatic cancer is the fourth leading cause of cancer death in males and females in the United States with a median overall survival rate of 2-5 months and a 5-year survival rate of 3-5%. Many epithelial tumors, including pancreatic cancer, overexpress the high molecular weight, transmembrane mucin MUC1 on the cell surface. MUC1 has three distinct domains: an extracellular domain (ECD) composed of a tandem repeat region rich in serine, threonine and proline; a transmembrane (TM) and a short cytoplasmic tail (CT) domain. Alternate splicing of MUC1 mRNA gives rise to MUC1 isoforms lacking either the transmembrane and cytoplasmic domains (MUC1/SEC) or the tandem repeat region (MUC1/Y). Overexpression and aberrant glycosylation of MUC1 makes tumor cells poorly adherent, increases drug resistance and promotes metastasis. Two independent approaches were used to attempt to reduce MUC1 expression in pancreatic cancer cell lines using drugs that activate Peroxisome Proliferator Activated Receptor- γ (PPAR γ), or inhibit the Epidermal Growth Factor Receptor (EGFR) tyrosine kinase activity. EGFR activation by Epidermal Growth Factor (EGF) modestly stimulated (1.5-2 fold) MUC1 expression in a human pancreatic adenocarcinoma cell line, HPAF II, while treatment with EGFR tyrosine kinase inhibitor, AG1478, reduces basal and stimulated MUC1 protein expression. Interestingly, Western blotting with an antibody directed against the MUC1 cytoplasmic tail (CT) that recognizes all cell associated forms of MUC1 differed markedly from blotting with a MUC1 antibody that recognizes an epitope in the MUC1-ECD. Specifically, inhibition of EGFR tyrosine kinase activity with AG1478 resulted in an increase in cell-associated MUC1-ECD reactive species whereas CT-reactive species were almost completely absent. Furthermore, preliminary studies with the PPAR γ agonist, rosiglitazone, demonstrated a marked (>90%) reduction of EGFR-driven MUC1 expression detected by both ECD and CT antibodies. Currently, we are examining the bases for differences in the ECD and CT reactivity in response to AG1478 as well as the molecular basis of EGFR stimulation of MUC1 expression using a combination of biochemical assays to detect differences in MUC1 glycoforms, mRNA splice variants and identification of the transcriptional pathways activated by EGFR. Understanding these biochemical and molecular mechanisms to reduce MUC1 expression may offer novel therapeutic avenues to improve cancer chemotherapies.

Sources of Research Support: Rice University Funding to Daniel D. Carson.

Nothing to Disclose: ND, PW, DDC

P3-115

Evaluating Protein Transduction as a Method of Cellular Reprogramming with Exogenous Transcription Factors.

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Background. The in vitro generation of patient-specific, therapeutic endocrine cells (such as insulin-secreting cells for the treatment of type I diabetes) requires technologies to reprogram an available cell type (such as skin fibroblasts) into the therapeutic cell type (such as beta-cells). Transcription factors that regulate cell lineage-specifying genes are promising molecular agents for such reprogramming. Overexpression of embryonic stem cell transcription factors SOX2 and OCT4 can reprogram fibroblasts into induced pluripotent stem cells, which have the potential to differentiate into nearly all tissue types. Protein transduction, which involves the use of cell-penetrating peptides (CPP's), is a promising approach for introducing exogenous transcription factors into cells in reprogramming applications.

Objectives. The purpose of our study is to prepare CPP-coupled SOX2 proteins for reprogramming cultured cells, evaluate the proteins' effectiveness as transducible transcription factors, and test conditions for improving their protein transduction efficiencies.

Methods. Two SOX2 fusion proteins with a polyarginine CPP, 9R-SOX2 and SOX2-9R, were prepared as recombinant proteins from bacteria. HeLa cells were transfected with an OCT4-overexpressing plasmid and a reporter plasmid expressing luciferase under the control of an enhancer from Fgf4 gene. The cells were incubated under various conditions with different concentrations of either SOX2 fusion protein added directly to the culture medium, and assayed for luciferase activity.

Results. In the presence of overexpressed OCT4, transduction with 20 nanomolar exogenous 9R-SOX2 protein activated the luciferase reporter in chloroquine pre-treated HeLa cells at 77% the level achieved by providing the SOX2 in the form of an overexpressing plasmid. Transduced SOX2-9R exhibited a comparable level of activity. Co-transduction with an HIV TAT domain-influenza HA2 fusogenic peptide greatly enhanced the reporter activating activity of 9R-SOX2 or SOX2-9R at lower protein concentrations.

Conclusions. We prepared two forms of transducible SOX2, measured their gene-activating activity using a HeLa cell reporter system, and identified conditions that enhanced their ability to activate transcription as exogenously supplied factors. Studies like this can help tailor the design and transduction conditions for each transducible transcription factor in therapeutic cellular reprogramming applications.

Sources of Research Support: NIH NIDDK, Juvenile Diabetes Resesarch Foundation.

Nothing to Disclose: PPL, KF

P3-116

Insulin-Like Growth Factor Binding Protein-3 (IGFBP-3) Improves Vascular Permeability by Reducing Acid Sphingomyelinase (ASM).

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Purpose IGFBP-3 has robust vascular protective effects including its ability to stimulate recruitment of endothelial progenitor cells (EPCs) (Kielczewski Circ Res 2009); however, the molecular mechanisms that mediate this effect are unclear. Since retinal injury activates ASM, a key inflammatory enzyme that results in ceramide generation and regulates permeability, we asked if IGFBP-3's protection of the blood retinal barrier (BRB) was mediated by reducing ASM.

Methods: Mice (n=18) underwent laser occlusion of retinal vessels and intravitreal injection with IGFBP-3-expressing or control plasmids (n=18) followed by sacrifice at 4 days post injection, time of peak IGFBP-3 expression. ASM mRNA was determined by qRT-PCR and activity by fluorescence. Immunohistochemistry was performed on retinal flatmounts for tight junction (TJ) proteins, occludin and VE-cadherin. In a second group of animals, vascular permeability was induced by intravitreal injection of VEGF (100 ng/μl). This was followed by intravitreal IGFBP-3 (non IGF-binding mutant)(100 ng/μl) given at 6 hrs (n=18) and 24 hrs (n=18) post VEGF injection. Intravitreal PBS served as control (n=18). Mice received a tail injection of FITC-albumin 2 hrs prior to sacrifice and retinas were harvested 48 hrs following initial VEGF injection. *In vitro* permeability across bovine retinal endothelial cells was assessed by measuring transepithelial resistance and fluorescent transendothelial flux over a 24 hr period.

Results: In mice receiving laser and control plasmid, ASM mRNA levels were elevated 4-fold compared to controls (p<0.05). However, in mice receiving laser and IGFBP-3 plasmid, a 4 fold decrease in ASM mRNA levels was observed (p<0.05), accompanied by a reduction in vascular permeability. ASM activity was decreased in IGFBP-3-treated eyes and this was accompanied by increased TJ staining. At 48 hrs, VEGF and IGFBP-3 together in uninjured mouse eyes resulted in a 1.5-fold reduction in retinal vascular permeability (p<0.05) compared to VEGF alone. However, *in vitro* IGFBP-3 stimulated increased permeability after 5 min exposure and to even a greater degree than VEGF alone, but by 24 hrs permeability was back to baseline.

Conclusion: IGFBP-3 dramatic effects on the BRB may be due to reduction of ASM. While IGFBP-3 increases permeability acutely, this may serve to facilitate leakage of serum factors for EPC recruitment. Chronically IGFBP-3 restores BRB function and stabilizes vessels following injury.

Sources of Research Support: NIH grants 2R01 EY012601-08 , 2R01 EY007739-17, R01 EY018358.

Nothing to Disclose: MBG, JLK, LW, JC, LCS, SLC, SMF, RCB, XQ, MEB

P3-117

IGF Binding Protein-3 Exerts Its IGF-Independent Action by Antagonizing BMP Action In Vivo.

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IGF binding protein (IGFBP)-3 is a secreted protein that binds and modulates IGF actions in extracellular environments. IGFBP-3 also has ligand-independent actions in cultured mammalian cells. In addition to its IGF binding domain (IBD), mammalian IGFBP-3 has a functional nuclear localization signal (NLS) and transactivation domain (TA). The specific roles of these structural domains of IGFBP-3 in mediating its *in vivo* actions are unclear. Here we show that these domains are conserved in zebrafish IGFBP-3. To study the functional roles of these domains, several zebrafish IGFBP-3 mutants were engineered. The IBD mutant failed to bind IGF-I but had normal nuclear localization. The NLS mutant had greatly reduced nuclear localization but showed normal IGF binding. The IBD+NLS double mutant had impaired IGF binding and nuclear localization. We also determined critical residues in the TA domain in IGFBP-3 and engineered a TA dead mutant. When expressed in zebrafish embryos, wild-type IGFBP-3, NLS, and TA mutants had strong dorsalizing effects. The activities of the IBD mutant and the IBD+NLS mutant were significantly lower, suggesting that IGFBP-3 acts in IGF-dependent and -independent manners and that the IGF-independent action is not related to its nuclear localization or transactivation activity. Since the phenotypes caused by IGFBP-3 expression resemble those of *bmp2b* mutants, we co-expressed zebrafish *Bmp2b* with IGFBP-3 and the IBD mutant. Both IGFBP-3 and IBD reduced the ventralizing effect of *Bmp2b* and abolished the *Bmp2a*-induced target gene expression *in vivo*. Furthermore, addition of human IGFBP-3 with BMP-2 into cultured cells inhibited BMP-2 actions, suggesting that IGFBP-3 exerts IGF-independent actions by antagonizing BMP signaling and this mechanism is evolutionarily conserved.

Sources of Research Support: University Innovation Project Seed Grant 070742, Chinese Ministry of Education and by US NSF Grant IOB 0110864.

Nothing to Disclose: YZ, JZ, YL, LL, DRC, CD

P3-118

Involvement of Insulin-Like Growth Factor Binding Protein-3 in the Effects of the Histone Deacetylase Inhibitor MS-275 in HepG2 Hepatoma Cells.

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Insulin-like growth factor binding protein-3 (IGFBP-3) expression is suppressed in some liver cancers, and can be reactivated by histone deacetylase (HDAC) inhibition. This study examines the role of IGFBP-3 in mediating effects of the HDAC inhibitor MS-275 in liver cancer cells, and identifies IGFBP-3-dependent proteins involved in regulation of proliferation and migration. In HepG2 cells, MS-275 inhibited DNA synthesis, cell cycle activity, and cell viability. These effects occurred concomitantly with increased binding of acetylated histone H3 to IGFBP-3 promoter sequences, and induction of IGFBP-3 expression. Downregulation of IGFBP-3 expression by siRNA significantly reversed the inhibition of cell viability and DNA synthesis by MS-275, indicating an intermediary role for IGFBP-3 in MS-275 effects. Induction of the cyclin-dependent kinase inhibitor p21 by MS-275 was attenuated by IGFBP-3 downregulation, explaining IGFBP-3-dependent effects of MS-275 on cell cycle activity. MS-275 stimulated HepG2 cell migration, and this effect was also inhibited by IGFBP-3 downregulation. MS-275-inducible genes showing IGFBP-3-dependence were sought using Affymetrix oligonucleotide arrays. Among genes induced at least 4-fold by MS-275 over 24 h, whose induction was attenuated at least 2-fold by IGFBP-3 downregulation, lymphatic vessel endothelial hyaluronan receptor-1 (LYVE1) and thrombospondin-2 (THBS2) were identified as possible mediators of IGFBP-3-dependent effects of MS-275. Downregulation of either LYVE1 or THBS2 by siRNA had no effect on the inhibition of HepG2 viability by MS-275, but reversed the stimulatory effect of MS-275 on cell migration. We conclude that among genes upregulated by MS-275, IGFBP-3 is a key mediator of effects on hepatoma cell growth and migration, and that these effects are likely to depend on the IGFBP-3-dependent proteins p21, for cell growth, and LYVE1 and THBS2, for cell migration. The enhanced cell motility that accompanies reactivation of IGFBP-3 expression in liver cancer by HDAC inhibition may be associated with an increased propensity for metastatic spread despite inhibited cell proliferation.

Nothing to Disclose: WHL, JLM, DJM, MMJ, RCB

P3-119

Global Deletion of IGFBP-2 Disrupts Hematopoiesis and Compromises Bone Marrow Engraftment in Lethally Irradiated Mice.

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IGFBP2, a transport binding protein for IGF-1, is an essential factor for *in vitro* propagation of human and mouse hematopoietic stem cells. We hypothesized that global deletion of IGFBP2 (BP2) might disrupt steady-state hematopoiesis and interfere with the engraftment of bone marrow donor cells. To test this hypothesis, peripheral blood and spleen cells from male (M) and female (F) WT and BP2^{-/-} mice were first analyzed by flow cytometry for the major white blood cell (WBC) populations. In peripheral blood, BP2^{-/-}F-mice had significantly lower CD3⁺ T cells compared to WT-F and BP2^{-/-} M (p=0.02) while BP2^{-/-}M had significantly increased CD3⁺ T cells (p=0.03), CD19⁺ B cells (p=0.00), F480⁺ macrophages (p=0.00), CD11b⁺ monocytes (p=0.00) and LY6G⁺ neutrophils (p=0.02) compared to both WT-M and BP2^{-/-}F. Intraperitoneal injection of IGFBP-2 heparin binding domain (HBD) peptide reduced the percentage of macrophages (p=0.01) and neutrophils (p=0.02) in peripheral blood of BP2^{-/-} M compared to PBS injected controls. BP2^{-/-}F splenic CD3⁺ T cells were increased (p=0.0) and CD19⁺ B cells were decreased (p=0.05) compared to WT-F, WT-M and BP2^{-/-}M. There was a significant increase in B220⁺ B cell progenitor cells in the spleens of BP2^{-/-} M compared to WT-M but no difference in the percentages of all other mature WBC populations. We next performed bone marrow transplants into lethally irradiated WT and BP2^{-/-} male recipients. In non-competitive transplants, the efficiency of donor engraftment was the same for both strains as $\geq 75\%$ of the repopulating cells in both WT and BP2^{-/-} were of donor origin (Ly5.1⁺). In competitive repopulation transplants, support donor cells from LY5.1⁺ mice were mixed with competitor donor cells from either WT or BP2^{-/-} in 2:3 and 1:3 ratios and transplanted into strain specific recipients. At 8 weeks post transplant, WT recipients in which 2/3 of the donor cells were WT (LY5.2⁺), 75% of bone marrow cells were of WT origin (LY5.2⁺) while in BP2^{-/-} recipients in which 2/3 of the donor cells were from BP2^{-/-} (LY5.2⁺) only 56% of bone marrow cells were LY5.2⁺ at 8 weeks post transplant representing 20% less (p=0.01) engraftment of BP2^{-/-} donor cells compared to WT. Future experiments will seek to rescue this deficiency with IGFBP2 HBD peptide. These data confirm that IGFBP2 may be an important regulator of the hematopoietic stem cell niche and support further investigation into the clinical importance of IGFBP2 in bone marrow transplantation.

Nothing to Disclose: ACB, MK, EG-D, DC, CJR

P3-120

Targeted Overexpression of IGFBP-6 in Osteoblasts Results in Impaired Bone Formation and Fracture Repair in Mice.

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We previously reported that IGFBP-6 (BP-6) inhibits osteoblast (Ob) marker gene expression and mineralization *in vitro* via an IGF-independent intracellular mechanism involving Lim Mineralization Protein (LMP)-1. Conversely siRNA knockdown of BP-6 mRNA and protein in MC3T3-E1 cells rapidly increased Ob marker gene expression. While our *in vitro* studies provide compelling evidence that BP-6 plays an important inhibitory role in Ob differentiation, the role of BP-6 on bone formation *in vivo* has not been established. To address this issue, transgenic (Tg) mice were generated with Ob targeted human (h)BP-6. The hBP-6 transgene was placed under the control of an Ob lineage restricted promoter that contained the proximal hCol1a2 promoter and a modified Ob enhancer from the hRunx2 promoter. Multiple founders were backcrossed to reduce effects of genetic background differences and establish independent Tg lines. hBP-6 mRNA was expressed in bones and calvarial cells from hBP-6 Tg mice at levels equivalent to or lower than endogenous BP-6 suggesting a moderate (2 fold) increase in skeletal tissue BP-6 levels. IHC revealed hBP-6 in preObs, Obs and osteocytes. Skeletal parameters were measured longitudinally in hBP-6 Tg and WT littermate controls by DEXA and micro-CT. Areal BMD was significantly reduced in the femurs of male (7%, $p < 0.01$) and female mice (14%, $p < 0.002$) in independent lines at 12-16 weeks. BP-6 Tg mice did not display differences in body weight or femur length compared to WT controls, suggesting that BP-6 does not influence the growth promoting effects of the IGFs that have been observed in other IGFBP-Tg mice. Micro-CT analysis of the distal metaphysis of Tg mice revealed a 30% ($p < 0.01$) reduction in bone volume fraction compared to WT. Because we found that BP-6 levels were decreased during fracture healing and LMP-1 overexpression promoted fracture healing, we proposed that moderately increased BP-6 expression in Obs would impair fracture healing in BP-6 Tg mice. Femur fractures were produced in BP-6Tg and WT mice by three-point bending. Assessment of fracture callus area and bone mineral content (BMC) at 17 days post fracture revealed a 40% ($p < 0.02$) reduction in callus size and a 30% reduction ($p < 0.01$) in BMC. Our data on BP-6 effects on Ob differentiation and bone formation *in vitro* and *in vivo* suggest that BP-6 is a negative regulator of bone formation that acts via an IGF-independent mechanism.

Nothing to Disclose: DDS, CR, CAS, MR, RT, JW, TAL, SM, CS, CdR

P3-121

Up-Regulation of IGF Binding Protein-6 by Hypoxia and Its Novel Role in Angiogenesis.

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Hypoxic stress causes metabolic and growth changes in all organisms requiring oxygen. It is also widely recognized as an important driving force for tumor angiogenesis by induction of many angiogenic factors. Although there are numerous reports on the expression of insulin-like growth factor (IGF) ligands, receptors, and binding proteins (IGFBPs) in the vascular tissues, their roles in angiogenesis and their relationship to hypoxia are not clear. In this study, we report that hypoxia up-regulates IGFBP-6 gene expression in vascular endothelial cells (VECs) and that the elevated IGFBP-6 inhibits angiogenesis in vitro and in vivo. IGFBP-6 is a high affinity binding protein for IGF-2 and regulates IGF-2 activity. IGFBP-6 also has IGF-independent activities. When cultured rat brain endothelial cells and human umbilical vein endothelial cells (HUVEC) were subjected to hypoxia (1% O₂), IGFBP-6 mRNA levels increased significantly. No significant change was detected in the levels of IGF-1 receptor and other IGFBPs. This hypoxic induction of IGFBP-6 expression is likely to be mediated by hypoxia inducible factor (HIF)-1, because CoCl₂, a compound that stabilizes HIF-1 α , caused a dose-dependent increase in IGFBP-6 mRNA expression. In vitro angiogenesis assay and cell migration assay results showed that addition of purified human IGFBP-6 to HUVEC inhibited angiogenesis in a dose-dependent manner. An IGFBP-6 mutant with impaired IGF binding had similar effect, suggesting this action is IGF-binding independent. These results suggest that induction of the anti-angiogenic IGFBP-6 may be part of a negative feedback loop in the hypoxia-induced angiogenesis. To further test this hypothesis in vivo, we expressed IGFBP-6 in flk1:GFP zebrafish embryos. flk1:GFP transgenic zebrafish exhibit green fluorescence in vascular endothelial cells, permitting visualization of the developing vascular system in vivo. Embryos overexpressed IGFBP-6 had significant lower number of inter somite vessels (ISV). This anti-angiogenic activity of IGFBP-6 appeared to be evolutionarily conserved because zebrafish IGFBP-6 had similar effects. Taken together, our results suggest that hypoxia up-regulates IGFBP-6 gene expression in VECs and the elevated IGFBP-6 inhibits angiogenesis via an IGF-independent mechanism.

Sources of Research Support: University Innovation Project Seed Grant 070742, Chinese Ministry of Education and by US NSF Grant IOB 0110864.

Nothing to Disclose: CZ, LL, XW, YL, PF, LB, CD

P3-122

The Role of IGFBP-3 on High Glucose Induced Apoptosis in Proximal Tubular Epithelial Cells.

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Purpose : Insulin-like growth factor binding protein-3(IGFBP-3), the major circulating carrier protein for IGFs, also acts as a potent antiproliferative agent, by blocking cell cycle and inducing apoptosis in various cell types⁽¹⁾. Recently, it was reported that IGFBP-3 mediates high glucose induced apoptosis in mesangial cells and podocytes⁽²⁾. Tubular cells are primary targets of hyperglycemia, and tubulointerstitial pathology, rather than glomerular pathology, correlates with renal dysfunction in diabetic nephropathy. This study was performed to investigate the role of IGFBP-3 on high glucose induced apoptosis in proximal tubular epithelial cells (PTEC).

Methods : IGFBP-3 expression in a PTEC line (LLC-PK1 cells) was measured by western blot, RT-PCR, and real time PCR, in low glucose (5.5mM) or high glucose (30mM) media. Apoptosis was quantified by Annexin V flow cytometry and DNA fragmentation ELISA. To investigate the role of IGFBP-3 on PTEC apoptosis, IGFBP-3 overexpressed PTEC line was established by transfection of IGFBP-3 cDNA. The effect of antioxidant and Poly(ADP-ribose) polymerases (PARP) inhibitor on IGFBP-3 expression was also evaluated to investigate the mechanism of increased IGFBP-3 expression by high glucose media.

Results: IGFBP-3 protein and mRNA expression was increased in high glucose media compared to low glucose media. Exposure to high glucose media for 72 hours increased PTEC apoptosis. High glucose induced PTEC apoptosis was prevented by preincubation with siRNA to IGFBP-3. IGFBP-3 overexpression increased PTEC apoptosis, which was also diminished by siRNA to IGFBP-3. High glucose media increased oxidative stress, but the siRNA to IGFBP-3 decreased oxidative stress. The increase in IGFBP-3 expression by high glucose media was prevented by N-acetyl-L-cysteine, an antioxidant or 3-aminobenzamide, a PARP inhibitor.

Conclusion : Our results suggest that increased IGFBP-3 expression may mediate PTEC apoptosis by high glucose. Increased IGFBP-3 expression in PTEC by high glucose seems to be related with increased oxidative stress and activation of PARP. Further investigation is required to verify the role of IGFBP-3 in the development of diabetic nephropathy.

(1)Jogie-Brahim S et al., Endocr Rev 2009;30:417

(2)Vasylyeva TL et al., Diabetes Res Clin Pract 2007;76:177

Nothing to Disclose: EGY, WJL, SEH, DHK, HSK

P3-123

Characterization of a Putative BH3-Like Domain Establishes IGFBP-3 as a Unique Intrinsic Pathway Activator That Integrates Cell Survival and Death Signals in the Mitochondrial Regulatory Network.

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In addition to other mechanisms, tumor suppression by IGFBP-3 occurs via direct apoptosis induction in vitro and in vivo. We have previously shown that IGFBP-3 activates the intrinsic apoptotic pathway via translocation of the nuclear receptors RXR α /Nur77 from the nucleus to the mitochondria. Additionally, we have demonstrated a functional nuclear export sequence in IGFBP-3 essential for its nucleo-cytoplasmic shuttling and apoptotic properties. This facilitates a mitochondrial interaction between IGFBP-3 and the pro-apoptotic Bcl-2 family member Bax during germ cell apoptosis. We hypothesized the existence of a BH3-like domain in IGFBP-3 between amino acid residues 100 and 114 based on sequence homology with a similar region in p53. Co-immunoprecipitation demonstrated IGFBP-3:Bax interaction in 22RV1 prostate cancer cells. Endogenous IGFBP-3 associated with higher order oligomeric Bax forms differentially present in nuclear versus cytoplasmic fractions. Large unilamellar vesicle release assays showed that co-incubation with IGFBP-3 increased the amount of Bax-induced permeabilization by 40%. The functional significance of IGFBP-3:Bax interaction was assessed by comparing HCT116 Bax wild-type and Bax null cells in a caspase 3/7 release assay. After 2.5 hours of incubation with 1 mcg/ml recombinant IGFBP-3 caspase 3/7 activity increased significantly in HCT116 Bax WT cells while Bax KO cells showed resistance to IGFBP-3 induced apoptosis. We created single and double BH3-like region mutants by conversion of residues 102 and 104 to alanine and functionally validated the significance of the BH3-like region for Bax, Nur77, and Humanin binding. These results establish IGFBP-3 as a unique activator of the mitochondrial intrinsic pathway, and a novel checkpoint in the mitochondrial regulatory network that integrates cell death and survival signals.

Sources of Research Support: Stein-Oppenheimer Foundation Lawson; Wilkins Pediatric Endocrinology Society; UCLA Prostate Cancer SPORE Career Development Award; National Institutes of Health Grant 2K12HD34610; Department of Defense award PC061077.

Nothing to Disclose: VP-V, JEC, KWL

P3-124

IGFBP3 Promoter Polymorphism -202A/C Associated with Postnatal Growth Pattern of Former Very Low Birth Weight Preterm Infants.

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Progress in neonatal intensive care medicine has improved survival and outcome of small preterm infants. Although the majority of these infants show catch-up growth, some remain short. Recently, we reported on a significant association of the GHRd3-isoform with postnatal catch-up growth of former very low birth weight preterm infants (JCEM 2007; 92, 4489-93). In this cohort (n=72; birth weight 350-1500 g) we now show a significant impact on postnatal growth for another common genomic variant, IGFBP3 -202A/C. Whereas auxological parameters at birth did not differ, length-SDS after correction for target height at a mean age of 6.0 yrs (range 4.2-8.0 yrs) were significantly higher in carriers of at least one -202A-allele (AA&AC -0.51 ± 1.02 SDS; CC -0.91 ± 0.91 SDS; $p=0.025$). Setting the threshold for catch-up growth to a current height-SDS of -1 SDS from parental target height, there was however a less clear discrimination (AA 86%, AC 68%, CC 67% children with catch-up growth, resp.) as compared to the GHR exon 3 isoform (d3/d3 100%, d3/fl 76%, fl/fl 53%). Follow-up of this cohort and the inclusion of further genomic variants will show to which extent polymorphisms in growth-related genes can explain the variability of postnatal growth in former very preterm infants.

Nothing to Disclose: FS, SS, RD, PB, BG, JW

P3-125

Novel Substrates of Insulin Receptor Isoform A Revealed by Unbiased Proteomic Analysis Using Stable Isotope Labeling by Amino Acid in Cell Culture.

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Mammalian cells express two insulin receptor isoforms (IR-A and IR-B), whose relative abundance is regulated by the development stage, tissue-specific factors and disease conditions. Studies addressing IR signaling have been usually performed in classical insulin target cells, which express both IR isoforms with a prevalence of the IR-B. It has been suggested, however, that the subtle structural differences showed by the two IR isoforms may account for important signaling differences.

Herein we applied a proteomic strategy based on stable isotope labeling aminoacid in cell culture (SILAC) in order to obtain an unbiased mapping of IR-A signalsome. To avoid the interference of the IGF-I receptor (IGF-IR), we used R-/IR-A cells, fibroblasts overexpressing the human IR-A and knocked-out for the IGF-IR. Cells labeled according to SILAC were stimulated with 10 nM insulin and phosphotyrosine enriched proteins subjected to LC-MS/MS analysis.

We identified 37 proteins as candidate IR-A substrates, which were then used to model a new IR-A substrate network by the STRING database. Only 12 of these substrates were known as insulin mediators while the remaining 25 were previously not directly related to insulin signaling.

Of these 37 substrates, 24 were positively regulated while 13 were negatively regulated by insulin. These novel IR-A mediators fell into the following functional categories: signal transduction (n=14), proliferation (n= 5), cell motility (n= 3), cytoskeleton rearrangement (n= 8), apoptosis (n=3), cell to matrix interaction (n= 4), cell transformation (n=2), DNA-repair (n=2), endocytosis (n=2), metabolism (n=3), angiogenesis (n=1), tyrosine kinase receptor (n=3).

The involvement of a subset of these mediators was validated by western blot.

In conclusion, we have identified novel IR-A signaling mediators. As IR-A is often aberrantly expressed in cancer, these data may help identifying new tumor biomarkers and molecular targets of anticancer therapies.

Sources of Research Support: Italian Association for Cancer Research (AIRC).

Nothing to Disclose: AM, MG, GP, GC, MRL, RV, AB

P3-126

The Mammalian Target of Rapamycin Complex-2 Modulates SGK1 Phosphorylation and Functional Activation.

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The serum- and glucocorticoid-induced kinase 1 (SGK1) plays a central role in hormone regulation of ENaC-dependent Na⁺ transport in the distal nephron. Its activity is well known to be tightly regulated by phosphorylation within a carboxy-terminal domain termed the hydrophobic motif (HM). However, until recently, the identity of the HM kinase was uncertain. We have shown that the mammalian target of rapamycin, mTOR, is essential for SGK1 HM phosphorylation and activation of ENaC. mTOR is organized into two distinct complexes, mTORC1 and mTORC2, which mediate widely divergent physiological effects. Our data show that mTOR in conjunction with rictor (mTORC2) phosphorylates SGK1 and stimulates ENaC. In contrast, when mTOR is assembled with raptor (mTORC1), it does not phosphorylate SGK1 or stimulate ENaC. In particular, mTOR inhibition by a small molecule competitive antagonist blocked both SGK1 HM phosphorylation and ENaC-mediated Na⁺ transport, while mTORC1-specific inhibition by rapamycin had no effect.

Furthermore, shRNA-mediated knockdown of rictor also inhibited SGK1 phosphorylation and Na⁺ current, while knockdown of raptor had no effect. In co-immunoprecipitation experiments, SGK1 interacted selectively with mTORC2, but not mTORC1. Truncation experiments localized this interaction to the N-terminal domain of SGK1, suggesting a molecular basis for specificity. mTORC2-dependent phosphorylation was found not only in ENaC-expressing kidney cells, but also in a variety of cell types of distinct tissue origins and of varying degrees of differentiation. We conclude that mTORC2 but not mTORC1 is the HM kinase for SGK1, possibly in all cell types. In ENaC-expressing cells, mTORC2 is required for SGK1-dependent activation of Na⁺ transport. These data provide new insight into the molecular mechanism(s) underlying the control of SGK1 activities, and in particular the signaling cascade required for the control of epithelial Na⁺ transport.

Nothing to Disclose: ML, JW, HI, L-JY, DP

P3-127

Rap1 Integrates cAMP Effector Pathways in Thyroid Cells.

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cAMP is a ubiquitous second messenger mediating a plethora of cellular functions. Particularly in the thyroid gland, it mediates the proliferative action of TSH. Both cAMP effectors, Epac1 and PKA, are required synergistically for TSH-mediated cell proliferation; however, the mechanisms involved in the integration of these effector pathways are largely unknown. We report here that the ERM member radixin complexes with both PKA and Epac1, thus delimiting a new functional compartment for cAMP action.

Epac1-radixin-PKA complex localizes in clusters present at a sub-membrane compartment. ERM binding domain (EBD) in Epac resides in its N-terminal 1-52 domain; (1-52)-dsRed localized in clusters indicating that Epac EBD is sufficient for proper targeting. Interestingly, a deletion D(1-52)-Epac protein is no longer associated with radixin in clusters but enriched in the nucleus. This result suggests that Epac-radixin interaction might be subjected to regulation, its dissociation unmasking a putative nuclear localization signal. Consistent with this, TSH stimulation induces a PKA-dependent translocation of Epac to the nucleus.

Dominant negative and shRNA approaches were utilized to disrupt Epac-radixin interaction; these reagents reduced cluster number accompanied by a concomitant inhibition of TSH mitogenic activity, indicating a critical functional role of this compartment in TSH biology. An shRNA resistant radixin construct was able to rescue TSH activity; however, when a L421P mutation disrupting its ability to bind PKA was introduced into this radixin construct, no rescue was observed. Thus, radixin-PKA interaction is strictly required for TSH biological action.

Rap1 is a substrate for both Epac1 and PKA; Rap1 could also rescue dominant negative and radixin shRNA-mediated inhibition, however, only in its constitutively active (GTP-bound) and phosphorylated state (i.e. G12V-S179D). This result is consistent with our previous observation that Rap activation and phosphorylation were required to efficiently transduce cAMP proliferative response.

We propose that radixin scaffolds both cAMP effectors in a functional cAMP-sensing compartment for efficient transduction, using Rap1 as a downstream signal integrator.

Sources of Research Support: NIH CA071649 to DLA.

Nothing to Disclose: DH, GB, FR-N, DLA

P3-128

Cooperative Regulation of Follicle-Stimulating Hormone β Subunit Transcription by SMADs and FOXL2.

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Activins selectively regulate follicle-stimulating hormone (FSH) synthesis by pituitary gonadotropes. We and others previously reported that activin A signals through SMAD proteins to induce FSH-beta (*Fshb*) subunit transcription, the rate limiting step in FSH synthesis (1, 2). More recently, we identified forkhead transcription factor FOXL2 as a critical mediator of activin A-regulated *Fshb* expression (3). Here, we close the loop by characterizing physical and functional interactions between SMADs and FOXL2. Ectopic FOXL2 expression conferred activin A responsiveness to the porcine *Fshb* promoter in heterologous cells (3). This effect was potentiated by co-expression of SMAD2, 3 or 4, which did not broker the activin A response on their own. The porcine *Fshb* promoter contains two *bona fide* FOXL2 binding elements (FBEs): a distal site (dFBE) unique to pig and a proximal site (pFBE) that is conserved across species (3). We demonstrated that SMAD3 and a SMAD2 splice-variant, SMAD2 ^{Δ exon3}, can bind a four bp SMAD binding element (SBE) juxtapose to and partially overlapping the dFBE, and that both FOXL2 and the dFBE are required for SMAD binding. We further observed that FOXL2 physically interacted with SMAD3 (see also ref. 4) and SMAD2 ^{Δ exon3}, though to a lesser extent. The murine *Fshb* promoter, which contains the pFBE, was regulated in a similar, but not identical manner. FOXL2 and SMAD2, 3 or 4 conferred activin A responsiveness to the murine reporter in heterologous cells, but only when co-expressed. This effect was significantly impaired by a mutation in SMAD3 (R74K) that impairs its DNA binding. In mouse, a four-bp SBE-like site is present juxtapose to the pFBE in an arrangement comparable to the dFBE and SBE elements in the pig promoter, but on the opposite strand. We have not yet been able to definitively demonstrate SMAD binding to this SBE-like site, even though mutating its component base-pairs impaired activin A or SMAD responsiveness. Mutation of the pFBE in the murine or porcine promoters caused a significant impairment of activin A or SMAD induction of reporter activity. Collectively, these and previous data suggest a model in which activin A induces the formation of SMAD complexes that accumulate in the nucleus where they interact with FOXL2 bound to at least one or two *cis*-elements in the proximal *Fshb* promoter. SMADs binding to adjacent SBEs stabilizes the complexes, facilitating induction of transcription.

- (1)Suszko MI et al., Mol Endocrinol 2005; 19(7):1849-1858
- (2)Lamba P et al., J Mol Endocrinol 2006; 36(1):201-220
- (3)Lamba P et al., Mol Endocrinol 2009; 23(7):1001-1013
- (4)Blount AL et al., J Biol Chem 2009; 284(12):7631-7645

Sources of Research Support: CIHR Grant MOP-89991 awarded to D.J.B.

Nothing to Disclose: PL, YW, DJB

P3-129

Nitric Oxide Signaling in the Endoplasmic Reticulum: Role of Caveolin 1, GRP94, and Calreticulin.

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Endothelial nitric oxide synthase (eNOS) can traffic between different intracellular organelles and its sub-cellular targeting can affect NO production. However, the molecular mechanisms involved in eNOS targeting are unresolved. We have shown that decreases in caveolin 1 correlate with reduced endothelial function in a mouse model of menopause. In this study, we explored the role of caveolin1 in regulating eNOS subcellular targeting. Utilizing SPOT technology and over-lapping caveolin 1 peptides we identified a novel region of caveolin 1 that binds eNOS. In order to determine the significance of this region in eNOS-caveolin-1 binding, bovine aortic endothelial cells were treated with either a TAT-tagged peptide comprising the identified region (TAT-CAV1) or a scrambled peptide (TAT-CAV1Scr). Our data indicate that TAT-CAV1 decreased eNOS-caveolin-1 interactions. This region encompasses the domain of caveolin 1 necessary for its exit from the endoplasmic reticulum (ER). We therefore next analyzed the effect of blocking eNOS-caveolin interactions on eNOS subcellular localization. Our data show that TAT-CAV1 results in eNOS accumulation in the ER and reduces its localization on the plasma membrane (PM). A concomitant increase in ER localized NO and a decrease in PM localized NO was also detected. The interaction of eNOS with calmodulin and HSP90 is well documented. In order to gain insight into the mechanism by which ER localized eNOS produces NO, its interaction with GRP94, an ER homologue of HSP90, and calreticulin, a calcium binding protein similar to calmodulin, was analyzed. The interaction of eNOS, with GRP94 and calreticulin was enhanced by TAT-CAV1. siRNA's targeted against GRP94 or calreticulin resulted in eNOS uncoupling corresponding to a reduction in ER localized NO and an increase in ER localized superoxide. Moreover, in vitro recombinant GRP94 or calreticulin potentiated NO production and attenuated superoxide production in human recombinant eNOS. In conclusion, our data demonstrate that eNOS traffics to the PM through its interaction with caveolin 1. Blocking this interaction results in eNOS accumulation in the ER, where eNOS activity is regulated by GRP94 and calreticulin. We speculate that the loss of caveolin 1 during menopause may lead to the redistribution of eNOS within the endothelial cell potentially altering ER protein function via enhanced S-nitrosylation.

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Nothing to Disclose: NS, SK, SMB

P3-130

Functional Interactions of Activin with PACAP and GnRH in Regulating Grass Carp LH beta Gene Transcription.

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Unlike mammals, in which activin is known to stimulate luteinizing hormone (LH) beta gene transcription, activin produced locally in the pituitary of fish models can inhibit LH transcript expression. However, the mechanisms for activin inhibition acting at the transcriptional level as well as its interaction with other hypothalamic releasing factors have not been investigated. Using grass carp as an animal model, we examined the transcriptional regulation of LH gene expression in fish by functional interactions of activin with gonadotropin-releasing hormone (GnRH) and pituitary adenylate cyclase-activating polypeptide (PACA). As a first step, the full gene of grass carp LH beta composed of two introns and three exons together with a 1.4 kb promoter was cloned by genome walking and the transcription initiation site was mapped by primer extension. The 1.4 kb promoter was subcloned into a luciferase-expressing pGL3 reporter for transfection studies in alpha T3 gonadotroph cell line. Using this cell model, activin treatment was shown to inhibit grass carp LH promoter activity through Smad2 and Smad3 activation. The inhibitory effect of activin, however, could be blocked by the activin-binding protein follistatin or by over-expression of Smad7, the feedback inhibitor for Smad2 and Smad3 activation. In contrast to activin, PACAP and GnRH were both effective in up-regulation of LH promoter activity via activation of the AC/cAMP/PKA and PLC/IP3/PKC pathways. Furthermore, the stimulatory effects of these two gonadotropin-releasing factors in fish could be suppressed or abolished by co-treatment with activin or by over-expression of Smad2 and Smad3, respectively. Similar inhibitory effects by activin and Smad2/Smad3 over-expression were also observed by substituting the membrane permeant cAMP analog for PACAP and GnRH as the stimulants for LH promoter activation. These results, as a whole, suggest that activin produced locally in the fish pituitary not only can suppress basal but also inhibit the stimulatory effect of PACAP and GnRH on LH gene transcription, presumably via Smad2/Smad3 inhibition of the stimulatory signals mediated through cAMP/PKA-dependent mechanisms.

Sources of Research Support: GRF grants from Research Grant Council (Hong Kong) to AOLW.

Nothing to Disclose: RSKF, CS, MH, AOLW

P3-131**Altered Insulin Processing by Hepatocytes from Insulin-Degrading Enzyme (IDE) Knockout (KO) Mice.**FG Hamel Ph.D.^{1,2}, GL Siford B.S.¹ and RG Bennett Ph.D.^{1,2}.¹Omaha Veterans Affairs Med Ctr Omaha, NE and ²Univ of Nebraska Med Ctr Omaha, NE.

IDE is a peptidase with high affinity for insulin, and is believed to be primarily responsible for the initiation of cellular degradation of insulin. We have used IDE KO mouse hepatocytes to examine the role of IDE in cellular processing and degradation of insulin. Hepatocytes were isolated by perfusion of the liver with Liberase. Cells were incubated with ¹²⁵I-iodoinsulin for up to 2h. Minimal degrading activity was found in the medium. At specific time points cells were separated from medium by centrifugation. Degradation was assessed by trichloroacetic acid precipitation of the medium. Cells were acid (pH 4) washed to separate surface insulin from internalized insulin. In additional experiments, insulin degradation products in the medium and cells were examined by reverse phase HPLC. Degraded insulin began to appear in medium after an approximately 15 min lag time, and proceeded linearly for 2 h. Figure 1 shows degradation by KO hepatocytes was about 75% that of wild type (WT). Binding of insulin (Figure 2) peaked at 30 min with WT cells and slowly diminished afterward. Binding by KO cells had a similar time course, but did not decrease out to 120 min and was 50% greater than in WT. Acid wash revealed all of the additional cell-associated insulin was due to accumulation of intracellular and not surface bound insulin. While HPLC analysis showed different product patterns for extracellular and intracellular insulin, no qualitative differences were seen between WT and KO. We conclude: 1) IDE is not the exclusive mechanism for the degradation of insulin in hepatocytes; 2) IDE does affect cellular processing of insulin; 3) the hyperinsulinemia in IDE knockout mice results from down regulation of receptors. Thus, the function of IDE may have significant effects on insulin action.

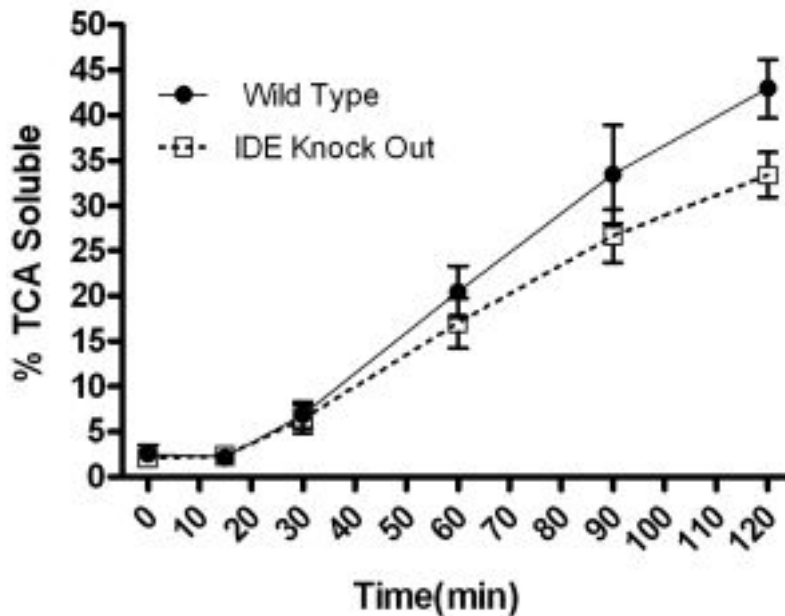
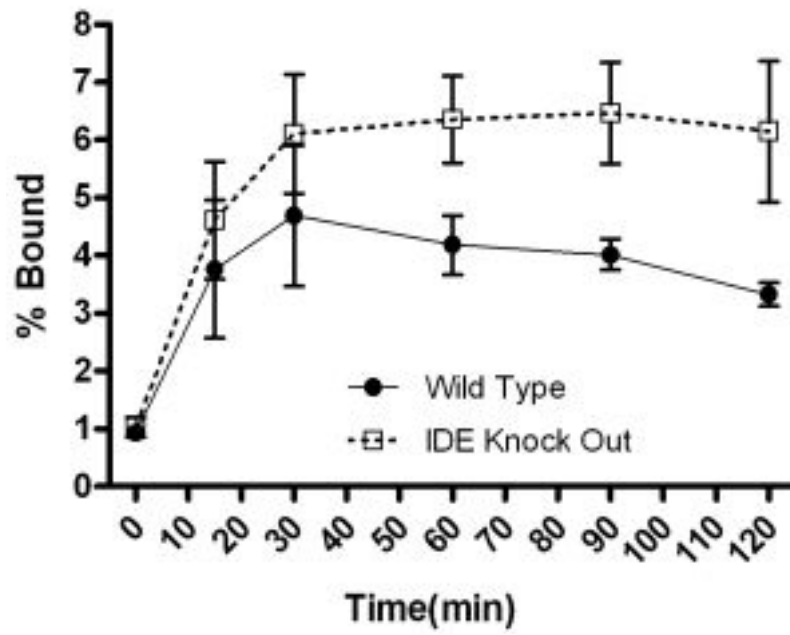
Figure 1. Insulin Degradation

Figure 2. Insulin Binding



Sources of Research Support: Department of Veterans Affairs Merit Review; Bly Memorial Research Foundation.

Nothing to Disclose: FGH, GLS, RGB

P3-132

Proteomic Identification of Novel FOXL2 Interacting Partners.

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FOXL2 is a member of the forkhead transcription factor family, with cell-specific expression in the ovary, pituitary, and developing eyelid. In murine pituitary gonadotrope cell lines, FOXL2 mediates activin-induction of gonadotropin-releasing hormone receptor (Gnrhr), follistatin (Fst), and follicle-stimulating hormone β subunit (Fshb) transcription. We previously demonstrated that ectopic FOXL2 expression confers activin A responsiveness to a porcine Fshb reporter gene in several heterologous cell lines. Activin A-regulated SMAD proteins physically and functionally interact with FOXL2 to induce Fshb transcription. However, the insufficiency of ectopic FOXL2 to confer activin-induction to Fshb in other (activin-responsive, SMAD-expressing) cell lines suggests that additional cell-specific factors contribute to the response. To address this possibility, we screened for novel FOXL2-interacting partners using tandem affinity purification (TAP) followed by liquid chromatography and tandem mass spectrometry (LC-MS/MS). Streptavidin and calmodulin-binding epitopes were fused to the N-terminus of murine FOXL2 (hereafter TAP-FOXL2). As with FOXL2 lacking the TAP tag, activin A stimulated porcine Fshb promoter activity in HEK293F cells transiently transfected with TAP-FOXL2. HEK293F cells were then stably transfected with TAP-FOXL2 and one stable line with FOXL2 protein expression comparable to that observed in homologous murine gonadotrope (L β T2) cells was selected for further characterization and analysis. TAP-FOXL2 protein was predominantly nuclear in its localization and could be co-immunoprecipitated with two known interactors, SMAD3 and FOXL2 itself, when co-expressed in these cells. Activin A stimulated porcine Fshb promoter-reporter activity in TAP-FOXL2, but not parental HEK293F, cells, and this response was abrogated with Foxl2 siRNAs, confirming the role of ectopically expressed FOXL2. TAP-FOXL2 and its interacting proteins were purified from unstimulated cells and identified by LC-MS/MS. We are currently confirming novel interactions and assessing their roles, if any, in activin A induction of Fshb in homologous and heterologous cells.

Sources of Research Support: ST and DJB are supported by fellowships from the FRSQ. The work was supported by operating funds from CIHR (MOP-89991).

Nothing to Disclose: ST, SA, DJB

P3-133

Modulation of the Inositol 1,4,5-Trisphosphate

Receptor-Dependent Ca²⁺ Wave Propagation Speed in Bovine Aortic Endothelial Cells.

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In recent decades, it has become evident that the endothelium is not only a passive inner lining of blood vessels, but is actively involved in vital functions of the cardiovascular system such as the modulation of arterial pressure and the maintenance of blood flow. Many of these functions are modulated by intracellular Ca²⁺ release from the endoplasmic reticulum via the inositol 1,4,5-trisphosphate receptor (IP₃R). Three isoforms of IP₃R (IP₃R-1, IP₃R-2 and IP₃R-3) have been identified and are expressed in different proportions among tissues. One way cells ensure the specificity and reliability of Ca²⁺ signals is by organizing them spatially in the form of waves that propagate throughout the cell or within a specific subcellular region. Using a videomicroscopic approach, we recently showed that the IP₃Rs are responsible for the initiation and propagation of agonist-induced repetitive Ca²⁺ waves in bovine aortic endothelial cells (BAEC) and that intact microtubule and microfilament networks are crucial for the propagation of these waves. In the present study, we focused on the speed of propagation of Ca²⁺ waves under different conditions that affect IP₃R function. When BAEC were stimulated with ATP via P2Y receptors, we observed that the waves speed depended on agonist concentration and was not significantly affected by the level of extracellular Ca²⁺. We measured an average speed of 17.3 ± 3.2 µm/s in response to 200 nM ATP (20.9 ± 1.8 µm/s in the presence of extracellular Ca²⁺), and 37.9 ± 4.9 µm/s in response to 1 µM ATP. Pretreatment of BAEC with 10 µM forskolin for 3 min, amplified the intracellular Ca²⁺ response to 200 nM ATP and increased the wave propagation speed to 26.3 ± 1.4 µm/s. At the opposite, pretreatment of BAEC with 2 µM PMA for 3 min decreased the wave propagation speed to 22.6 ± 1.1 µm/s in response to 1 µM ATP. A 5 min pretreatment of BAEC with 10 µM rapamycin, also decreased the wave propagation speed to 26.8 ± 2.5 µm/s in response to 1 µM ATP. These results show that the same conditions known to affect the amplitude of Ca²⁺ increases within BAEC also modulate the speed at which Ca²⁺ waves propagate. The speed of propagation of IP₃R-dependent Ca²⁺ waves may represent an important aspect of the wide diversity of spatiotemporal Ca²⁺ signals generated in living cells.

Sources of Research Support: Heart and Stroke Foundation of Quebec; Canadian Institutes of Health Research.

Nothing to Disclose: EB, GG

P3-134

Vasoinhibins Prevent Bradykinin-Induced Endothelial Cell Proliferation *Via* Regulation of Ca²⁺ Homeostasis and Nitric Oxide Production.

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Vasoinhibins are a family of peptides derived from prolactin that reduce excessive retinal vasopermeability associated with diabetes and that antagonize the vasorelaxing effect of bradykinin (BK), a factor found to be overactivated in vitreous of patients with proliferative diabetic retinopathy. As BK also promotes endothelial cell proliferation, we here study whether and how vasoinhibins could block BK-induced increase of proliferation of bovine umbilical vein endothelial cells (BUVEC). Vasoinhibins inhibit BK-induced activation of phospholipase C, inositol triphosphate (IP3) production, and Ca²⁺ release from endoplasmic reticulum. Neither prolactin nor an inactive mutant of vasoinhibins could reproduce these effects. Vasoinhibins also inactivate endothelial nitric oxide synthase (eNOS) by preventing its phosphorylation at Ser1179 through protein phosphatase 2A activation, since okadaic acid, a protein phosphatase 2A activator counteracts vasoinhibin effect on eNOS. Inhibition of both intracellular Ca²⁺ mobilization and eNOS activity may mediate the anti-angiogenic action of vasoinhibins, since EGTA and the NO donor DET-ANONOate prevent vasoinhibins from blocking BK-induced proliferation of BUVEC. Our data support the subsequent intracellular signaling pathway: vasoinhibins prevent BK-induced phospholipase C activation, IP3-mediated intracellular Ca²⁺ store release, eNOS activity, and thereby, BK promotion of BUVEC proliferation.

Sources of Research Support: Grants from DGAPA (IN202209-20).

Nothing to Disclose: ST, DA, CG, CG, LV, GDLE, CC

P3-135

A C-Terminal Fragment of Inositol 1,4,5-Trisphosphate Receptor Type 2 Forms a Leaky Ca²⁺ Channel.

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Inositol 1,4,5-trisphosphate receptors (IP₃Rs) are intracellular IP₃-gated Ca²⁺ release channels that play important roles in the regulation of numerous Ca²⁺-dependent cellular processes. Three isoforms of IP₃R (IP₃R-1, IP₃R-2 and IP₃R-3) have been identified and are expressed in different proportions among tissues. IP₃Rs consist of three domains: a ligand-binding domain, a regulatory domain and a membrane-spanning domain. The membrane-spanning domain has six transmembrane domains and a short hydrophobic loop between the fifth and sixth transmembranes, which constitute the Ca²⁺ pore. We have created a mutant IP₃R-2 (CTR2) containing the C-terminal portion of the protein up to the fifth transmembrane domain. Western blots revealed that CTR2 was efficiently expressed in HEK293 cells. Immunocytofluorescence studies revealed that CTR2 was exclusively expressed in the endoplasmic reticulum, with no significant staining at the nucleus. CTR2 decreased angiotensinII-induced Ca²⁺ release and ionomycin-induced Ca²⁺ release in HEK293 cells incubated in a Ca²⁺-free medium. Under the same conditions, CTR2 time-dependently increased the basal capacitative Ca²⁺ entry in HEK293 cells. Aspartate 2502 is known to participate to the selectivity filter of IP₃R-2, an essential feature for the transport of Ca²⁺. The mutant D2502A-CTR2 in which aspartate 2502 was substituted for an alanine, did not modify angiotensinII- or ionomycin-induced Ca²⁺ release. D2502A-CTR2 did not modify the basal capacitative Ca²⁺ entry in HEK293 cells. These results suggest that CTR2 is a constitutively open channel that causes a constitutive leak of Ca²⁺ from the endoplasmic reticulum.

Sources of Research Support: Heart and Stroke Foundation of Quebec; Canadian Institutes of Health Research.

Nothing to Disclose: AM, BC, GG

P3-136

Regulation of Preoptic Regulatory Factor-2 Expression by Insulin and IGF-1 Signaling Pathway.

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Preoptic regulatory factor-2 (PORF-2) is a putative growth regulating factor whose cDNA was originally found in the preoptic region of hypothalamus. PORF-2 mRNA is highly expressed in regions with continual cell divisions including hippocampus, skin, testis and placenta. Functional study of the gene by knocking down shows the proapoptotic and antiproliferative function of PORF-2 gene in neural stem cells. In vivo diabetic Zucker rat studies show PORF-2 declines in rats treated with antioxidant diet which decreases kidney damage compared to rats on regular diet. In addition, PORF-2 expression is down regulated by insulin and IGF-1. To further investigate the major intracellular pathways that affect PORF-2 expression, we used Fisher Rat Thyroid Cell Line-5 (FRTL-5). To study the effect of several kinase inhibitors on PORF-2 mRNA in the presence of 100 ng/ml IGF-1, we used LY294002 and Wortmannin to inhibit PI3 kinase and used Akt Inhibitor IV to block AKT, both of which are essential kinases downstream of insulin and IGF-1 receptor. We also used *Raf Kinase Inhibitor* to block Raf kinase and PD 98059 to block ERK, both of which are major kinases in another pathway downstream to the above receptors. The results show that LY294002 at 6 μ M reverses the effect of IGF-1 on PORF-2 expression while 1.2 μ M LY294002 partially reverses the effect. A similar dose response effect is seen with Wortmannin, which reverses IGF-1 effect at a concentration of 200 nM with partial blockage at 100 nM, and no effect was seen at 50 and 5 nM. The use of Akt Inhibitor IV at both 625 nM and 1.25 μ M only achieves partial blockage of IGF-1 effect. Raf kinase inhibitor at a concentration of 500nM partially inhibits the IGF-1 effect while PD 98059 at 25, 50, 100 and 200 μ M has no inhibitory effect. Taken together, these results indicate the effect of IGF-1 on PORF-2 expression is mainly regulated through the PI3K pathway with partial regulation through MAPPK pathway.

Sources of Research Support: Ohio University Diabetes Research Initiative.

Nothing to Disclose: ZW, FVN

P3-137

Oxidized LDL-Induced CREB Down-Regulation and Protein Oxidation in Vascular Smooth Muscle Cells.

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Purpose of Study: Cardiovascular disease is the leading cause of morbidity and mortality in the US and worldwide. A large void is present in our understanding of the molecular mechanism of atherogenesis. Of the many participants in atherosclerotic plaque formation vascular smooth muscle cell (VSMC) activation and oxidant generation are integral components. The transcription factor cAMP Response Element Binding Protein (CREB), is essential to maintenance of VSMC quiescence and the defense of cellular integrity. Oxidized LDL (oxLDL) exposure activates VSMC proliferation, migration and inflammation. OxLDL and palmitic acid in vitro as well as diet induced dyslipidemia in vivo lead to loss of VSMC CREB protein expression, but the exact molecular mechanism requires further delineation. We tested the hypothesis that oxLDL induced CREB down-regulation and generation of reactive oxygen species (ROS) through cytosolic and mitochondrial mechanisms.

Methods: Primary VSMC were serum-deprived for 24 hours and then exposed to oxLDL (0.05-0.1 mg/ml), or glucose (25mM) for 24 hours. Protein extracts were examined for CREB, phospho-CREB, phospho-ERK (extracellular signal-regulated kinase) and MnSOD (manganese superoxide dismutase). Oxidized proteins purified from cell extracts with NEM-biotin were separated on 12% SDS-PAGE and subjected to immunoblot analysis using a biotin-alkaline phosphatase conjugated antibody. General ROS production was measured with dihydroethidium dye and specific generation of hydrogen peroxide with Amplex red. Immunoprecipitation was used to evaluate CREB ubiquitination, and CREB localization was performed using immuno-histochemistry.

Summary of Results: A significant dose-dependent loss of CREB and increase in ERK activity was demonstrated in VSMC following treatment with oxLDL. No significant increase in cytosolic or mitochondrial ROS was demonstrated in VSMC exposed to oxLDL, whereas in glucose treated VSMC (positive control) ROS was significantly increased. In VSMC, oxLDL induced protein oxidation in a dose-dependent manner, increased MnSOD and ERK phosphorylation, and induced nuclear export and ubiquitination of CREB.

Conclusion: Oxidized LDL led to total and activated CREB down-regulation, induction of ERK and MnSOD, but did not induce generation of cytosolic or mitochondrial reactive oxygen species. These data support protein oxidation as a contributor to oxLDL induced mechanisms of oxidant stress in vascular smooth muscle cells.

Nothing to Disclose: MB, LK, PAW, JEER

P3-138

EGF-Induced Vacuolar ATPase Assembly: A Linkage to mTOR Activation and Mitogenesis.

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The vacuolar ATPase (vATPase) is a multimeric complex, responsible for the acidification of intracellular organelles such as endosomes and lysosomes. Using proteomic and immunofluorescent methods we demonstrated that EGF induced a rapid recruitment of extrinsic V1 subunits of the vATPase (1.5 to 4.3-fold increase) to intrinsic V0 subunits in rat liver endosomes and lysosomes. This was accompanied by a reduction in vacuolar pH. The antibiotic bafilomycin is known to bind to and inhibit vATPase function, and was shown to inhibit EGF-stimulated DNA-synthesis. In parallel bafilomycin inhibited mTOR activation as indicated by the inhibition of EGF-stimulated 4E-BP1 phosphorylation, and p70S6K phosphorylation and kinase activity. Bafilomycin had no corresponding inhibitory effect on EGF-induced AKT and ERK activity. The effects of Bafilomycin were mimicked by chloroquine a compound neutralizing vacuolar pH. Bafilomycin did not inhibit the association of TOR with Raptor nor did it affect AMPK activity. Rather the intracellular concentrations of essential (viz. leucine and phenylalanine) but not nonessential amino acids were decreased by bafilomycin in EGF-treated primary rat hepatocytes. Cycloheximide, which is known to elevate intracellular amino acid levels, reversed the inhibitory effects of bafilomycin on mTOR activation. These results indicate that EGF, by augmenting vacuolar acidification, promotes autophagocytosis. In the face of augmented protein synthesis, this appears to be required to maintain stable intracellular amino acid levels and hence assure mTOR activation .

Sources of Research Support: The Canadian Institutes for Health Research; Genome Canada and Genome Quebec.

Nothing to Disclose: YX, AP, ER, VD, EBM, SC, BIP

P3-139

Globular Adiponectin Activates AKT in Osteoblasts.

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Adiponectin is synthesized and secreted mainly by adipose tissue and acts on insulin-target cells to promote insulin actions. Adiponectin binds and activates 2 receptors, AdipR1 and AdipR2, which are the products of two different genes. Adiponectin receptors are 7 transmembrane spanning receptors, with an intracellular amino-terminus and an extracellular carboxy-terminal tail and do not couple to G proteins. They activate intracellular signaling through a cascade of protein-protein interactions. Recently, osteoblasts were shown to modulate glucose and fat metabolism through humoral factors. Also, adiponectin has been shown to stimulate proliferation, differentiation and mineralization of osteoblastic MC3T3-E1 cells. In this study, we examined if adiponectin activates AKT in osteoblastic cells. We screened several osteoblastic cell lines: ROS 17/2.8, MC3T3-E1, MC3T3-E1 clone 4 (MC4), SaOS-02, and primary cultured osteoblasts for responsiveness to globular adiponectin. Confluent cells were serum-deprived for 2 hours and then challenged with 100 nM globular rat adiponectin (gAdp) (BioVision, Mountain View, CA), hPTH(1-34) (hPTH) (Bachem, Torrance, CA), gAdp and hPTH, or vehicle for 15 minutes. Cell lysates were subjected to Western Blot with antisera against pAKT (Ser⁴⁷⁴), pCREB(Ser¹³³) and β -actin. Interestingly, gAdp increased pAKT in all the cell lines tested. PTH did not increase pAKT but increased pCREB, as expected. The most dramatic effect to gAdp was observed in the MC3T3-E1 and primary cultured osteoblasts. Next, the response to gAdp in MC3T3-E1 cells was further characterized in term of a time course for the effects of 100 nM gAdp at 1, 5, 10, 15, 20, 30 and 60 minutes and in term of a dose-responsiveness using 1, 3, 10, 30, 100 and 300 nM gAdp at 5 min. The effect of 100 nM gAdp was maximal between 5-10 min and did not return to basal line by the end of the 60 min incubation. The minimum effective gAdp concentration was 3 nM. The effects were maximal at 100 nM gAdp. The fact that adiponectin increases pAKT levels in several osteoblastic cell lines and in primary cultured osteoblasts suggests that adiponectin (gAdp) regulates osteoblast functions and cell growth via AKT phosphorylation at Ser⁴⁷⁴ in osteoblasts. Thus, adiponectin may be an important regulator in the process of bone formation.

Sources of Research Support: NIH/NIDDK and Institutional funding.

Nothing to Disclose: BT, SG, BC, TAS, AAS, NSD, ABA-S

P3-140

cAMP Stimulation of Interleukin 11 in Decidualized Immortalized Human Endometrial Stromal Cells Is PKA-Independent.

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Successful embryo implantation requires decidualization of the uterine endometrium, a process involving complex morphologic and biochemical changes. Understanding of the regulation of the decidualization process in humans is very limited. In mice, the multifunctional cytokine interleukin 11 (IL-11) is required for decidualization and implantation. IL-11 is produced by human endometrium, but its regulation is poorly understood. In various cell types, IL-11 stimulation appears to occur via multiple signaling pathways. The current study tested the hypothesis that increased intracellular cAMP stimulates IL-11 production in decidualized human endometrial stromal cells using a PKA-dependent pathway. A telomerase-immortalized human endometrial stromal cell line, an excellent model of human endometrial decidualization, was used in this study. For each experiment, replicate wells of cells were incubated in phenol red-free media in the absence (n=6) or presence (n=6) of 0.5mM 8-bromo-cAMP. Media were replenished every 48 hours for 21 days. On day 21, replicate wells in the cAMP-treated group were incubated for an additional 24 hours with 0.5mM 8-br-cAMP in the absence (n=3) or presence (n=3) of 10µM Rp-cAMPS, a specific PKA inhibitor. Media IL-11 levels were measured by specific enzyme-linked immunosorbent assay. Since prolactin (PRL) is a well-established marker of decidualization, media PRL levels were measured by radioimmunoassay. The morphology of the cAMP-treated cells changed from stromal to epithelioid over the course of the protocol, and media PRL levels were strongly detectable (>25ng/ml) at day 21. In contrast, untreated cells remained stromal in appearance with undetectable day 21 media PRL levels (<5ng/ml). Incubation with 8-br-cAMP stimulated day 22 IL-11 to levels 8.3-fold higher than levels from untreated cells (3577pg/ml and 431pg/ml respectively, n=2 experiments, each in triplicate). No effect of inhibition of PKA was seen. Day 22 media IL-11 levels from cAMP-treated cells cotreated with the PKA inhibitor were not different than those in media from cells treated with 8-br-cAMP alone (3454pg/ml and 3577pg/ml respectively, n=2 experiments, each in triplicate). Thus, increased intracellular cAMP, by a PKA-independent pathway, stimulates IL-11 production in decidualized human endometrial cells. These findings advance our understanding of the regulation of a factor critical for the establishment of pregnancy.

Nothing to Disclose: AJF, SSM, DMC, ASW, GW, LTG

P3-141

Increased Bone Mass by Simultaneous Deletion of the FoxO1, 3, and 4 Genes from Committed Osteoblast Progenitors (Osterix Expressing Cells).

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Diversion of the limited pool of β -catenin from Wnt/TCF- to FoxO-mediated transcription in the setting of increased oxidative stress suppresses osteoblastogenesis and is associated with the old age-dependant decrease in osteoblast number and bone formation in the murine skeleton. To elucidate the significance of the antagonism of Wnt signalling by FoxOs in skeletal homeostasis we deleted FoxO1, 3, and 4 from committed osteoblast progenitors. This was accomplished by crossing mice with conditional alleles of FoxO1, 3, and 4 (Mx-Cre;FoxO1,3,4^{L/L}) with mice expressing the Cre recombinase under the control of the murine osterix promoter (Osx-Cre). FoxO1, 3, and 4 mRNAs were substantially decreased in bone and isolated osteoblasts but remain unchanged in the liver or spleen of Osx-Cre⁺;FoxO1,3,4 mice, confirming the specificity of the deletion. Female or male Osx-Cre⁺;FoxO1,3,4 mice exhibited a 20% increase in vertebral and femoral bone mineral density (BMD) by DEXA at 3, 5 and 8 month of age. Micro-CT analysis confirmed the increase in BMD and revealed an increase in cancellous bone volume, as well as an increase in trabecular number and connectivity, in both the vertebrae and the femur. Osx-Cre⁺;FoxO1,3,4^{L/L} mice also exhibited an increase in femoral cortical thickness; and this was associated with an increase in the external diameter of the femurs. However, the internal diameter of the femur was unchanged indicating that the increase in cortical thickness was not the result of decreased endosteal resorption; and this was confirmed by the unchanged expression of osteoclast specific genes in the femoral shafts. On the other hand, consistent with the contention that FoxOs antagonize Wnt/TCF signaling, the expression of the β -catenin/TCF target genes axin2 and cyclin D1 was elevated, as was the expression of the osteoblast specific gene osteocalcin — an indicator of osteoblast number or activity. Nonetheless, the number of colony forming unit (CFU)-osteoblasts was not changed by the triple FoxO deletion, indicating that the number of mesenchymal stem cells was not affected. Collectively, these findings demonstrate that FoxO-mediated antagonism of Wnt/ β -catenin signalling in committed osteoblast progenitors is a negative regulator of bone mass. Therefore, interference with FoxO-mediated transcription in such progenitors results in bone anabolism.

Sources of Research Support: NIH (P01 AG13918, R01 AR49794); the Department of Veterans Affairs.

Nothing to Disclose: EA, CAO, LH, RLJ, RSW, MA, SCM

P3-142

Activation of PKC β /p66^{shc} Is a Key Mechanism for the Promotion of Apoptosis and NF-kB Activation by Oxidative Stress in Osteoblastic Cells and the Molecular Target of the Anti-Oxidant Properties of Sex Steroids.

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The age-dependant decrease in bone mass and osteoblast/osteocyte survival is temporally associated with increased levels of reactive oxygen species (ROS) and phosphorylation of p66^{shc} — an adapter protein that amplifies mitochondrial ROS generation and influences apoptosis and lifespan in mice. The same increases in oxidative stress and p66^{shc} phosphorylation are caused by the removal of the gonads in young female or male C57BL/6 mice, and in female mice bearing an estrogen receptor α knock-in mutation that prevents binding to DNA. Based on the above, we have sought mechanistic evidence causally linking p66^{shc} to the adverse effects of oxidative stress and sex steroid deficiency on bone homeostasis, using uncommitted osteoblast progenitors (C2C12) and osteoblastic cells (OB-6, UAMS-32). We report that silencing of p66^{shc} abrogated H₂O₂- but not etoposide-induced activation of apoptosis, as determined by caspase 3 activity. Moreover, silencing of p66^{shc} reduced NF-kB activation in response to H₂O₂ as determined by the phosphorylation of I κ B, the activity of an NF-kB reporter construct. p66^{shc} silencing also abrogated the H₂O₂-induced increase in the expression of the NF-kB-target genes IL-6 and TNF α . The effects of H₂O₂ on both apoptosis and NF-kB activation were also attenuated by the specific PKC β inhibitors hispidin or LY333531. On the other hand, PMA, a potent inducer of PKC, had similar effects to H₂O₂ on both apoptosis and NF-kB activation. These effects of PMA were abrogated in the absence of p66^{shc} or in the presence of the PKC β inhibitors, as were the case with H₂O₂, indicating that the H₂O₂- or PMA-induced changes are indeed dependent on PKC β . 17 β -estradiol (E₂) or the non-aromatizable androgen dihydrotestosterone (DHT) prevented H₂O₂- or PMA-induced p66^{shc} phosphorylation, apoptosis, and NF-kB activation. Lastly, a polymeric form of E₂ that is not capable of stimulating the nuclear-initiated actions of ER α reproduced the effects of E₂ on all of these parameters. We conclude that p66^{shc} is an essential mediator of the effects of oxidative stress on apoptosis, NF-kB activation, and cytokine production by osteoblastic cells. Estrogens or androgens attenuate these effects by suppressing PKC β -induced p66^{shc} phosphorylation via a mechanism that does not require the nuclear-initiated actions of the ER α .

Sources of Research Support: NIH (P01 AG13918, R01 AR49794); the Department of Veterans Affairs.

Nothing to Disclose: MA, LH, EA, CAO, SCM

P3-143

The Effect of ER α and ER β Specific Agonists on Cell Proliferation and Energy Metabolism in Human Cultured Bone Cells.

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We have previously reported that in cultured human bone cells (Obs) estradiol-17 β (E2) stimulated DNA synthesis, dose-dependently, as well as the specific activity of creatine kinase BB (CK). We now investigate the response of Obs to ER α and ER β specific agonists compared to E2 in different parameters. Obs either primary human cells or SaOS2 and hFOB cell lines were treated with E2, 2,3-bis (4-hydroxyphenyl)-propionitrile (DPN;ER β specific agonist) and 4,4',4''-[4-Propyl-(1H)-pyrazol-1,3,5-triyl]tris-phenol (PPT;ER α specific agonist) and the effects on DNA synthesis, CK, the expression of mRNA for ERs, 12 lipooxygenase (12LO), 15 lipooxygenase (15LO), 12 and 15HETEs formation and ROS production were analysed. Treatment with E2, DPN or PPT increased DNA synthesis. Both PPT and DPN similar to E2 stimulated CK. Raloxifene (Ral), a specific ER α antagonist, inhibited the stimulation of DNA synthesis or CK by E2 or PPT, but not by DPN. The LO inhibitor baicalein did not affect PPT stimulations but inhibited E2 and DPN effects. Real time PCR in SaOS2 revealed that E2 had no effect on ER α mRNA expression whereas DPN and PPT stimulated it. All compounds stimulated ER β mRNA expression. All compounds stimulated ER α with no effect on ER β in human female bone cells. E2 as well as DPN and PPT modulated the expression of both 12 and 15LO and HETEs production in human bone cells. All free hormones stimulated ROS production in SaOS2 but the protein bound ones failed to do so. ROS production inhibitor; DPI did not significantly affect hormonal induced cell proliferation and CK. In conclusion, we provide herein evidence for the separation of mediation via ER α and ER β pathways in the different effects of E2 on Obs. The exact mechanisms and the precise role of ROS formation in these effects is the subject of future studies.

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

P3-144

Protein Kinase A Regulates Caspase 1 Via Ets1 Proto-Oncogene Activation in Mouse and Human Bone Tumors Derived from Bone Stem Cells.

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Prkar1a and prkaca encode the type 1A regulatory subunit (R1 α) and type A catalytic subunit (C α) of protein kinase A (PKA). Humans with PRKAR1A mutations develop bone tumors that are known as osteochondromyxomas (OCM). Interestingly, Prkar1a^{+/-} Prkaca^{+/-} mice develop more bone tumors than Prkar1a^{+/-} mice, also starting at earlier ages (1). Tumor cells from Prkar1a^{+/-} Prkaca^{+/-} mice express early osteogenic and bone stem cell (BSC) markers, show high PKA activity and cAMP levels and an alternate pattern of PKA catalytic subunit expression. Tumors from Prkar1a^{+/-} Prkaca^{+/-} mice have radiologic and histological similarities to both OCMs and bone lesions from humans with neonatal-onset multisystemic inflammatory disease (NOMID). Activating mutations in NLRP3, the gene encoding cryopyrin protein, are found in 60% of NOMID patients. Cryopyrin forms a complex termed "inflammasome" that activates caspase 1, which cleaves pro-interleukin-1 β in its bioactive form (IL-1 β). Caspase 1 is regulated by the Ets1 proto-oncogene. We investigated Ets1 and inflammasome involvement in OCMs and the Prkar1a^{+/-} Prkaca^{+/-} mice. Ets1, cryopyrin, caspase 1 and IL-1 β were significantly highly expressed at mRNA and protein levels in Prkar1a^{+/-} Prkaca^{+/-} bone tumors and in human OCMs. NOMID tumor cells showed higher PKA activity, cAMP levels, and β catalytic subunit (C β) expression, when compared to normal chondrocytes isolated from the same NOMID patient. Flow-cytometry analysis demonstrated that NOMID tumor cells express human BSC markers CD44 and CD146. siRNA down-regulation of C α decreased Ets1 and caspase 1 protein expression in mouse pre-osteoblastic (MC3T3) cells, whereas PRKACA-transfected cells showed a significant increase in Ets1 and caspase 1 expression. Simultaneous transfection of Ets1 siRNA and PRKACA gene blocked the increase in caspase 1 expression. Ets1, caspase 1 and IL-1 β expression were stimulated by forskolin and inhibited by PKA inhibitor H89 at mRNA and protein levels. Both mouse and human bone tumor cells showed high PGE2 levels, a well-known stimulator of cAMP/PKA pathway and inhibitor of chondrocyte differentiation. In conclusion, PKA regulates caspase 1 expression through Ets1 activation in mouse and human bone tumors derived from BSCs; this is the first demonstration of cAMP-signaling being involved in caspase 1 and inflammasome regulation in any condition; it also implicates PGE2 in OCM and NOMID, pointing to new therapeutic targets for these conditions.

(1) Tsang et al, in submission 2010

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Nothing to Disclose: MQA, KMT, XW, JCG, MN, JAC, RG-M, CAS

P3-145

The Role of the C-Type Natriuretic Peptide Pathway in Formation of Cranial Foramina in the Chick Embryo.

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The CNP signalling pathway has known roles in endochondral ossification. CNP binds to the GC-B receptor, activating PKGII, prolonging Sox9 activity and therefore chondrogenesis. Loss of function mutations and knockout of CNP (Nppc), GC-B and PKGII reveal achondroplasia phenotypes, including acromesomelic dysplasia type Matreaux (AMDM). In contrast, CNP binding to NPR-C receptor, which has no internal guanylyl cyclase domain prevents chondrogenesis. NPR-C mutant mice display bony overgrowth phenotypes. Therefore this pathway is critical in controlling the extent of skeletogenesis in the embryo.

Examples where skeletal growth is tightly controlled in a highly localised manner include cranial foramina formation. These are holes that form in the skull allowing entry/exit of major blood vessels and nerves. Prevention of skeletogenesis in specific areas is critical for their formation. Failure to form or closure of foramina can result in raised intracranial pressure and loss of cranial nerve function, which may be fatal. We have examined the expression of CNP ligands and receptors in developing cranial foramina in the chick embryo.

Using section insitu hybridisation and immunohistochemistry, we find high levels of CNP-1, CNP-3 and NPR-C expression in non-skeletogenic areas. However both GC-B and PKGII expression is absent, which could explain why skeletal tissue does not form in this specific location. Some studies suggest high levels of CNP may up-regulate NPR-C and down-regulate GC-B expression and these feedback mechanisms may play a role in restricting skeletogenesis in the cranial foramina.

We have used nerve ablation techniques to investigate the role of the cranial nerves in controlling expression of components of the CNP pathway. Removal of the optic nerves results in smaller and malformed optic foramina and we observe an increase in CNP-1 expression. In contrast, CNP-3 is weaker. This suggests that the cranial nerve may play a role in regulating expression of CNP ligands in developing cranial cartilages.

These data suggest that the CNP pathway may be playing a critical role in restricting skeletogenesis adjacent to major cranial nerves. Furthermore the expression of CNP-1 and CNP-3 suggest they have differing roles in this process. Interestingly, mutations in genes of the CNP pathway in humans are also associated with stenosis of the jugular and hypoglossal foramina, which is further evidence of the importance of this pathway in foramina formation.

Sources of Research Support: Anatomical Society of Great Britain and Ireland.

Nothing to Disclose: SEA, IRT, APP, RCF, IMM

P3-146

Regulation of Osteoclast Differentiation by the IGF Binding Protein IGFBP-2.

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Male IGFBP-2 knockout mice (IGFBP-2 ^{-/-}) have impaired bone turnover, compared with wild-type mice (IGFBP-2 ^{+/+}). The aim of this study was to elucidate the molecular mechanism by which IGFBP-2 regulates osteoclast differentiation. Bone marrow cells (BMC) were isolated from IGFBP-2 ^{+/+} and ^{-/-} male mice at 8 weeks of age. BMC were cultured in medium containing rmM-CSF (25 ng/ml) and rhRANKL (50 ng/ml) for 7-10 days to induce osteoclast differentiation and fusion. Cells were either fixed in 10% formalin and stained for tartrate resistant acid phosphatase (TRAP) or lysed and proteins visualized by western immunoblotting.

The ^{-/-} BMC formed 84% fewer osteoclasts than ^{+/+} BMC. The addition of IGFBP-2 was sufficient to induce osteoclast formation to the extent in the wild-type BMC. Using a bone resorption "pit" assay we detected an 80% reduction in bone resorption by RANKL stimulated ^{-/-} BMC compared with the wild-type BMC.

Previous studies have shown that there is an inverse relationship between IGFBP-2 levels and PTEN levels. We confirmed this relationship in BMC cultures. The addition of exogenous IGFBP-2 was sufficient to suppress the increase in PTEN. The levels of PTEN are regulated by its phosphorylation status. The increased PTEN associated with the ^{-/-} BMC was highly phosphorylated. The phosphorylation of PTEN has been associated with increased stability thus presumably accounting for the increase in PTEN protein in the absence of IGFBP-2. PTEN is a negative regulator of the PI-3 kinase/AKT signaling pathway essential for BMC survival and osteoclast differentiation. There was a significant reduction in the amount of phosphorylated AKT that could be detected in the ^{-/-} BMC but the addition of IGFBP-2 was sufficient to restore AKT phosphorylation. We detected significantly increased PARP cleavage in the ^{-/-} cultures suggesting the lack of AKT signaling lead to reduced cell survival. To determine whether the phosphorylation of PTEN was related to the inhibition of osteoclastogenesis we used an inhibitor of casein kinase 2 (CK2), a kinase implicated in PTEN phosphorylation. When two different inhibitors of CK2, DMAT and TBB were added to ^{-/-} BMC PTEN phosphorylation was reduced AKT phosphorylation was elevated and there was osteoclast maturation in the ^{-/-} BMC. Our data suggest that IGFBP-2 regulates osteoclast maturation by suppressing PTEN phosphorylation thereby enhancing AKT activation contributing to survival of the maturing osteoclast.

Nothing to Disclose: LAM, CW, TC, CR, DC

P3-147

Changes in Gene Expression Occurring in Periosteum during Juvenile Growth Deceleration Resemble Those in Non-Skeletal Tissues.

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In mammals, somatic growth is rapid in early postnatal life but slows with age. This growth deceleration occurs in both skeletal and many non-skeletal tissues. We previously showed evidence that growth deceleration in kidney, heart, lung, and liver is due in part to a common genetic program involving declining expression of multiple growth-promoting genes, including *Ezh2*, *Mest*, *Plagl1*, *Mdk*, *Gpc3*, *Peg3*, and *Igf2*. In growth plate, the changes in gene expression that occur during growth deceleration have some similarity to non-skeletal tissues (e.g. declining expression of *Peg3* and *Igf2*), but primarily involve a different set of genes, including declining expression of *Wnt4* and *Frzb* and increasing expression of *Igfbp7* and *Pycard*.

The rate of periosteal bone growth also slows with increasing age. This deceleration helps determine the adult cortical width and therefore bone strength. We asked whether changes in gene expression occurring during periosteal growth deceleration resemble changes occurring in the growth plate and/or those occurring in non-skeletal tissues. We dissected periosteum from parietal bones of male mice at 1, 4, and 8 wk of age, isolated RNA, and used real-time PCR to assess changes in gene expression with age. From 1 to 8 wk of age, expression declined for *Ezh2* (2.6-fold, $P = 0.006$), *Mest* (16-fold, $P < 0.001$), *Plagl1* (8-fold, $P = 0.003$), *Mdk* (3.2-fold, $P = 0.02$), *Peg3* (3.4-fold, $P = 0.02$) and *Igf2* (13-fold, $P < 0.001$). Expression of *Gpc3*, *Wnt4*, *Frzb*, *Igfbp7*, *Pycard*, and *Gapdh* did not change significantly with age. The findings suggest that the slowing of periosteal bone growth with age involves a genetic program, which includes decreasing expression of multiple growth-promoting genes, similar to that occurring in non-skeletal organs.

Sources of Research Support: Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development.

Nothing to Disclose: KMB, AD, JB

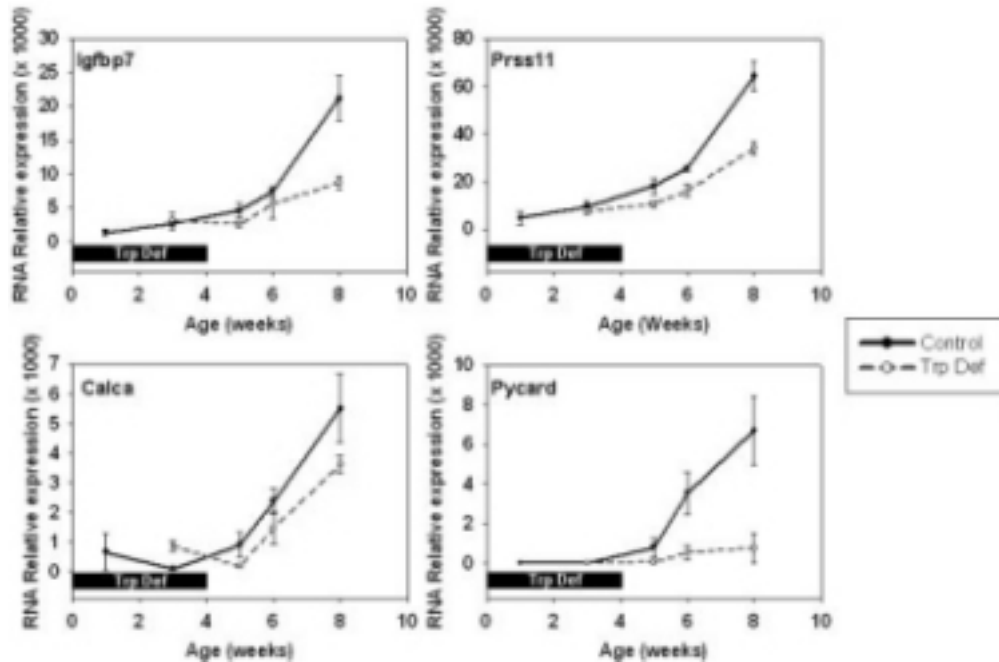
P3-148

Growth Plate Senescence Is Not a Function of Time *Per Se* but of Growth.

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During juvenile life, the growth plate undergoes programmed senescence, which causes skeletal growth to slow with age. We have reported that hypothyroidism slows growth plate senescence in juvenile rats, suggesting that senescence is driven, not simply by time, but by growth itself. To determine if this delay is a specific consequence of hypothyroidism or a general result of growth inhibition, we studied a second growth-inhibiting condition. We inhibited skeletal growth in juvenile rats by inducing tryptophan deficiency (which causes anorexia) for the first 4 weeks of life and then allowed the animals to recover. We then studied growth plate proliferative zone using real-time PCR to assess the effect of prior tryptophan deficiency on multiple molecular markers of growth plate senescence. We found that expression of *Igfbp7*, *Prss11*, *Calca*, *Cdkn2a* and *Pycard* increased between 1- and 8-weeks of age in the control group ($P < 0.01$) as expected. In animals that had previously been tryptophan deficient, the increase in expression (at 5-, 6- and 8-weeks of age) was delayed significantly ($P < 0.01$) for *Igfbp7*, *Prss11*, *Calca* and *Pycard* (Figure), but not *Cdkn2a*. *Asb4* and *RxRg* decreased with age ($P < 0.01$), but did not show a significant delay in their gene expression after tryptophan deficiency.



A similar pattern of significant delay ($P < 0.05$) was observed after PTU-induced hypothyroidism during the first 5 weeks of life for *Igfbp7*, *Prss11*, *Calca*, *Pycard*, *Asb4*, but not for *Cdkn2a* or *RxRg*. In the tryptophan deficiency model, we also used quantitative histological methods to analyze structural changes of growth plate senescence. For all structural markers that showed a significant decline with age in control animals, prior tryptophan deficiency delayed this decline significantly ($P < 0.05$): overall growth plate height, proliferative zone height, number of proliferative zone chondrocytes per column, and number of resting zone chondrocytes, as has been reported following hypothyroidism. These findings suggest that delayed growth plate senescence is a general consequence of growth inhibition and therefore that senescence is driven by growth and not simply by time.

Nothing to Disclose: PF, JCL, KMB, RM, ACA, JB, ON

P3-149

Chondrocytes Express the Thyroid Hormone Transporter Monocarboxylate Transporter 10 (MCT10).

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Thyroid hormone action is essential for normal growth and development. Since most thyroid hormone action and metabolism takes place intracellularly, thyroid hormone requires transport across the plasma membrane. Recent studies have revealed that thyroid hormone transporters mediate cellular uptake and efflux of iodothyronines. Up to date, three thyroid hormone transporters highly specific for thyroid hormone, namely MCT8, MCT10, and OATP1C1 have been identified. However, it is unknown how these and other transporters contribute to thyroid hormone pathophysiology in specific tissue/cell types.

The discovery that loss-of-function mutations in MCT8 lead to the Allan-Herndon-Dudley syndrome (AHDS), characterized by severe psychomotor retardation and elevated triiodothyronine levels, has greatly increased interest in this area of research. We have previously reported an AHDS patient with a c.1649delA mutation (counted from the first ATG) and another with a c.1201G>A (p.G401R) substitution. Though these patients exhibit severe psychomotor retardation similar to that seen in patients with endemic infantile hypothyroidism, unlike the latter disorder, we and other investigators have shown that growth is within normal range unless severely malnourished due to feeding problems. Given that thyroid hormone is essential for normal proliferation and differentiation of chondrocytes, the growth plate of AHDS patients would have to be thyroid hormone replete. We thus hypothesized that chondrocytes utilize transporters other than MCT8 for thyroid hormone uptake.

Since little is known about thyroid hormone transporters in chondrocytes, we comprehensively analyzed thyroid hormone transporter mRNA expression in chondrogenic ATDC5 embryonal carcinoma cells. cDNA was prepared from total RNA extracts. Out of 20 putative thyroid hormone transporters, 11 were detected by RT-PCR. The detected transporters were subsequently quantified by real-time PCR using the LightCycler System (Roche). MCT10 was the most abundant of the three transporters highly specific for thyroid hormone with 146,114 copies/ng RNA. Expression levels of MCT10 did not change throughout chondrocyte differentiation. These results suggest that MCT10 might be the major thyroid hormone transporter in chondrocytes and that growth is preserved in AHDS patients due to thyroid hormone influx via this transporter.

Nothing to Disclose: NN, SA, MK, KO

P3-150

Alternate Protein Kinase A Activity Identifies a Unique Population of Stromal Cells in Adult Bone.

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A population of stromal cells that retains osteogenic capacity in adult bone (adult bone stromal cells or aBSCs) exists and is under intense investigation. aBSCs may be different from their embryonic or neonatal counterparts, and influenced by species- and age-specific, as well as anatomic site of origin, membranous or endochondral ossification of the source, proximity to cortical or trabecular bone, and other factors. Mice heterozygous for a null allele of *prkar1a* (*Prkar1a*^{+/-}) the gene that codes for the primary receptor for cyclic adenosine monophosphate (cAMP) and regulator of protein kinase A (PKA) activity in cells, developed bone lesions that were derived from cAMP-responsive, osteogenic cells and resembled fibrous dysplasia (FD). *Prkar1a*^{+/-} mice were now crossed with mice that were heterozygous for catalytic subunit α (*Prkaca*^{+/-}), the main PKA activity-mediating molecule, to generate mouse models with double heterozygosity for *prkar1a* and *prkaca* (*Prkar1a*^{+/-}*Prkaca*^{+/-}). Unexpectedly, *Prkar1a*^{+/-}*Prkaca*^{+/-} mice developed a great number of osseous lesions starting at 2-3 months of age that varied from the rare chondromas in the long bones and the ubiquitous osteochondrodysplasia of tail vertebral bodies to the occasional sarcoma in older animals. Cells from these lesions were fibroblast- and FD-like, and almost always originated from an area of mostly trabecular bone, proximal to the growth plate and the adjacent endosteal surface of the periosteum; they expanded gradually in the bone marrow space. These cells expressed osteogenic cell markers, showed higher PKA activity that was mostly type II (PKA-II), an alternate pattern of catalytic subunit expression, and surprisingly higher cAMP levels. Markers of both bone synthesis and lysis were increased. Gene expression profiling confirmed an early (progenitor) osteoblastic nature for these cells but also showed a signature that was indicative of mesenchymal-to-epithelial transition and increased Wnt signaling, brachyury expression in particular. These studies show that a specific subpopulation of aBSCs can be stimulated in adult bone by PKA-II and altered α activity, generating the only available germline mutant mouse model of a disorder that has similarities to human FD. Along with previous data, these studies also suggest that the effects of cAMP signaling on osteogenesis and stromal cell maintenance and proliferation in mice are age-, bone-, site- but also PKA-type and catalytic subunit-specific.

Sources of Research Support: U.S. National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development intramural project Z01-HD-000642-04 (to Dr. C.A. Stratakis).

Nothing to Disclose: KT, MFS, MN, SAB, TW, MQA, MH, AL, MTC, CC, ELM, SL, LSK, PR, CAS

P3-151

Reambulation Reverses Disuse-Stimulated Bone Loss Via Normalization of Mesenchymal Cell and Osteoclast Differentiation.

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It is well established that musculoskeletal disuse leads to a significant loss of bone mass and strength in the load-bearing limbs of both young and old animals and humans. We previously demonstrated that after 2 wks of hindlimb suspension (HS) there is an increase in adipogenesis (AD), a decrease in proliferation and differentiation of osteoblast (OB) progenitors, an associated loss of bone and architecture, and increased OB apoptosis. These disuse-induced changes are further enhanced in aged rats (32 months of age). Here, we determined the extent to which osteoclast (OCL) differentiation is affected by HS, and the extent to which 2 wks of reambulation (RE) can alleviate the deleterious effects of 2 wks of HS in both adult (6 mo) and aged (32 mo) rats on bone cell differentiation and osteoblast activity. Bone marrow cultures from femurs were established to determine the capacity of cells to be recruited into the OB/AD lineage and to undergo osteoclast (OCL) differentiation. In 6 mo rats, OCL number was increased 3X by HS, and restored to control levels after 2 wks of RE. Age alone increased OCL 5X, which was further enhanced 2X by HS. As in the young rats, RE restored the OCL number back to the aged control levels. HS did not affect the percentage of cells recruited into the OB/AD lineage at either age, as indicated by the percentage (%) of CFU-F colonies stained for alkaline phosphatase (AP), but RE increased the % AP+ CFU-F in 6 mo rats. The RE-stimulated progenitors were committed to AD differentiation, based upon their increased sensitivity to the pro-adipogenic factor, Rosiglitazone (Rosi). HS enhanced mesenchymal progenitor commitment to the AD adipogenic lineage (Rosi-stimulated CFU-AD) in 32 mo rats, and the HS effect was again neutralized by RE. Together, these data demonstrate that the age- and HS-dependent increases in OCL formation can exacerbate the bone loss due to enhanced AD differentiation at the expense of OB cell differentiation, survival, and bone formation. Importantly, for the bone loss associated with disuse in humans, these data suggest that normal ambulation (RE) may be sufficient to reverse the deleterious effects of HS on bone formation in part by decreasing AD and OCL numbers to normal levels, even in the elderly population.

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Nothing to Disclose: TWF, KMN, NSA, EED-V, LJS, DG

P3-152

Overexpression of CXCR4 in Mesenchymal Stem Cells Inhibits Osteogenic Differentiation through Inducing Stress to Endoplasmic Reticulum.

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CXCR4 is the specific receptor for stromal -derived factor-1 (SDF-1), a chemokine required for homing of progenitor cells to tissues. To date, there were plenty of successful attempt to enhance the efficacy of stem cell therapy using genetically modified mesenchymal stem cells (MSCs) overexpressing CXCR4. In this study we examined the contribution of CXCR4 signaling to osteogenic differentiation of mouse mesenchymal stem cells induced by Wnt-3A. Retrovirally transduced MSCs constitutively expressing CXCR4 (CXCR4-GFP) showed slight increase of cell viability (~1.2 folds). Analysis of alkaline phosphatase activity showed that CXCR4-GFP reduced Wnt-3A induced osteogenic differentiation compared with the control group. Electron microscopic evaluation revealed that rough endoplasmic reticulum (ER) is engorged in CXCR4-GFP cells suggesting that cells were under ER stress. Elevated a phosphorylated form of eukaryotic translation initiation factor (eIF)-2a and augmented response to thapsigargin induced X-box binding protein (XBP)-1 splicing also support the existence of ER stress in CXCR4-GFP cells. Our observations suggest that overexpression of CXCR4 inhibits osteogenic differentiation in mouse mesenchymal stem cells. ER stress induced by CXCR4 could affect these cell differentiation.

Nothing to Disclose: HJC, SWC, JHA, YL, KWK, HSJ, SWK, SYK, CSS

P3-153

Akt1: A Negative Regulator of Osteogenic Differentiation.

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Mesenchymal stem cells play a central role in repair of bone and other skeletal tissues, and can be directed toward the osteoblast lineage by actions of bone morphogenetic proteins (BMPs), which induce expression of osteogenic transcription factors, including Dlx3, Dlx5, and Runx2. BMP-2-stimulated osteoblast differentiation additionally requires sustained activity from the PI3-kinase - Akt pathway via actions of the insulin-like growth factors. Here we find that Akt1 is a negative regulator of osteogenesis. Targeted siRNA-mediated knockdown of Akt1 in mouse bone marrow stromal cells in primary culture or in a mesenchymal cell line, or genetic knockout of Akt1, did not alter BMP-2-mediated signaling (Smad 1, 5, 8 phosphorylation) or modify expression of Smad-activated target genes (Sox9, JunB), but resulted in enhanced and precocious osteoblast differentiation coincident with increased production of Runx2 mRNA and protein. The augmented rate and extent of osteogenesis was manifested by up-regulation of other bone-specific genes, including osterix and osteocalcin, by a 2-3-fold rise in alkaline phosphatase activity over controls beginning by day 3 of differentiation, and by a ~2-fold increase in extracellular mineralization at day 10. Moreover, restoration of Akt1 in Akt1 null cells by acute lentiviral delivery reduced the accelerated pace of osteogenic differentiation down to control levels. As loss of Akt2 in the same model systems prevented BMP2-mediated osteogenesis by blocking Runx2 gene expression (Mukherjee, Wilson, and Rotwein, *Molecular and Cellular Biology* 30: 1018-1027, 2010), our results document potentially adversarial roles for the two-highly related Akts in skeletal tissues.

Nothing to Disclose: AM, PR

P3-154

Tp53inp2, a Newly Discovered Nuclear Co-Factor for Thyroid Hormone (TH) Receptor, Modulates TH Effects on Osteoblast Differentiation.

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Although TH is known to exert important effects on the skeleton, the molecular pathways by which TH mediates its effects on target gene expression in osteoblasts (Obs) remain poorly understood. Because the replacement of co-repressor by a co-activator complex in the promoters of TH responsive genes is essential for transcriptional activation of TH receptor regulated genes, it is important to identify and evaluate the role of nuclear factors that constitute the TH receptor co-activator complex in Obs. A recent study demonstrated that the actions of TH on myoblast differentiation are dependent on DOR (Diabetes and Obesity Related Protein), also known as Tp53inp2. We recently found Tp53inp2 expression increased during in vitro differentiation of bone marrow stromal cells into Obs and that treatment of MC3T3-E1 mouse Obs with TH significantly upregulated Tp53inp2 expression. In the present study, we sought to test the hypothesis that Tp53inp2 acts as a modulator of TH action during Ob differentiation by examining whether knockdown of Tp53inp2 expression using lentiviral shRNA impairs the ability of TH to induce Ob differentiation. Seventy-two hours of treatment with TH increased ALP activity in scramble control shRNA treated MC3T3-E1 cells by 2-fold ($P < 0.05$). However, TH induced increase in ALP activity was dramatically reduced (70%, $P < 0.01$) in MC3T3-E1 cells treated with Tp53inp2 shRNA compared to scramble shRNA control. The consequence of Tp53inp2 knockdown on Ob differentiation was also evaluated in primary cultures of mouse calvaria Obs. As expected, ALP activity increased in a dose dependent manner in scramble control shRNA treated Obs with a maximal increase of 78% at 10 ng/ml of TH ($P < 0.01$). In contrast, TH treatment failed to elicit an increase in ALP activity in Tp53inp2 knock-down cells. The gene expression of Ob specific markers in response to TH treatment was also examined in MC3T3-E1 cells expressing scramble control shRNA and Tp53inp2 shRNA. Expression of ALP and Runx2 increased 3-fold and 2-fold respectively in scramble control shRNA treated cells in response to TH treatment. However, TH treatment failed to induce ALP and Runx2 gene expression in Tp53inp2 knock-down cells. Based on these data, we conclude that Tp53inp2 plays a critical role in mediating TH effects on Ob differentiation.

Sources of Research Support: NIH AR48139.

Nothing to Disclose: GRL, WX, STC, SM

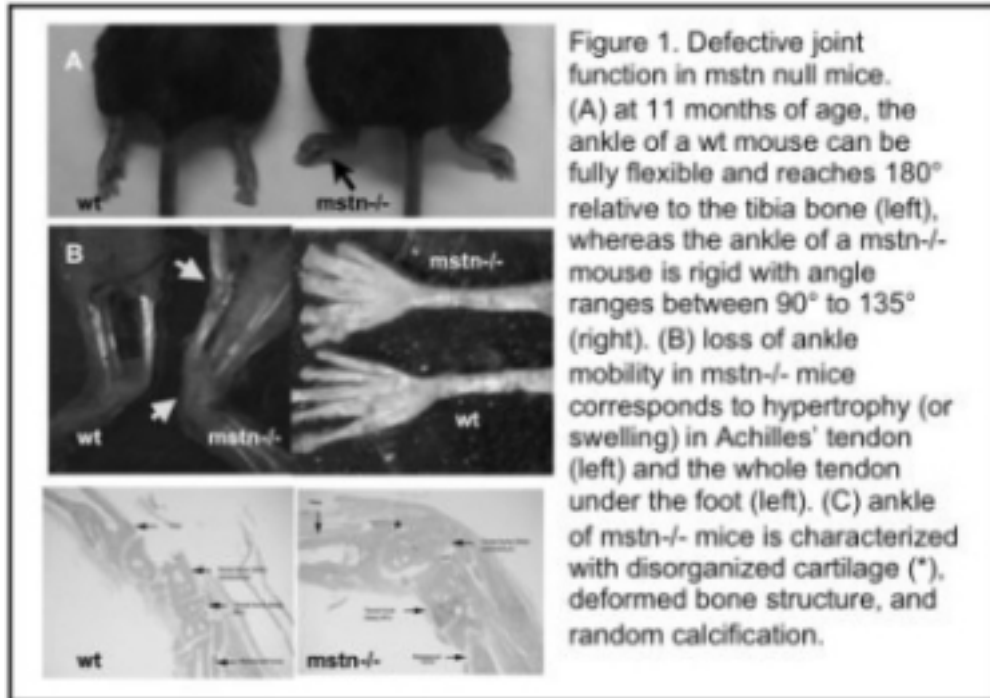
P3-155

Age-Related Changes in Bone, Tendon, and Cartilage in Myostatin-Null Mice.

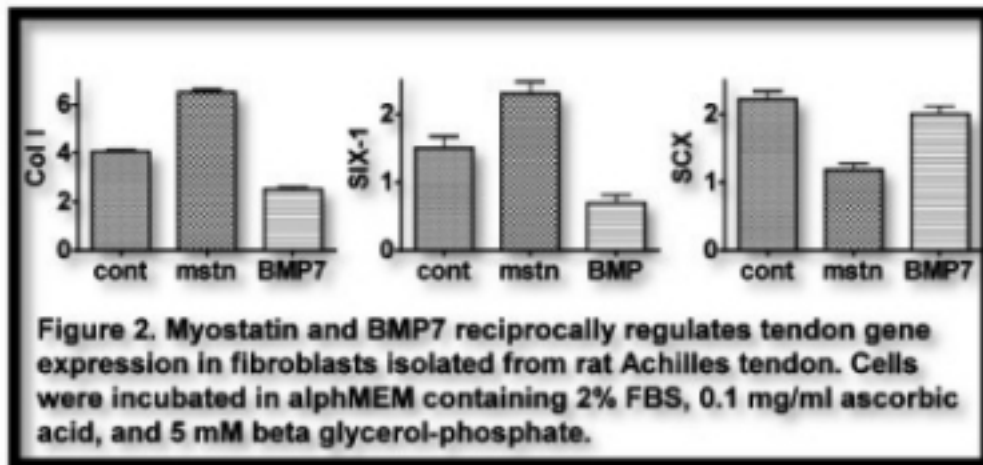
MS Yee BS, MA¹, S Bhasin MD¹, AD Miller DVM², KS Wong BS¹, A Lee¹ and W Guo PhD¹.

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Myostatin (mstn) belongs to the TGFβ family and is best-known as an inhibitor of muscle growth. Hypermuscularity in mstn^{-/-} mice is widely recognized and numerous mstn inhibitors are developed to treat sarcopenia associated with aging and chronic illness. To determine the health impact of long-term mstn deficiency, we studied mstn^{-/-} mice at different ages (6,12,16 months). We found that, beginning at middle age (9-12 months), mstn^{-/-} mice gradually lost their ankle mobility and displayed overt tendon swelling, irregular cartilage, ectopic calcification, and disorganized bone structure.



Compared to wild-type animals, mstn^{-/-} mice had higher plasma alkaline phosphatase activity (2 fold), and higher plasma concentrations of osteopontin (6.8 fold), osteoactivin (5 fold), osteoprotegerin (1.5 fold), as well as selected MMPs and TIMPs (2 - 4 fold). These findings suggest an active bone remodeling process in the mstn^{-/-} mice, which may be responsible for the derangement of surrounding soft tissue causing impaired ankle joint function. To test the hypothesis that proper balance between mstn and BMP activity is important for soft tissue health, we incubated tendon fibroblasts in an osteogenic medium for 9 days with mstn (50 ng/ml) or BMP7 (500 ng/ml). qPCR analysis showed that mstn increased the expression of tendon phenotype-related genes such as collagen I and Six-1 and suppressed expression of SCX, a transcription factor that promotes bone ridge formation at tendon insertion site. As expected, the effect of BMP7 on gene expression was opposite to that of mstn.



These data suggest that an imbalance between mstn and BMP signaling may contribute to tendon and bone defects in aged mstn^{-/-} mice. Further investigation of the mechanisms of these defects may provide important molecular targets

for treatment of joint disorders.

Sources of Research Support: NIH Grant Number: 5P3,AG,031679-02.

Nothing to Disclose: MSY, SB, ADM, KSW, AL, WG

P3-156

Towards Patterning Muscle-Tendon Interfaces.

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Musculoskeletal tissue interfaces, which serve to connect different tissues as well as facilitate the efficient transfer of mechanical loads (1), comprise multiple cell types and extracellular matrix components within spatially-graded microenvironments. Presently, repair and regeneration of injuries to these tissue interfaces is limited and represents a clinically relevant target for therapeutic development. Towards this goal, a novel bio-inkjet printer was used to create persistent patterns of exogenous growth factors (GFs) to spatially direct a single stem cell population toward multiple cell types present in muscle, tendon, and bone phenotypes *in vitro* in register with these patterns. Initially, a screen was conducted to identify tendon-promoting GFs. This screen identified FGF-2 and FGF-4 as being capable of inducing C3H10T1/2 mesenchymal stem cells, C2C12 myoblasts and primary muscle-derived stem cells towards a tendon lineage by upregulating the tendon marker scleraxis (Scx). Quantitative PCR studies showed that FGF-2 directs stem cells towards a tendon lineage via members of the *Ets* family of transcription factors such as *pea3* and *erm*. Following this, persistent patterns of BMP-2 and FGF-2 were created by printing these GFs on fibrin-coated glass coverslips. These immobilized patterns of BMP-2 and FGF-2 directed C2C12 myoblasts towards an osteoblast (bone) and tenocyte (tendon) fates, respectively, with myotubes (muscle) forming randomly off-pattern. Having demonstrated control of multi-cell lineage specification in a spatially-defined manner, a novel nanofibrous scaffold (Spinneret-based Tunable Engineered Parameters or STEP scaffold) developed by Nain *et al.* 2008 was used in conjunction with inkjet printing of FGF-2 to control cell alignment as well as cell differentiation to engineer a muscle-tendon interface. In such studies, C2C12 myoblasts spontaneously fused to form elongated myotubes which aligned along the nanofiber axis while printed patterns of FGF-2 induced cells towards a tendon fate, highlighting the versatility of using GF bioprinting with different scaffolds to obtain the desired cell behavior(s). This work illustrates spatial control of cell differentiation in conjunction with cell alignment and may have applications for engineering multi-tissue units in regenerative medicine.

(1) Yang, P. J. and J. S. Temenoff (2009). "Engineering Orthopedic Tissue Interfaces." *Tissue Eng Part B Rev.*

(2) Nain, A. S., J. A. Phillippi, et al. (2008). "Control of cell behavior by aligned micro/nanofibrous biomaterial scaffolds fabricated by spinneret-based tunable engineered parameters (STEP) technique." *Small* 4(8): 1153-9.

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Nothing to Disclose: DFEK, ASN, BC, JAP, BG, JH, LW, PGC

P3-157

Bone Mineral Density in Transitional Endocrine Clinic in a UK Teaching Hospital.

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Introduction:

Endocrinopathies during childhood can cause secondary osteoporosis and little is known of the extent of this condition in young adult life.

Methods:

In order to assess the bone mineral density in endocrinopathies in young adults, a retrospective analysis of 25 transitional clinic patients who underwent dual energy X-ray absorptiometry (DEXA scan) was done using case notes and the hospital database.

Results:

23 patients were male and the mean age was 19 years.

Results

| | |
|---|----------|
| Disease distribution | |
| Growth hormone deficiency | 16 |
| Hypogonadism | 6 |
| Long term steroids | 4 |
| Abnormal DEXA scan results | 20 (80%) |
| Osteoporosis | 1 (4%) |
| Low BMD | 19 (76%) |
| Low BMD Distribution according to primary disease | |
| Sex hormone deficiency | 100% |
| Childhood leukaemia | 100% |
| Growth hormone deficiency | 87.5% |
| Radiotherapy | 81% |

some had multiple hormonal deficiency

16 patients had growth hormone deficiency, 6 had sex hormone deficiency, and 4 patients were on long term steroids. Endocrinopathies were due to post-operative radiotherapy for brain tumours in 12/25, due to cranial & total body irradiation following Leukaemia in 4/25. Histiocytosis X and Klinefelter's syndrome accounted for 2 each.

1(4%) patient had osteoporosis with a significant spinal fracture history and low bone mineral density (BMD). 19 (76%) patients had reduced BMD.

All patients who had sex hormone deficiency and all childhood leukaemia survivors had low BMD. 87.5% of patients with GHD and 81% of the patients who had radiotherapy had low BMD.

15 out of 20 patients (75%) with abnormal DEXA scan result were on calcium, Vitamin D3 and bisphosphonates. Z-scores were not available in 21 patients (due to absence of age-matched controls).

Conclusions:

Low bone mineral density was present in 80% of at risk patients in our transitional endocrine clinic. The patients with hypogonadism, childhood leukaemia survivors and children GH insufficiency were at highest risk of reduced BMD as young adults, even if they were on hormone replacement in childhood.

It's still not clear whether replacement of the deficient hormone along with calcium and Vitamin D3 supplementation is adequate in these young adults, or whether additional bisphosphonate therapy with potential side-effects is required. The health consequences of early-onset BMD problems in childhood endocrinopathies needs to be carefully monitored.

Nothing to Disclose: GS, IC, AA, MD

P3-158

FRAX Fails To Change Prescribing Behavior Despite Increased Recommendation To Treat.

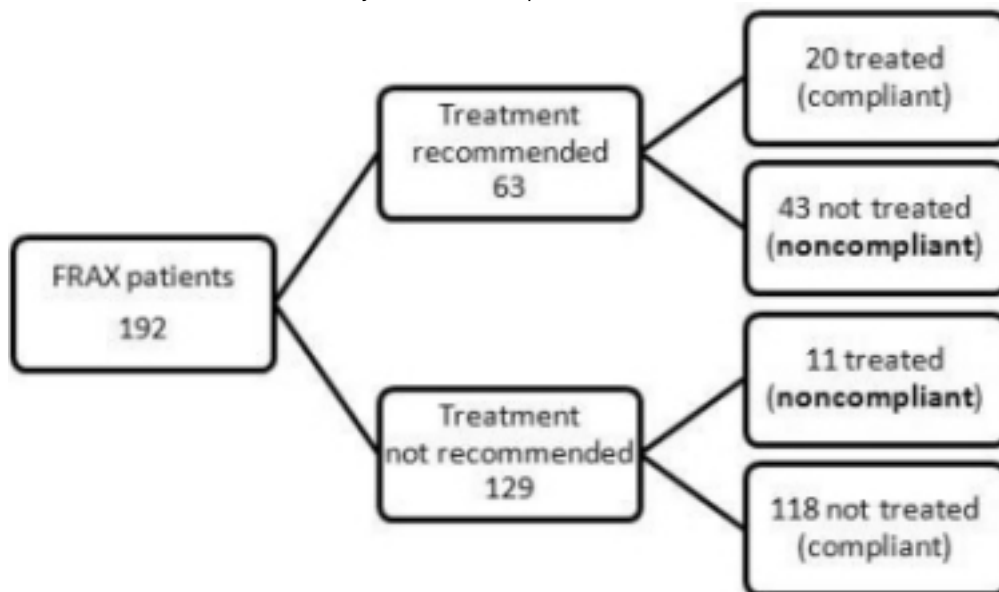
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Prevention of osteoporotic fractures using FDA approved drugs is desirable to decrease morbidity, mortality, and health care costs. The WHO FRAX tool was expected to enhance physician decisions on treatment by capturing high risk patients that would have been otherwise missed using primarily bone density T scores. We sought to determine compliance with FRAX and its impact on prescribing behavior in an outpatient setting.

Methods: We retrospectively reviewed compliance of clinicians to FRAX recommendations in a group of patients that underwent bone densitometry 7 months before and 7 months after FRAX was introduced into the DXA reports. We focused on osteopenic patients (DXA $-1.0 > T > -2.5$) since this group would be most influenced by FRAX. Exclusions: <50 years old, known osteoporosis. Demographic information, provider type, DXA/FRAX results and the decision to prescribe an FDA approved drug within 6 months of DXA were captured. Data was analyzed using chi square.

Results: 1346 charts were reviewed and 368 met inclusion criteria (176 pre-FRAX, 192 FRAX). The FRAX report recommended treating 63 patients with osteopenia who might not have been treated using DXA alone. Of these, only 20 (31.8%) were treated. Conversely, 11 out of 129 patients who did not meet FRAX treatment criteria were treated.



In the pre-FRAX group, 26 patients (14.8%) were treated contrary to DXA recommendation.

There was no difference in total number treated between the FRAX group and the pre-FRAX group (16.2% versus 14.8%; $P = 0.682$). However, 20/31 (67%) of those treated in the FRAX group was in compliance with recommendation compared to 0/26 in the pre-FRAX group.

Overall, compliance of clinicians to DXA recommendation was 85.2% while compliance to FRAX recommendation was 71.9% ($P = 0.0016$).

Conclusions: Despite the potential benefit of using the FRAX tool, we found that (1) clinicians had poor compliance with FRAX, (2) prescriptions were not increased with FRAX but treatment was more appropriate when given, (3) FRAX had limited benefit to patients since it was mostly ignored. There is therefore a need for increased awareness and use of FRAX to guide clinical decision making.

Nothing to Disclose: KEI, NA, JB-S, VT, MSN

P3-159

Self-Reported Risk Factors for Bone Loss: Utility of a Patient Questionnaire Completed at Time of Bone Mineral Density Testing.

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Objective: We examined the prevalence and association of self-reported risk factors for bone loss with the bone mineral density (BMD) values in a cohort of patients undergoing DXA scanning in our clinical center.

Methods: 201 consecutive patients (post-menopausal females and men >50 years of age) underwent BMD testing and were included in our study. Each patient completed a standardized questionnaire developed by the Canadian Panel of International Society of Clinical Densitometry (ISCD). The DXA scans of patients were reviewed to evaluate BMD and the respective T and Z scores. Information on serum vitamin D was obtained from the patient's record when available.

Results: Patients were predominantly female (87%) and white (87%) with a mean age of 65 years. Patients reported the following: frequent falls (13%), steroid use (20%), chemotherapy (5%), smoking (15%), family history of hip fracture (9%), fragility fracture (42%), and use of epilepsy medications (7%). Inadequate 25-hydroxy vitamin D levels were found in 47 percent (N=61 of 132 available). We found no association between a diagnosis of osteopenia or osteoporosis by DXA and any of the self reported risk factors. We found current smokers had significantly lower BMD (10% lower BMD; $p < 0.001$) and Z scores (33% lower score; $p = 0.02$), but there was no significant association with T scores. These results were unaffected by adjustment for age, gender, and race using multiple linear regression.

Conclusion: We found that most of the ISCD self-reported risk factors for bone loss in our patient population were not associated with lower BMD or the respective T and Z scores. Perhaps this lack of association is related to a lower accuracy of the survey in our patient population. Nevertheless, these risk factors were previously established based on the outcome of osteoporotic fracture, which was not measured in our study. Further study of the ISCD questionnaire is needed to determine if it can be utilized in diverse patient populations.

Nothing to Disclose: SYK, KQC, TWG, AY

P3-160

Cost-Effective Laboratory Screening in Osteoporosis Investigation.

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Involution osteoporosis is the most common cause of osteoporosis, but it is extremely important to search for undiagnosed disorders that could contribute to bone loss, in order to optimize the treatment of osteoporosis. However, there is yet no consensus on the best laboratory screening to be done.

We aim to analyze the prevalence of laboratory alterations and its cost-effectiveness in osteoporosis investigation.

A cross sectional study was conducted in 62 osteoporotic patients from a general endocrine clinic at Felício Rocho Hospital, in Brazil. Patients with renal failure, liver diseases, taking medication or having previously diagnosed disorders that could affect bone metabolism were excluded. The laboratory tests included serum (55) and urinary calcium levels (35), PTH (45), 25OHD (44), TSH (53) and hemogram (62). Protein electrophoresis (15), salivary or urinary cortisol (5), IGF1 (3) and celiac profile (3) were done when patients had a very serious osteoporosis for the age or if clinically suspected

Sixty two subjects, 58 women, average age 68.33 years (range 45-92), average BMI 24.37, were included in this study. Among the tests analyzed we found a variable percentage of alterations and 54 new diagnoses were identified within this population. Hyperthyroidism was diagnosed in one patient, and there were six patients with elevated TSH, eventually confirmed as Hashimoto thyroiditis. A patient with Cushing syndrome and three acromegalic patients were also identified and subjected to surgical treatment. In spite of living in a tropical country, we found a great percentage of vitamin D insufficiency (32.23%). Serum calcium (20%), urinary calcium (17%), and protein electrophoresis (33%) were also commonly altered.

When minimal screening tests were done (serum and urinary calcium, PTH, 25OHD and TSH) the total cost was U\$90 for each patient and U\$170 for diagnosis. Further tests, e.g. cortisol, protein electrophoresis or IGF1 had a much higher cost and if all patients underwent these tests regardless the clinical suspicion, the number of new diagnoses would be low, and the approach would not be cost-effective.

We conclude that minimal screening tests here proposed are important to be done for all osteoporotic patients since these tests do have favorable cost effectiveness. More complex tests should be restricted to selected patients, after a complete history and physical examinations, leading to a specific clinical suspicion.

Nothing to Disclose: BCGM, BCCS, EPD, LADM, MMSS

P3-161

Differences of Structural Trends in the Proximal Femur by Age and Gender: Population -Based Study.

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Introduction : Indices of bone geometry are known to affect the bone strength. Differences of bone geometry according to age and gender can bring the differences in fracture rate.

Methods : Dual X-ray absorptiometry scans were obtained of 1504 men and 2076 women, aged between 19 and 92 years (mean age, 18.46 for men and 49.46 for women) and were analyzed further to calculate the hip axis length(HAL), neck shaft angle(NSA), femur neck width(FNW), femur neck cross-sectional area(CSA), section modulus and femur neck buckling ratio with a structural analysis program at the proximal femur. Age trends of BMD and femoral geometry in men and women were studied. Moreover, we investigated the differences of the age associated changes of femoral geometry by each age group and gender.

Results : BMD, cortical thickness and CSA of femur neck showed similar age trends in both men and women. The large decreases of femur neck BMD and cortical thickness occurred in 30~39 years and 50~59 years group in men and postmenopausal 50~59 years group in women. Femur neck width showed slight increase at age 60~69 years group of men (0.019cm/year, $p<0.001$) and 70~79 years group for women (0.016cm/year, $p=0.008$), but in general, no significant changes were observed according to age in both men and women. Hip axis length and neck shaft angle also did not show any significant age trends in both men and women. Femur neck buckling ratio showed linear increase according to age with largest increase in 60~69 years group in men (0.217/year, $p<0.001$). In contrast, femur neck buckling ratio in women showed exponential rise with aging and the most apparent increase occurred in 70~79 years group (0.239/year, $p=0.011$). In age, height and weight adjusted correlations between BMD, the indices of geometry and body composition, 25(OH)D correlated positively with BMD, cortical thickness and CSA($r=0.12, 0.115, 0.17, p<0.001$ for all) but negatively correlated with buckling ratio($r=-0.089, p<0.001$). Moreover, cortical thickness had positive correlation with leg lean mass($r=0.165, p<0.001$) and negative correlations with leg fat mass and leg fat(%)($r=-0.129, -0.162, p<0.001$ for all).

Conclusions : Fracture rates differ greatly from age and gender. These differences result from not only the loss of BMD but also the deterioration of bone geometric parameters. For the prevention of fracture, we need to recognize the accelerated phase of the most determinant geometric parameters.

Nothing to Disclose: KMK, KJK, HSC, YR, S-KL

P3-162

Bone Mineral Density in Grand-Multiparous Premenopausal Women of Ashkenazi Jewish Descent.

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Introduction: Pregnancy and lactation may be associated with at least transient bone loss (3-5). Effects of grand-multiparity and length of lactation on BMD are not clearly determined due to conflicting results of previous research (1,2,6).

Objective: To evaluate the effect of multiple pregnancies and lactation on BMD in a group of grand-multiparous (GM) Ashkenazi-Jewish (AJ) women.

Methods: We conducted a cross-sectional study comparing BMD of 21 pre-menopausal GM women of AJ descent with >4 deliveries to 11 BMI- and age-matched AJ women with 2 or fewer full-term pregnancies (C). Data included age, height, weight, age of menarche, number of births, length of lactation, weight before first pregnancy, family history of osteoporosis, smoking and alcohol intake, past medical and medication history. All subjects had dual energy x-ray absorptiometry (DXA) testing of L1-L4 AP-spine and proximal non-dominant femur performed by the same certified technologist with Hologic QDR 4500 DXA scanner. Mean values of BMD and SD were calculated for both groups. Group comparison was done with Student's t-test for independent samples. Simple regression analysis was performed using L-spine, femoral neck (FN) and total hip (TH) average Z-scores against number of pregnancies, lactation time, and weight before first pregnancy.

Results: Study groups did not differ in age, BMI, weight before first pregnancy, history of smoking, alcohol use, family history of osteoporosis. In GM women mean BMD of L-spine was 0.949 (+/- 0.131) g/cm², FN - 0.758 (+/-0.087) g/cm², TH - 0.918 (+/- 0.100)g/cm². In C women mean BMD of L-spine was 1.009 (+/- 0.099)g/cm², FN - 0.791(+/- 0.086)g/cm², TH - 0.977 (+/- 0.107)g/cm², with no significant difference in mean BMD of spine (P = 0.190), FN (P =0.284) and TH (P = 0.071) between groups. There was an inverse correlation between length of lactation and BMD of L-spine in combined study group (P = 0.013), but no correlation with BMD of FN and TH. There was negative correlation between the age of menarche and BMD of FN(P=0.037) and TH (P=0.02) in combined study group regardless of parity.

Conclusions: We discovered no significant difference in BMD in ethnically homogeneous, well matched premenopausal GM AJ women and AJ women with two or fewer term pregnancies. Inverse relationship was noted between length of lactation and spine BMD, and between age of menarche and BMD of FN and TH, independent of parity. Our findings suggest preservation of BMD in GM women.

1. Henderson III PH, Sowers MF, Kutzko KE, Jannausch ML. Bone mineral density in Grand multiparous women with extended lactation. *Am J Obstet Gynecol* 2000;137:1-77.
2. Chesney RW, Specker BL, Mimouni F, McKay S. Mineral metabolism during pregnancy and lactation. In: Coe FL, Favus MJ, editors. *Disorders of bone and mineral metabolism*. London: Raven Press; 1992. p. 383-93.
3. Kalkwarf H, Specker B, Bianchi D, Ranzj, Ho M. The effect of calcium supplementation on bone density during lactation and after weaning. *N Engl J Med* 1997;337:523-8. Kent GN, Price RI, Gutteridge DH, Smith M, Allen JR, Bhagat CI, et al. Human lactation: forearm trabecular bone loss, increased bone turnover, and renal conservation of calcium and inorganic phosphate with recovery of bone mass following weaning. *J Bone Miner Res* 1990;5:361-9.
4. Sowers MF, Corton G, Shapiro B, Jannausch ML, Crutchfield M, Smith ML, et al. Changes in bone density with lactation. *JAMA* 1993;269:3130-5.
5. Prentice A, Jarjou L, Stirling D, Buffenstein R, Fairweather-Tait S. Biochemical markers of calcium and bone metabolism during 18 months of lactation in Gambian women accustomed to a low calcium intake and those consuming a calcium supplement. *J Clin Endocrinol Metab* 1998;83:1059-66
6. Walker ARP, Richardson B, Walker F. The influence of numerous pregnancies and lactations on bone dimensions in South African Bantu and Caucasian mothers. *Clin Sci* 1972;42:189-96.

Nothing to Disclose: EIK, RK, HA, GS

P3-163

Rate of Bone Loss in Spine & Hip BMD and the Predictive Factors in Asian Women during Menopausal Transition.

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Objective: To investigate the rate of change in spine and hip BMD and predictive factors in Asian women during menopausal transition. Material & Methods: A total of 161 community dwelling treatment-naïve Chinese women aged 45-55 years were followed annually for 5years. Serial BMD, clinical data, serum E2 & FSH were obtained. Menstrual status at each visit was determined according to Stages of Reproductive Aging Workshop (STRAW) of the Practice Committee of American Society for Reproductive Medicine. Results: At baseline, 80.1% of the subjects were premenopausal, 19.3% perimenopausal and 0.6% postmenopausal. At the end of study, 12.4% remained premenopausal, 30.4% changed from pre- to perimenopausal, 35.4% from pre- through peri- to post-menopausal and 21.2% from peri- to postmenopausal. The annualized BMD loss at the spine, femoral neck and total hip was $1.3 \pm 1.3\%$, $1 \pm 1.7\%$ and $0.7 \pm 1.1\%$. The rate of bone loss according to menopausal transition and STRAW stagings is shown in Tables 1&2.

Annual BMD changes in percentage (%) with S.D.

| menopausal stage | Annualized bone loss L1-4 | Annualized bone loss neck of femur | Annualized bone loss total hip |
|------------------|---------------------------|------------------------------------|--------------------------------|
| pre-pre | -0+/-0.8%* | -0.6+/-1.3% | -0+/-0.7% |
| pre-peri | -1+/-1.1%* | -0.8+/-1.6% | -0.5+/-0.9% |
| pre-peri-post | -1.6+/-1.1% | -1+/-1.6% | -0.8+/-1.2% |
| peri-post | -1.7+/-1.5% | -1.6+/-2%# | -1.2+/-1.4%# |

*p</=0.01(ANOVA) with all other groups, #p</=0.05 with pre-pre and pre-peri groups

Annual BMD changes according to different Straw stagings in percentage with S.D.

| Straw staging | annualized bone loss of L1-4 | annualized bone loss of femoral neck | annualized bone loss of total hip |
|------------------|------------------------------|--------------------------------------|-----------------------------------|
| within -4 and -3 | -0.2+/-1.6% | -0.2+/-2.1% | +0.2+/-1.2% |
| -4/-3 to 2/-1 | -1.6+/-3.5% | -1.6+/-3.5% | -1.3+/-1.7% |
| within -2 and -1 | -2.7+/-2.5% | -2.1+/-3.3% | -1.6+/-2.3% |
| -2/-1 to 0/1/2 | -2.5+/-3% | -1.5+/-2.8% | -1.3+/-2.7% |
| within 0/1/2 | -1.6+/-1.5% | -1.5+/-2.2% | -0.9+/-1.8% |

Maximum bone loss was seen in the pre-peri-post and peri-post group. Body weight, change in menstrual pattern, menopausal age and FSH level correlate with the rate of bone loss. In multiple logistic regression, only the change in menstrual pattern and menopausal age were independent predictors. Conclusion: The maximum bone loss occurs at the perimenopausal stage. Strategies to prevent bone loss should best be reinforced during this stage. FSH represents the change in menstrual pattern and by itself is not an independent predictor of rate of bone loss.

Nothing to Disclose: EYC, AWK

P3-164

The Differential Relationship between Fat Mass and Bone Mineral Density by Gender and Menopausal Status.

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Introduction Osteoporosis and obesity are important public health problems in aging society. Most previous studies regarding the relationship between osteoporosis and obesity did not adjust for the mechanical loading effects of body weight on bone. Furthermore, there are gender differences in body fat distribution and the relative contribution of adipose tissue to produce estrogen is not equal according to gender or menopausal status. Therefore, to establish the precise relationship between bone and fat, men and pre- and post-menopausal women should be analyzed separately. The purpose of this study was to investigate the differential impacts of fat on bone mineral density (BMD) according to gender and menopausal status after adjusting body weight.

Methods We analyzed the baseline data of an ongoing observational cohort study, including a total of 502 healthy subjects 20-88 years of age (144 men, 159 premenopausal women, 199 postmenopausal women). Body composition and fat mass were measured using computed tomography (CT) and dual energy X-ray absorptiometry (DXA). BMD was measured using DXA.

Results In men and postmenopausal women, there was no significant correlation between fat and bone parameters after adjusting for age and body weight. However, in premenopausal women, BMD had significant negative correlations with waist circumference ($r=-0.20$; $p=0.02$), total fat area ($r=-0.26$; $p<0.01$), subcutaneous fat area ($r=-0.30$; $p<0.01$), appendicular fat mass ($r=-0.32$; $p<0.01$) and percentage fat mass ($r=-0.32$; $p<0.01$) even after adjusting for both age and body weight. Furthermore, only in premenopausal women, the subjects with the highest quartile of percentage fat mass had the lowest BMD even after adjusting for confounding factors including age, body weight, physical activity, alcohol use and smoking history. Multiple linear regression analysis showed that only body weight was a significant determinate factor for BMD in men. In postmenopausal women, age and body weight were conclusive factors for BMD. However, percentage fat mass, independent of body weight and age, was a significant negative decisive factor for BMD in premenopausal women.

Conclusions Our study showed the differential relationship between fat mass and BMD according to gender and menopausal status. Only in premenopausal women did fat mass have a significant negative effect on bone mass. This study suggests the importance of reducing fat mass in order to achieve peak bone mass in young adult women.

Nothing to Disclose: SJY, HYC, HJY, YJK, CRE, JHK, HYK, JAS, SGK, NHK, KMC, SHB, DSC

P3-165

Thyroid Hormone Status Is Associated with Bone Mass and Fracture Risk in Healthy Young Men.

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Context: Thyroid hormone is a regulator of bone metabolism. Thyrotoxicosis and hypothyroidism are known to have deleterious effects on the bone, both in growing children as in adulthood, and have been associated with fracture risk. However, little is known about the effect of variance in thyroid hormone status, within the physiological range, on bone geometry and density.

Objective: We aimed to investigate thyroid hormone concentrations and TSH in relation to parameters of bone geometry and bone density and fracture risk.

Design and setting: Healthy male siblings (n=677) at the age of peak bone mass (25-45 yrs) were recruited in a cross-sectional, population-based study. Trabecular and cortical bone parameters of the radius and cortical bone parameters of the tibia were assessed using peripheral quantitative computed tomography (pQCT). Areal bone mineral density (BMD) was determined using DXA. Thyroid hormones were determined using immunoassays and fracture prevalence was assessed using questionnaires. Statistical analysis was performed using linear mixed-effects modeling and adjusted for age, length, weight and current smoking. Men with positive thyroid autoimmunity (n=32) were excluded from further analysis.

Results: Free triiodothyronine (fT3) was inversely associated with bone mineral density and bone mineral content at the hip ($\beta = -0.08 \pm 0.03$, $p = 0.02$ and $\beta = -0.08 \pm 0.03$, $p = 0.02$ respectively), the spine ($\beta = -0.10 \pm 0.04$, $p = 0.008$ and $\beta = -0.08 \pm 0.03$, $p = 0.02$) and the total body ($\beta = -0.09 \pm 0.03$, $p = 0.007$ and $\beta = -0.08 \pm 0.03$, $p = 0.006$). Using pQCT we observed an inverse relationship between free thyroxine (fT4) and trabecular density at the distal radius ($\beta = -0.08 \pm 0.04$, $p = 0.04$). Finally, we found that with decreasing TSH (OR: 1.20/SD decrease, $p = 0.037$), odds ratios for prevalent fractures increased.

Conclusion: In healthy young men, variations in thyroid hormone concentrations within the physiological range can influence bone density and fracture risk.

Nothing to Disclose: GLR, SG, HGZ, YET, JMK

P3-166

Bone Mineral Density in Korean Men.

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Male osteoporosis is a serious health problem that cannot be overlooked. To determine the hormonal and lifestyle risk factors for low BMD in Asian men, we studied 1452 community-dwelling Korean men aged 30 years and above. Medical history and lifestyle habits were obtained with a structured questionnaire. Dietary calcium, educational status, smoking and alcohol habits, weekly activities were assessed by a semi-quantitative questionnaire. Geometry and BMD at the spine and hip were measured by dual-energy X-ray absorptiometry(DXA). Fasting blood was analyzed for 25(OH)D, parathyroid hormone (PTH), lipid profile, HbA1c and other laboratory parameters. The mean age of the cohort was 48.3±16.1 (30-92) years.

Table 1. Baseline characteristics (mean ± SD) of the all subjects (n = 1452)

| Parameter | | |
|---------------------------------------|-------|------------------------------|
| Age (years) | | 48.3 ± 16.1 |
| Age ≥ 50 (n) | | 657 (45.2 %) |
| Weight (kg) | | 68.34 ± 10.95 |
| Height (m) | | 1.69 ± 0.07 |
| Waist (cm) | | 84.02 ± 9.12 |
| BMI (kg/m ²) | | 23.78 ± 3.18 |
| | < 18 | 37 (2.5 %) |
| | ≥ 25 | 483 (33.3 %) |
| | ≥ 30 | 49 (3.4 %) |
| Smoker (current/history) | | 688 (45.9 %) / 1222 (81.6 %) |
| Drinker | | 1358 (93.9 %) |
| Osteoporosis | | 272 (18.1 %) |
| Severe osteoporosis | | 1 (< 1 %) |
| Osteoporotic fracture | Wrist | 0 (0 %) |
| | Hip | 0 (0 %) |
| | Spine | 1 (< 1 %) |
| Recognized osteoporosis | | 8 (4 %) |
| Osteoporosis treatment | | 4 (2 %) |
| Fall in past 1year | | 169 (11.3 %) |
| Limitation of activity | | 229 (15.3 %) |
| Back pain | | 377 (25 %) |
| Parental history of osteoporosis | | 227 (15.1 %) |
| Arthritis | | 168 (11.2 %) |
| Osteoarthritis | | 147 (9.8 %) |
| Rheumatoid arthritis | | 23 (1.5 %) |
| L1-4 BMC (g) | | 63.84 ± 14.42 |
| L1-4 BMD (g/cm ³) | | 0.97 ± 0.14 |
| T score L1-4 | | -0.46 ± 1.17 |
| L1-L4 area (cm ²) | | 65.72 ± 9.76 |
| Femoral neck BMC (g) | | 4.49 ± 0.81 |
| Femoral neck BMD (g/cm ³) | | 0.81 ± 0.14 |
| Femoral neck area (cm ²) | | 5.55 ± 0.36 |
| Total hip BMC (g) | | 40.92 ± 6.80 |
| Total hip BMD (g/cm ³) | | 0.96 ± 0.13 |
| T score total hip | | 0.17 ± 0.95 |
| Total hip area (cm ²) | | 42.50 ± 4.00 |
| Serum PTH (pg/mL) | | 57.96 ± 23.16 |
| Serum 25(OH)D (ng/mL) | | 24.46 ± 27.20 |
| Serum ALP (IU/L) | | 230.73 ± 68.17 |

We divided subjects into 2 different groups based on the BMD.

Table 2. Comparison of the variables (mean \pm SD with or without osteoporosis (n= 1450)

| Parameter | Control (n=1300) | Osteoporosis (n=270) | p-value |
|---------------------------------------|------------------------|-----------------------|---------|
| Age (year) | 45.7 \pm 15.6 | 50.3 \pm 15.8 | <0.001 |
| Age \geq 65 (n) | 409 (31.4%) | 104 (38.5%) | <0.001 |
| Weight (kg) | 70.00 \pm 13.00 | 69.75 \pm 13.05 | 0.721 |
| Height (cm) | 1.70 \pm 0.07 | 1.67 \pm 0.07 | <0.001 |
| Peak height (cm) | 1.70 \pm 0.06 | 1.67 \pm 0.06 | <0.001 |
| Waist (cm) | 84.68 \pm 8.80 | 80.20 \pm 9.30 | 0.003 |
| BMI (kg/m ²) | 24.22 \pm 3.09 | 25.85 \pm 3.04 | 0.190 |
| < 18 | 15 (1.2%) | 22 (8.2%) | |
| > 25 | 389 (30.0%) | 31 (11.5%) | 0.001 |
| > 30 | 49 (3.8%) | 9 (3.3%) | 0.903 |
| Current smoker | 102 (7.8%) | 113 (41.7%) | 0.000 |
| Previous smoker | 386 (29.7%) | 120 (44.4%) | 0.000 |
| Smoking time (year) | 27.40 \pm 13.00 | 30.40 \pm 13.04 | <0.001 |
| Smoking quantity (cigarettes/day) | 14.77 \pm 9.21 | 16.34 \pm 10.7 | 0.479 |
| Smoking quantity (packs/year) | 206.00 \pm 108.20 | 209.02 \pm 105.05 | 0.879 |
| Drinker | 1109 (85.2%) | 234 (87.2%) | <0.001 |
| Drinking time (year) | 27.20 \pm 14.20 | 26.60 \pm 15.74 | <0.001 |
| Drinking quantity | 34.20 \pm 14.00 | 32.75 \pm 17.04 | <0.001 |
| Personal history of osteoporosis | 177 (13.6%) | 11 (4.1%) | 0.04 |
| Asthma | 110 (8.5%) | 46 (16.9%) | <0.001 |
| Cholesterolemia | 80 (6.2%) | 43 (15.9%) | <0.001 |
| Diagnosed arthritis | 17 (1.3%) | 5 (1.9%) | 0.420 |
| Joint scores | 9.34 \pm 7.04 | 8.8 \pm 8.20 | 0.014 |
| Limitation of activity | 121 (9.3%) | 77 (28.5%) | <0.001 |
| Work grade | 272 (20.9%) | 43 (15.9%) | 0.006 |
| Public | 122 (9.4%) | 70 (25.9%) | 0.000 |
| Public frequency (within 2 year) | 3.2 \pm 14.4 | 2.4 \pm 2.32 | 0.200 |
| Income (10 thousand Won) | 4195.20 \pm 43145.43 | 3199.07 \pm 2031.21 | 0.007 |
| Education | 6.44 \pm 3.01 | 5.36 \pm 3.02 | <0.001 |
| Education (years) | 2.49 \pm 1.01 | 2.2 \pm 1.10 | <0.001 |
| Drinking time per day | 6.40 \pm 2.00 | 6.02 \pm 2.20 | 0.100 |
| Alcohol ^a | 2.40 \pm 1.71 | 2.9 \pm 0.92 | 0.432 |
| Exercise days per week | 2.2 \pm 1.77 | 3.04 \pm 1.61 | 0.001 |
| Energy intake (kcal/day) | 2102.2 \pm 400.70 | 1891.01 \pm 713.00 | <0.001 |
| Calcium intake (mg/day) | 519.20 \pm 141.20 | 498.70 \pm 103.70 | 0.002 |
| Serum PTH (ng/dL) | 34.24 \pm 20.17 | 32.31 \pm 20.07 | 0.864 |
| Serum 25(OH)D (ng/dL) | 24.41 \pm 19.40 | 24.00 \pm 8.22 | <0.001 |
| Serum ALP (U/L) | 226.20 \pm 42.04 | 226.05 \pm 42.14 | <0.001 |
| L1-4 BMC (g) | 47.24 \pm 12.98 | 46.07 \pm 9.70 | <0.001 |
| L1-4 BMD (g/cm ³) | 1.01 \pm 0.12 | 0.97 \pm 0.09 | <0.001 |
| T score L1-4 | -0.22 \pm 0.86 | -0.51 \pm 0.80 | <0.001 |
| L1-4 area (cm ²) | 40.00 \pm 9.24 | 41.27 \pm 10.47 | <0.001 |
| Femoral neck BMC (g) | 4.70 \pm 0.72 | 4.38 \pm 0.47 | <0.001 |
| Femoral neck BMD (g/cm ³) | 0.95 \pm 0.12 | 0.91 \pm 0.08 | <0.001 |
| Femoral neck area (cm ²) | 5.00 \pm 0.26 | 5.12 \pm 0.26 | 0.275 |
| Total hip BMC (g) | 40.00 \pm 6.01 | 38.01 \pm 4.79 | <0.001 |
| Total hip BMD (g/cm ³) | 1.00 \pm 0.11 | 0.97 \pm 0.09 | <0.001 |
| T score total hip | 0.42 \pm 0.81 | -0.51 \pm 0.81 | <0.001 |
| Total hip area (cm ²) | 40.00 \pm 5.00 | 40.00 \pm 4.00 | 0.001 |
| Arm length | 52.46 \pm 5.07 | 52.29 \pm 5.41 | 0.004 |

Univariate logistic regression analysis showed that age, body mass index (BMI), Audit score, cigarette smoking, energy intake, weight bearing exercise, level of education, all of geometry data independently associated with osteoporosis. In the linear regression model, weight, age, body mass index (BMI) were significant determinants of total hip and femur neck BMD. Body mass index being the most important determining factor. After adjusting for age and body mass index (BMI), weight bearing exercise was identified as additional determinants of total hip and femur neck BMD. In the linear regression model, weight, age, body mass index (BMI) were significant determinants of total hip BMD. Strategies to prevent bone loss and osteoporosis in Asian men should include lifestyle modification such as weight bearing exercise regularly and quit smoking.

Nothing to Disclose: KJK, EYL, KMK, HSC, YMR, SKL

P3-167

Osteoporosis and Osteopenia in Both Early and Late Parkinson's Disease in Men.

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Introduction:

Osteoporosis and osteopenia are common in neurologic diseases that affect physical activity and mobility. In Parkinson's disease (PD), decreased motor function and postural instability increase the risk for osteoporosis/osteopenia, falls, and potentially fractures.

Objectives:

- 1) To describe bone health-related characteristics of male PD subjects
- 2) To compare male PD subjects with PD less than 5 years (early PD group) versus 5-10 years (late PD group) in terms of bone health-related behavior and bone mineral density (BMD).

Methods:

Medical history was obtained. Bone health questionnaires were administered. BMD was obtained using DEXA. Comparisons between groups were performed using t-test for continuous variables and chi-square for discrete variables.

Findings:

Sixty-four males were included.

Comparison between subjects with Late and Early PD

| Variable | All subjects with late PD | All subjects with early PD | P value | Late PD subjects with BMD | Early PD subjects with BMD | P value |
|--|---------------------------|----------------------------|---------|---------------------------|----------------------------|---------|
| N | 38 | 26 | | 19 | 17 | |
| Age ± SD (yrs) | 67.8 ± 11.1 | 64.6 ± 10.1 | 0.249 | 67.9 ± 11.9 | 66.1 ± 7.5 | 0.577 |
| Duration of PD from time of diagnosis ± SD (yrs) | 7.2 ± 1.5 | 2.7 ± 1.5 | <0.001 | 7.3 ± 1.2 | 2.6 ± 1.5 | <0.001 |
| Duration of PD from time of first symptom ± SD (yrs) | 9.4 ± 5.0 | 5.8 ± 3.5 | 0.001 | 10.9 ± 6.5 | 5.5 ± 2.8 | 0.003 |
| % taking calcium supplements | 52.6 | 34.6 | 0.155 | 52.6 | 35.3 | 0.296 |
| % taking vitamin D supplements | 52.6 | 38.5 | 0.265 | 52.6 | 35.3 | 0.296 |
| % on medications specific for osteopenia/osteoporosis | 7.9 | 3.8 | 0.511 | 10.5 | 0.0 | 0.169 |
| % with previous bone density | 15.8 | 15.4 | 0.965 | 10.5 | 11.8 | 0.906 |
| Spine BMD (g/cm ²) | | | | 1.031 ± 0.217 | 1.137 ± 0.173 | 0.129 |
| Femoral neck BMD (g/cm ²) | | | | 0.724 ± 0.083 | 0.797 ± 0.13 | 0.053 |
| Total hip BMD (g/cm ²) | | | | 0.893 ± 0.089 | 0.964 ± 0.126 | 0.055 |
| % with osteopenia or osteoporosis on at least one site | | | | 84.2 | 58.8 | |

Discussion:

Among this PD cohort, 72% of subjects with BMD had osteoporosis/osteopenia. Though the 2008 National Osteoporosis Foundation guidelines recommend BMD testing of men ≥70 years, or men 50-70 years at risk for bone loss, only 15% of the subjects had previously undergone BMD. Preventive and treatment measures were suboptimal. Bone loss was present even in those with early PD, suggesting the need for early osteoporosis screening in male subjects with PD.

Bezza A, Ouzzif Z, Naji H, Achemlal L, Mounach A, Nouijai M, Bourazza A, Mossadeq R, Elmagharoui A. Prevalence and risk factors of osteoporosis in patients with Parkinson's disease. *Rheumatol Int* 2008;28:1205-1209.

Fink HA, Kuskowski MA, Talor BC, Schousboe JT, Orwoll ES, Ensrud KE, for the Osteoporotic Fractures in Men (MrOS) Study Group. Association of Parkinson's disease with accelerated bone loss, fractures and mortality in older men: the

Osteoporotic Fractures in Men (MrOS) Study. *Osteoporosis Int* 2008;19:1277-1282.

Nothing to Disclose: SKD, MSO, MCL

P3-168

Fracture Risk Predictors in Men with Prostate Cancer.

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Introduction: Androgen-deprivation therapy for prostate cancer has been shown to decrease bone mineral density (BMD) and increase the risk of fracture. However, whether duration of gonadotropin releasing hormone (GnRH) agonist use, height loss, prostate specific antigen (PSA), testosterone, estradiol, and 25 hydroxy vitamin D can predict decline in BMD and increase in fracture risk of men with prostate cancer has not been well studied.

Methods: This was a retrospective study of 65 male patients (mean age 77; 47 whites, 15 African-Americans, 3 Hispanics, and 1 Spanish) with prostate cancer who reported height loss of 1 inch or greater, and had radiographic imaging of both thoracic and lumbar spine. Subjects were patients at Veterans Affairs Medical Center in Dallas, Texas. The primary question of the study was to elucidate strongest predictors of declining BMD and increased fracture risk in this patient population. Descriptive statistics were performed using the Wilcoxon Rank Sum Test and Chi-Square analysis stratified by fracture history. Multivariable logistic regression was performed to evaluate predictors of fracture, and multivariable linear regression analysis was used to evaluate the predictors of BMD.

Results: Of the 65 subjects, 23 had fractures, of which 13 had compression fractures in the thoracic and/or lumbar spine. Interestingly, only two of the 13 patients with compression fractures were known to have compression fractures before spine films were taken during first osteoporosis clinic visit. In our study population, neither fracture nor BMD was significantly associated with height loss, testosterone, estradiol, and 25 hydroxy vitamin D levels. In multivariable logistic regression models higher PSA was significantly associated with fractures even after adjusting for height loss, BMD, alkaline phosphatase, and testosterone levels. Moreover, the effect of GnRH therapy duration on the relationship of PSA and fractures was independent of testosterone level.

PSA was also positively correlated with alkaline phosphatase level.

Conclusions: Height loss or BMD did not predict fractures in our study population. Yet by doing spine films at first visit in men with 1" height loss, 11 additional compression fractures were found. This finding significantly changed treatment strategy in those men.

Due to this finding, it may be advisable to check spine radiographs for all men with prostate cancer, especially those with elevated PSA.

Sources of Research Support: University of Texas, Southwestern Medical Center Summer Student Award.

Nothing to Disclose: KN, AYC, UG

P3-169

Lean Mass, but Not Fat Mass, Predicts Bone Mineral Density in Middle-Aged Individuals with Non-Insulin-Requiring Type 2 Diabetes Mellitus.

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Individuals with type 2 diabetes mellitus (T2DM) typically have higher bone mineral density (BMD), but higher fracture risk, than non-diabetics. Fat mass, lean mass and regional muscle strength may contribute separately to BMD in non-diabetics; the effect of body composition components and strength on BMD in T2DM is unclear.

Purpose: To investigate the relationship between BMD and body composition, strength, medications and glycemic control in T2DM.

Methods: Subjects for this cross-sectional analysis were men (n=78) and women (n=53) aged 40-65 years (56 ± 6 yrs) with non-insulin-requiring T2DM. Medications were recorded; HbA1C and leptin were measured. Total body fat and lean mass, as well as total body, hip, and lumbar spine BMD were measured with dual energy x-ray absorptiometry. Abdominal visceral, subcutaneous (SQ) and total abdominal fat were measured with magnetic resonance imaging. Total, upper, and lower body strength were evaluated as the sum of 1-repetition maximum weight lifted in resistance exercises.

Results: Participants had normal BMD at all sites and were overweight (BMI 29 to 42 kg/m²) with well controlled T2DM (HbA1C in women $6.6 \pm 1.2\%$, men $6.7 \pm 1.5\%$). Bivariate analysis revealed that, among women, lean mass was positively associated with total body, hip, femoral neck, and spine BMD. Fat mass, abdominal total and abdominal SQ fat were associated with total body and hip BMD. Upper body strength correlated with femoral neck BMD (all p-values <0.05). Among men, lean mass was positively associated with total body, total hip and femoral neck BMD. Fat mass, total abdominal and SQ fat were associated with total body BMD. Total, upper and lower body strength correlated with BMD at the hip and femoral neck. After stepwise regression analysis, lean mass was the only independent positive predictor of total body, hip, and femur BMD in both sexes (R² range, 0.14-0.30). Thiazolidendione use and fasting leptin did not correlate with BMD, while HbA1C was an independent positive predictor of total body, hip and femoral neck BMD in men only (R² range, 0.03-0.08).

Conclusion: In middle-aged men and women with non-insulin-requiring T2DM, lean mass, but not fat mass, was an independent predictor of BMD. These data suggest that maintenance of lean mass may preserve BMD in those with T2DM who lose weight to improve insulin sensitivity. Strengthening exercises may be an intervention for fracture prevention in T2DM.

Nothing to Disclose: KFM, DAD, KJS, SMJdB, DES

P3-170

Cost-Effectiveness of Oral Bisphosphonates for Osteoporosis at Different Ages and Levels of Life Expectancy.

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Context: Osteoporosis treatment decisions can be difficult at the extremes of age and life expectancy.

Objective: To evaluate the cost-effectiveness of oral bisphosphonate therapy for osteoporosis in women at different ages and life expectancies.

Methods: A Markov simulation model using a societal perspective and lifetime horizon. The base case involved treatment of osteoporosis (T score = -2.5) with an oral bisphosphonate for 5 years compared to no intervention. Women at each age were divided into life expectancy quartiles: the lowest 25th percentile of life expectancy ('sickest' group); the median quartiles (25th to 75th percentile); and the highest 25th percentile of life expectancy ('healthiest' group). Monte Carlo simulations were performed for hypothetical cohorts of white women at 5 year intervals between 50 to 90 years and for each life expectancy group. Data sources include published fracture rates, costs, utility values and mortality risks.

Main Outcome Measures: Costs per quality-adjusted life years (QALY) gained for bisphosphonate therapy compared to no treatment. Cost-effectiveness was defined at a willingness-to-pay of \$US 70,000.

Results: In the median quartiles of life expectancy, lifetime costs per QALY were less than \$27,000 for patients at all ages; treatment became cost-saving at age 75 years, and remained so until age 85. In the healthiest quartile, all costs were less than \$18,000 per QALY and treatment was cost-saving from ages 75 to 85 years. Even among the sickest quartile, although osteoporosis treatment was not cost saving, it remains cost-effective through age 90 with lifetime costs less than \$43,000 per QALY.

Conclusion: Treatment with an oral bisphosphonate for 5 years in women with osteoporosis was cost-effective for all ages and for all quartiles of life expectancy. Advanced age and multiple co-morbidities should not necessarily prevent consideration of osteoporosis treatment based on cost effectiveness.

Disclosures: TJW: Principal Investigator, Amgen, Lilly USA, LLC, Novartis Pharmaceuticals; Speaker, Novartis Pharmaceuticals, Procter & Gamble, GlaxoSmithKline. CSC-E: Consultant, Novartis Pharmaceuticals, Amgen; Clinician, Pfizer, Inc.

Nothing to Disclose: ANP, SKD, LCW

P3-171

Effect of Bisphosphonate and Teriparatide Therapy on Bone Loss in Small Bowel Transplant Recipients - A Longitudinal Analysis.

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Limited data are available on effective treatment (Rx) to maintain skeletal health of small bowel transplant (SBTx) recipients. We examined bone mineral density (BMD) changes in patients following SBTx on no pharmacologic treatment (No Rx), bisphosphonates (Bis) or teriparatide (PTH). We retrospectively identified 41 adult SBTx recipients at University of Pittsburgh Intestinal Rehabilitation and Transplant Center. BMDs at three clinical sites (total hip, femoral neck, lumbar spine), and measures of bone mineral metabolism were assessed pre- and post-SBTx. Ten (24%) patients received Bis, 5 (12%) PTH and 26 (64%) No Rx for osteoporosis. All patients were on calcium and vitamin D. Fourteen (34%) were male. At baseline, mean±standard error (SE) age was 43.7±1.8 years, calcium 8.91± 0.10 mg/dl, parathyroid hormone 63.67±7.28 pg/ml and 25 hydroxy-vitamin D 16.66±1.39 ng/dl. There were no significant differences between groups. Baseline Z-scores (SD comparison to age and gender matched) were significantly lower ([§] p<0.05) at all sites in Rx groups and lower at hip in No Rx group. Z-scores were lower in Rx groups compared to No Rx group (# p<0.05 between groups). Duration between BMD assessments was similar between groups (mean 605.7±59.5 days). There was significant loss of bone density at hip and femoral neck in No Rx group (see table; *p<0.05 for within-group change). Percent decrease in BMD was less in the group treated with Bis compared to the group receiving No Rx (#p<0.05). We conclude there is significant decrease in BMD at hip and femoral neck after SBTx in patients on No Rx. Treatment with Bis and PTH may decrease bone loss in SBTx recipients.

Baseline BMD and change in BMD following Small Bowel Transplantation

| | Group 1: No Rx(n=26) | Group 2:Bis(n=10) | Group 3:PTH(n=5) | p-value | | |
|-------------------------------|-------------------------|-------------------------|-------------------------|---------------------|---------------------|--------|
| | Mean±SE | | | 1 vs 2 | 1 vs 3 | 2 vs 3 |
| Baseline Z-Scores (SD) | | | | | | |
| Hip | -0.45±0.17 [§] | -1.81±0.27 [§] | -2.58±0.48 [§] | <0.001 [#] | <0.001 [#] | 0.13 |
| Femoral Neck | -0.38±0.19 | -1.59±0.21 [§] | -2.02±0.26 [§] | <0.001 [#] | <0.001 [#] | 0.37 |
| Lumbar Spine | -0.45±0.29 | -1.84±0.36 [§] | -1.84±0.41 [§] | <0.01 [#] | 0.04 [#] | 1.0 |
| % Change in BMD | | | | | | |
| Hip | -14.14±2.54* | -4.03±2.55 | -7.98±8.02 | 0.03 [#] | 0.35 | 0.59 |
| Femoral Neck | -14.79±2.58* | -0.35±4.57 | -9.82±4.60 | <0.01 [#] | 0.49 | 0.24 |
| Lumbar Spine | -3.46±1.93 | 10.06±8.33 | -2.47±3.76 | 0.02 [#] | 0.43 | 0.37 |

Sources of Research Support: 2K24DK062895-06 (K24 Award): Greenspan (PI).

Disclosures: SLG: Principal Investigator, Merck & Co., Lilly USA, LLC, Sanofi-Aventis; Consultant, Amgen, Merck & Co.
Nothing to Disclose: NG, JLR, SP, DM, DK, KMA-E

P3-172

Effects of Risedronate on Bone Mineral Density and Bone Metabolic Markers in Patients with Liver Transplantation.

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¹12 de Octubre Univ Hosp Madrid, Spain.

Introduction: Bisphosphonates have shown to prevent the rapid bone loss observed during the first 3-6 postransplant months. This is the first study with Risedronate (RSN) in liver transplant (LTx).

Objectives: To analyze the effect of oral RSN on bone loss and bone resorption markers in patients with low bone mass after LTx.

Patients and Methods: This is a randomized open-label single centre prospective study. 81 LTx patients (62 males, 19 females; 56 ± 8 years) with osteopenia or osteoporosis have been included. In the first month after transplantation, patients were randomly assigned to Group 1 (G1) or Group 2 (G2). G1 (n=45) received oral RSN (35 mg once weekly) plus calcium (1000 mg/day) and vitamin D (800 IU) daily; G2 (n=44) received calcium and vitamin D. Primary endpoint was bone mineral density (BMD) change at lumbar spine and femoral regions (measured at 0, 6 and 12 months after LTx) (Hologic QDR 4500w densitometer); secondary endpoints include changes in serum β -CTX, P1NP and 25-OH vitamin D₃. Spine X-rays were obtained in all patients at months 0 and 12.

Results: At baseline 39% of patients had densitometric criteria of osteoporosis, and 31% had prevalent vertebral fractures. Spine BMD increased in G1 at 6 months ($2.82 \pm 6.92\%$; $p=0.014$) and 12 months ($4.81 \pm 8.81\%$; $p=0.001$). In G2 change in spine BMD was significant at 12 months ($3.35 \pm 5.76\%$, $p=0.001$). At femoral and intertrochanter regions, BMD increased in G1 ($2.65 \pm 7.10\%$; $p=0.04$) and ($3.80 \pm 5.31\%$; $p=0.001$) respectively, but only per protocol analysis. There were not significant differences in BMD change between groups during the study. β -CTX decreased in both groups after 3, 6 and 12 months ($p<0.01$). Differences between groups were only observed in per protocol analysis ($p<0.003$ at 3, 6 and 12 months). P1NP increased in G2 after 6 months of treatment ($p<0.05$). P1NP levels were higher in G2 than in G1 at 6 and 12 months ($p<0.05$). At baseline, 91% of patients had 25-OH vitamin D₃ < 30 ng/ml. In both groups 25-OH vitamin D₃ levels increased at three months ($p<0.001$), maintaining this level throughout the study. At 12 months 52% of patients showed 25-OH vitamin D₃>30 ng/ml. 12 patients had incident fracture, 4 in G1 and 8 in G2.

Conclusions: RSN in recent LTx patients and low bone mass prevents bone loss at spine level since 6 months treatment, and at femoral level after 12 months. RSN effectively reduces bone resorption in these patients. LTx patients have a high prevalence vitamin D insufficiency.

Sources of Research Support: Mutua Madrileña Foundation (2005-072).

Nothing to Disclose: SG, GM, CV, RS, MP, EM, FH

P3-173

Change in Hip Bone Mineral Density with Testosterone and Growth Hormone Administration in Older Men.

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PURPOSE: We hypothesized that supplementation of testosterone (T) & recombinant human growth hormone (GH) in older men will increase hip bone mineral density (BMD). **METHODS:** A multicenter, placebo controlled, double-blind, factorial study assessed T & IGF-1 response & BMD changes after 16wks of treatment in 65-90 yr old men with wk0 IGF-1 in lower adult tertile & T in 200-500 ng/dL range. 122 men were randomized to 6 groups & received transdermal T (5 or 10g/day) & GH (0, 3, or 5ug/kg/day). 4 BMD variables of the hip were assessed by DEXA at wk0 & 17 in 76 participants who completed the scans. **RESULTS:** Intertrochanteric & total hip BMD increased for groups C & F, & trochanteric BMD for group E. Pearson correlation showed an association between increased IGF-1 levels & increased intertrochanteric BMD($r=0.27$; $p=0.03$). **CONCLUSION:** These data suggest that 16 wks of T & GH administration in older men increases site-specific hip BMD; increases in IGF-1 may be central in achieving these effects.

| | T5 | | | T10 | | |
|-------------------|-------------|---------------|-----------------------|--------------------|----------------------|----------------------|
| Group(N) | A(13) GH0 | B(13) GH3 | C(13) GH5 | D(13) GH0 | E(13) GH3 | F(13) GH5 |
| IGF-1 (<0.001**) | 5±21 | 44±49; 0.005* | 113±67; <0.001* | 15±30 | 80±33; <0.001* | 113±44; <0.001* |
| T (0.01**) | 54±294 | 257±459 | 30±294 | 544±491; 0.002* | 419±525; 0.02* | 557±554; 0.003* |
| Intertrochanteric | 0.003±0.03 | -0.016±0.09 | 0.032±0.03; 0.003* | 0.007±0.03 | 0.008±0.03 | 0.014±0.20; 0.03* |
| Femoral Neck | 0.002±0.03 | -0.107±0.39 | 0.005±0.02 | 0.003±0.02 | 0.005±0.03 | 0.008±0.03 |
| Trochanteric | -0.001±0.01 | -0.0684±0.25 | 0.004±0.02 | 0.006±0.02 | 0.015±0.02; 0.02* | 0.011±0.02 |
| Total Hip | -0.001±0.02 | -0.050±0.19 | 0.027±0.03; 0.01* | 0.010±0.02 | 0.012±0.03 | 0.012±0.01; 0.02* |

*paired t-test; **one way ANOVA; significance $p<0.05$

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Nothing to Disclose: LJA, DNE, MK, JH, ETS

P3-174

The Effects of Testosterone and Growth Hormone Administration on Spine Bone Mineral Density in Older Men.

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Purpose: The combined effects of testosterone (T) and recombinant human growth hormone (GH) supplementation on spine bone mineral density (sBMD) in older men is not well studied. We hypothesized that augmenting T and GH to youthful levels in older men would increase sBMD. **Methods:** This was a placebo controlled, double-blind, factorial study to assess T & GH dose levels in hyposomatotropic (IGF-1 in lower 3rd), low/hypogonadal (T 200-500 ng/dL) 65-90 yr old men for 16wks. 122 men were randomized to 6 treatment groups with 76 completing all scans. Men received transdermal T (5 or 10g/day) and GH (0, 3, or 5ug/kg/day) to investigate the separate and interactive effects on sBMD (see table below). sBMD was assessed by DEXA at wk0 and wk17. Data were analyzed by paired t tests, one-way ANOVA across groups and by Pearson's correlations. Significance was set at $p \leq 0.05$. **Results:** The Table shows the change in T and IGF-1 levels, and sBMD for the 6 groups. Lumbar spine values did not significantly increase for any of the treatment groups. There was a significant correlation between change in IGF-1 and sBMD ($r=0.35; p=0.02$) with the correlation between change in T and sBMD nearing significance ($r=0.22; p=0.06$). **Conclusion:** These data suggest that 16wks of T and GH supplementation was not sufficient to alter sBMD in older men. A possible reason for this finding may include the short treatment period. However correlation data suggests that increasing IGF-1 levels leads to an increase in sBMD. Further studies are needed to elucidate the role of IGF-1 on sBMD.

| | T 5 | | | T 10 | | | |
|----------------------------|------------|------------|------------|------------|-------------|------------|---------|
| GH | 0 | 3 | 5 | 0 | 3 | 5 | |
| Group (N) | A(13) | B(13) | C(13) | D(13) | E(11) | F(13) | P |
| sBMD (gm/cm ²) | 0.003±0.04 | 0.001±0.03 | 0.014±0.05 | 0.011±0.03 | 0.0003±0.03 | 0.014±0.05 | 0.88 |
| P | 0.82 | 0.92 | 0.30 | 0.17 | 0.97 | 0.34 | |
| T (ng/dl) | 54±293.5 | 257±458.6 | 30±294.0 | 544±490.5 | 419±525.1 | 557±554.3 | 0.007** |
| P | 0.52 | 0.06 | 0.72 | 0.002* | 0.024* | 0.003* | |
| IGF-1 (ng/ml) | 5±20.5 | 44±49.1 | 113±67.2 | 15±30.0 | 80±32.9 | 113±44.0 | 0.001** |
| P | 0.39 | 0.005* | 0.001* | 0.09 | 0.001* | 0.001* | |

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Nothing to Disclose: DEE, LJA, MK, JH, ETS

P3-175

Phase 3 Fracture Trial of Odanacatib for Osteoporosis - Study Design.

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The number of patients who benefit from osteoporosis therapy is growing steadily. Most current anti-resorptive drugs are either bisphosphonates or act via the estrogen receptor. Odanacatib selectively and reversibly inhibits cathepsin K, a collagenase secreted by osteoclasts. In a 2-year, phase 2, dose-finding study and its 1-year extension of postmenopausal women with low BMD, oral odanacatib 50 mg once-weekly increased BMD progressively over 36 months compared to placebo, and was similar to placebo in terms of safety and tolerability. The incidence of cutaneous adverse experiences was similar among treatment groups. A randomized, double-blind, phase 3 trial has enrolled postmenopausal osteoporotic women to receive odanacatib 50 mg or placebo once weekly with or without food for approximately 36 months. Participants will also receive vitamin D₃ 5600 IU weekly. They are ≥ 65 years old, with or without a prior vertebral fracture (BMD T-score at either total hip or femoral neck ≤ 1.5 and ≤ 2.5 , respectively). Participants without prior vertebral fracture were enrolled so that at least 2/3 were ≥ 70 years old. This trial has three primary endpoints: morphometric vertebral fracture, hip fracture, and non-vertebral fracture (with controls for elevation of the false-positive error rate due to having multiple primary endpoints). Fractures will be adjudicated centrally via clinical history, radiology reports, and/or x-rays. Secondary endpoints include clinical vertebral fractures, BMD, height loss, bone turnover markers, and safety and tolerability. Collection of trans-iliac bone biopsies from some participants, archived serum and urine samples, and pharmacogenomic data will provide additional information about osteoporosis and the effects of odanacatib from this large trial. This event-driven trial will be completed after pre-specified numbers of fractures have occurred. Enrollment at approximately 380 centers world-wide was completed in November 2009 with approximately 16,200 patients randomized. The results of this trial will determine whether once-weekly odanacatib 50 mg is safe and effective in reducing the risk of osteoporotic fractures in postmenopausal women with osteoporosis.

Sources of Research Support: Merck & Co., Inc.

Disclosures: HB: Research Funding, Merck & Co. DD: Research Funding, Merck & Co. JAE: Research Funding, Merck & Co. SG: Research Funding, Merck & Co. MM: Research Funding, Merck & Co. TN: Research Funding, Merck & Co. SP: Research Funding, Merck & Co. JS: Research Funding, Merck & Co. AL: Employee, Merck & Co. AS: Employee, Merck & Co. NV: Research Funding, Merck & Co. ER: Employee, Merck & Co. AL: Employee, Merck & Co.

P3-176

Effect of Intravenous Bisphosphonate Therapy among Boys with Duchenne Muscular Dystrophy and Osteoporosis: Clinical Outcomes.

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Introduction: Boys with Duchenne Muscular Dystrophy (DMD) may develop symptomatic vertebral fractures. Bisphosphonates have been used to treat the spine fragility; however, detailed analyses of the response to therapy are lacking. The objective of this study was to assess the efficacy and safety of IV bisphosphonate treatment in boys with spinal osteoporosis due to DMD.

Methods: This was a one-year, retrospective observational study of 7 boys (age 8.5-14.3 years) with DMD who had received either IV pamidronate (9 mg/kg/year) or zoledronate (0.1 mg/kg/year) to treat painful vertebral fractures. The primary outcome was change in vertebral morphometry at 12 months post-bisphosphonate initiation. Secondary outcomes included back pain status, changes in lumbar spine volumetric bone mineral density (vBMD), and adverse events.

Results: A description of the cohort is presented in the Table. All but one had received glucocorticoid therapy prior to treatment initiation, and all but one was non-ambulatory. There were 27 fracture events noted in the 7 patients at baseline; 15/27 were in the T4-T9 region. Grade 2 (moderate) and Grade 3 (severe) vertebral fractures reconstituted at 12 months, and this was associated with either improvement or complete resolution of back pain. The median spine vBMD also increased. First-dose side effects were present in 4 patients and included fever (N=2), nausea (N=2), myalgias (N=3) and hypocalcemia (N=2).

Conclusion: In boys with spinal osteoporosis and DMD, IV bisphosphonate therapy administered over 12 months was associated with improved vertebral morphometry and density; similarly, there was amelioration in back pain. The therapy was generally well-tolerated.

Table. Clinical Parameters Pre- and 12 Months Post-Treatment

| Clinical Characteristics | Results 12 Months Post Bisphosphonate Initiation (N=7) | |
|--|--|-------------------|
| | Pre-Treatment | 12 Months Post |
| Anthropometry | | |
| Height Z-score | -1.7 (-4.2, -0.5) | -2.0 (-3.5, -0.1) |
| Weight Z-score | 0.4 (-2.4, 1.8) | -1.7 (-1.9, 1.9) |
| Vertebral Morphometry | | |
| Genant Grade for VF Events, N (%) | | |
| Grade 0.5 = 15-19.9% loss in VH | 5 (18%) | 11 (41%) |
| Grade 1 = 20-25% loss in VH | 4 (15%) | 7 (26%) |
| Grade 2 = 25.1-40% loss in VH | 14 (52%) | 9 (33%) |
| Grade 3 = >40% loss in VH | 4 (15%) | 0 (0%) |
| Lumbar Spine Volumetric BMD (Z-score) | -1.0 (-3.0, 0.9) | -0.1 (-2.6, 1.4) |

Values reported are median (min, max) unless otherwise specified. VH=Vertebral height, BMD= Bone mineral density.

Disclosures: LMW: Consultant, Novartis Pharmaceuticals.

Nothing to Disclose: AMS, FR, VK, MT, PJ

P3-177

Effects of Pamidronate Treatment in Non-Ambulatory Children with Spastic Quadriplegia and Other Neuromuscular Disorders.

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Objectives: 1) To examine the bone mineral density (BMD) and biochemical marker responses to intravenous pamidronate in non-ambulatory children with various neuromuscular disorders. 2) To determine if there are differences in BMD pre and post treatment in 2 groups of patients classified by muscle tonicity: spasticity group (spastic quadriplegia) and hypotonicity group. Our hypothesis is that the patients with hypotonicity with decreased osteogenic signals from the muscles that normally stimulate bone formation may have more severe low bone density and less response to pamidronate 3) To assess laboratory variables at baseline that may impact BMD response to pamidronate.

Methods: Forty (mean age 10.5 years, 27 females) non-ambulatory patients with low BMD and/or history of fractures were studied. Thirty-two (80%) was spastic quadriplegia, 8 was hypotonicity group (5 myelomeningocele, 3 spinal muscular atrophy). Intravenous pamidronate, 1 mg/kg/day was administered 3 consecutive days, repeated at 3 month intervals for 1 year. Lumbar BMD (Hologic DEXA), Z score (adjusted for weight and pubertal status) and biochemical values at baseline and 1 year were analyzed.

Results: Mean BMD and Z score increased significantly from baseline to 1 year in spasticity group (0.44 to 0.51 gm/cm², $p < 0.001$; Z score -3.5 to -2.6; $p = 0.008$). In hypotonicity group, BMD increased significantly at 1 year (0.39 to 0.43 gm/cm²; $p = 0.004$), with an upward trend in Z score (-4.2 to -3.7, $p = 0.06$). There were no differences in BMD response between 2 groups. There were no differences in serum calcium, phosphorus, alkaline phosphatase, 25-OH vitamin D, 1,25(OH)₂ vitamin D, PTH between baseline and 1 year and between groups. Urine pyridinoline and deoxypyridinoline decreased from baseline (293 and 66 $\mu\text{mol/molCr}$) at 1 year (274 and 46 $\mu\text{mol/molCr}$), but the decrease was not statistically significant. Serum 25-OH vitamin D was found to be negatively correlated with BMD Z score response ($p = 0.04$).

Conclusion: Pamidronate treatment is effective in improving BMD and BMD Z score in children with neuromuscular disorders, both spasticity and hypotonicity groups. There were no differences in BMD response between 2 groups. There were no differences in biochemical markers between these 2 groups and between pre and post treatment. Lower vitamin D at baseline resulted in more response in BMD Z score at 1 year. Further study is needed to examine the effects of vitamin D in treatment response.

Nothing to Disclose: SAB, AB, WW, JDM

P3-178

Comparison of the Clinical Characteristics of Bisphosphonate-Related Osteonecrosis of the Jaw (BRONJ) with Non-BRONJ in Osteoporotic Patients.

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Backgrounds: Oral bisphosphonates are commonly used as a treatment for osteoporosis. Recently, there have been increasing reports of osteonecrosis of the jaw (ONJ) in osteoporotic patients using oral bisphosphonates. ONJ could occur in conditions with oral infections or benign diseases. We investigated the differences of bisphosphonate-related ONJ (BRONJ) with non-BRONJ.

Methods: We reviewed medical records with key words of osteonecrosis, sequestrum, or dead bone of mandible or maxilla from January 2003 to July 2009 in Ajou University Hospital. We found 22 cases and reviewed clinical characteristics. By using criteria of BRONJ from the American Association of Oral and Maxillofacial Surgeons, 11 cases were diagnosed with BRONJ and 11 cases non-BRONJ. The non-BRONJ group matches BRONJ criteria except exposure history of bisphosphonates.

Results: Mean age of BRONJ group was 74.3 (SD=5.4) years-old and 10 of 11 (91%) were women. In BRONJ group, 6 of 11 (55%) oral lesion occurred in the mandible, 6 of 11 (55%) took medication for diabetes mellitus, 3 of 11 (27%) had glucocorticoid. All of them had oral bisphosphonates and mean duration of administration was 4.0 (SD=0.7) years. Mean age of non-BRONJ group was 50.1 (SD= 21.6) years-old, and 6 of 11 (55%) were women. In non-BRONJ group, 6 of 11 (55%) patients had their lesion in mandible, 2 of 11 (18%) patients had diabetes mellitus, but none had glucocorticoid.

Conclusion: BRONJ patients had tendency to have old age, female predominance, concomitant diseases including diabetes mellitus, and glucocorticoid use compared with non-BRONJ.

Nothing to Disclose: Y-JC, M-SL, E-KK, H-KK, E-JH, H-JK, K-WL, Y-SC, SA, Y-JJ

P3-179

Bone Turnover Markers and OPG/sRANKL Levels in Women with Thyroid Cancer.

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AIM: Thyroid hormones play an important role in bone remodeling. The aim of the present study was to evaluate the changes of bone turnover markers and osteoprotegerin (OPG)/receptor activator of nuclear factor κ B ligand (RANKL) levels during follow-up in a group of women with thyroid cancer.

MATERIAL AND METHODS: Twenty euthyroid women with newly diagnosed differentiated thyroid cancer were enrolled into the study (39.25 ± 13.46 yrs, 6 postmenopausal). Blood and urine samples were collected before thyroidectomy (euthyroid state), in postoperative period, just before radioactive iodine (RAI) administration (hypothyroid state) and under thyroxin suppressive therapy (subclinical hyperthyroid state). Change of bone turnover markers [Bone alkaline phosphatase (BALP), osteocalcin (OC), type 1 procollagen amino-terminal propeptide (P1NP), urine deoxypyridinoline (DPD)], OPG and sRANKL levels were evaluated. Homocysteine (HCY) levels were also measured in a subgroup of patients ($n=15$). Statistical analysis was performed on SPSS 15.0. The study was approved by local Ethical Committee and informed consent was obtained from each subject.

RESULTS: The level of all bone formation markers decreased in hypothyroidism when compared to euthyroid period (BALP, 10.5 ± 3.5 IU/L vs 11.9 ± 5.7 IU/L, $p < 0.05$; OC, 3.4 ± 1.7 ng/ml vs 6.5 ± 3.7 ng/ml, $p < 0.01$; P1NP, 38.3 ± 18.2 ng/ml vs 53.1 ± 33.6 ng/ml, $p < 0.05$), but no significant change was observed in urine DPD. In subclinically hyperthyroid state, bone formation markers (BALP 13.9 ± 7.7 IU/L; OC 5.3 ± 3.1 ng/ml; P1NP 42.5 ± 19.3 ng/ml) increased and reached to the levels of euthyroid state. OPG levels increased in hypothyroid state ($p < 0.001$) and returned to comparable levels in subclinically hyperthyroidism (4.3 ± 1.1 pmol/L in euthyroid period, 7.2 ± 2.3 pmol/L in hypothyroidism, 5.1 ± 1.7 pmol/L in subclinically hyperthyroidism). sRANKL levels did not change during follow-up. Multiple regression analysis showed a significant positive association between OPG and TSH. In addition, there was a correlation between OPG and HCY, if the regression model not included creatinine clearance.

CONCLUSION: Our results indicated that there is a decrease in bone formation and an increase in OPG levels in hypothyroidism, but no change in short subclinically hyperthyroid period. The increased OPG levels in hypothyroidism and significant positive correlations between OPG, TSH and HCY were thought to be related to increased cardiovascular risk of hypothyroidism.

Sources of Research Support: Research Fund of Istanbul University (project no:514).

Nothing to Disclose: SS, AKU, FA, HB, NCO, BO, RT, FA

P3-180

Do DNA Variants of the CaSR Play a Role in Developing Familial Hypocalciuric Hypercalcemia (FHH) in Different Ethnic Populations: The University of Mississippi Experience.

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Heterozygous, inactivating mutations (mut) of the CaSR cause autosomal dom FHH, whereas homozygous mut are seen with autosomal recessive FHH and neonatal HPT. DNA variants (SNPs) of the CaSR with unclear functional effects are identified in various ethnic populations.

Objective: Report geno-and phenotypes of pts with HPT and CaSR SNPs in MS.

Design: Direct sequence analysis of the CASR gene, clinical and biochem analyses.

Results: Table 1.

Demographic, Biochemical, and Genetic Data

| Race/Gender | S-Calcium (mg/dl) | Intact PTH | 25-OH Vitamin D | Fractional excretion of Ca | CaSR SNPs | AA change |
|-------------|-------------------|------------|-----------------|----------------------------|-----------------|--------------|
| AA-F | 10.2 | 82 | 28 | 0.002 | 2244C>G,3031C>G | P748P,Q1011E |
| AA-F | 11.4 | 187 | 14 | 0.003 | 2244C>G,3031C>G | P748P,Q1011E |
| AA-F | 10.9 | 89 | 31 | 0.002 | 2244C>G,3031C>G | P748P,Q1011E |
| AA-F | 11.3 | 91 | 45 | 0.001 | 2411C>A | A804D |
| AA-F | 11 | 93 | 34 | 0.006 | negative | |
| AA-F | 10 | 128 | 22 | 0.006 | negative | |
| C-F | 11.9 | 122 | 12 | 0.01 | negative | |
| C-F | 10.7 | 98 | 47 | 0.003 | negative | |
| C-F | 10.1 | 116 | 41 | 0.001 | negative | |
| C-F | 10.7 | 93 | 15 | 0.005 | negative | |
| C-F | 10.7 | 78 | 108 | 0.005 | 2968A>G | R990G |
| C-F | 8.7 | 84 | 50 | 0.001 | 2968A>G | R990G |
| | | | | | 1608+52G>A | intron 5 |

6 were unrel white (C) and 6 unrel African American (AA) women with case 1 (1) being an immigrant from Nigeria and osteoporotic. None had renal stones. In the AA group, the Q1011E SNP, although rarely reported in Asians and Caucasians (2), was present in 3 of 6 AA pts. The 2244C>G(P748P) heterozyg SNP present concomitantly with SNP Q1011E in these pts was considered not disease-associated (DA). One AA pt had a heterozyg SNP 2411C>A-p.A804D considered to be DA. Interestingly, the Nigerian pt had the same heterozyg SNPs (2244C>G, 3031C>G) as cases 2 and 3 from MS, supporting the idea of the same genetic heritage which originates from West Africa. Heterozyg R990G SNPs were present in 2 C pts. One had bilateral cystosarcomata phylloides of the breast at age 11y. Both had osteopenia.

Conclusion: Ethnic and geographic aspects should be considered when interpreting homozyg and heterozyg SNPs of the CaSR and clinical phenotype. R990G genotype is in significant linkage disequilibrium (2), seen in Arab-Bedouin descent (3), and not always associated with hypercalciuria and renal stones (4). Previously reported "silent" SNPs of the CaSR may actually have functional effects.

1. Mohammed R, Bray MB, Koch CA, Uwaifo GI. Spontaneous rib fractures in a black woman with familial hypocalciuric hypercalcemia. *Med Sci Monit.* 2008 Oct;14(10):CS102-6.
2. Yun FHJ, Wong BYL, Chase M, et al. Genetic variation at the calcium-sensing receptor (CASR) locus: Implications for clinical molecular diagnostics. *Clinical Biochemistry* 2007;40:551-561
3. Lietman SA, Tenenbaum-Rakover Y, Jap TS, et al. A novel loss-of-function mutation, Gln459Arg, of the calcium-sensing receptor gene associated with apparent autosomal recessive inheritance of familial hypocalciuric hypercalcemia. *J Clin Endocrinol Metab* 2009;94:4372-4379
4. Vezzoli G, Terrangera A, Arcidiacono T, et al. R990G polymorphism of calcium-sensing receptor does produce a gain-of-function and predispose to primary hypercalciuria. *Kidney International* 2007;71:1155-1162
5. Hendy GN, D'Souza-Li L, Yang B, Canaff L, Cole DEC. Mutations of the calcium-sensing receptor (CASR) in familial hypocalciuric hypercalcaemia, neonatal severe hyper-parathyroidism, and autosomal dominant hypocalcaemia. *Hum Mutat* 2000;16:281-96.

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Nothing to Disclose: GIU, EM, SG, KP, EKK

P3-181

Acquired Hypocalciuric Hypercalcemia Unresponsive to Glucocorticoid Therapy in a Patient with Autoantibodies Against the Calcium-Sensing Receptor.

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Introduction: Autoantibodies directed against the calcium-sensing receptor (CaSR) have been reported in several individuals with hypocalciuria and acquired PTH-mediated hypercalcemia. We have previously shown that glucocorticoid therapy decreased the CaSR autoantibody titers and normalized the hypercalcemia in a patient with acquired hypocalciuric hypercalcemia. These findings raised the possibility that glucocorticoid responsiveness might be an important feature of autoimmune hyperparathyroidism with possible diagnostic and therapeutic implications.

Methods: We evaluated a patient with acquired hypocalciuric hypercalcemia (Ca 11.3 mg/dL, nl \leq 10.5; iCa 1.46 mmol/L, nl \leq 1.30; PTH 110 pg/mL, nl \leq 60; 24h urine Ca 26 mg, nl \geq 100; Ca:Creatinine clearance ratio 0.003, nl \geq 0.010) and elevated anti-nuclear antibody (ANA) titers for the presence of autoantibodies against the CaSR. Sera from the patient and from 20 healthy controls were tested in immunoprecipitation and flow cytometry assays using the CaSR expressed in HEK293 cells. The patient's biochemical response to a trial of glucocorticoid therapy was also analyzed with measurement of Ca, iCa, PTH, and urinary calcium excretion on and off therapy.

Results: The patient was found to have autoantibodies directed against the CaSR as determined by immunoprecipitation and flow cytometry assays. No immune reactivity against the CaSR was identified in any of the control subjects. Treatment with glucocorticoids had no effect on the patient's calcium or PTH levels. In contrast, urinary calcium excretion increased with glucocorticoid treatment. The peak 24h urine Ca excretion was 102 mg and the peak Ca:Cr clearance ratio was 0.013 while the patient was on prednisone.

Conclusions: We report a patient with markedly elevated ANA titers, acquired hypocalciuric hypercalcemia, and autoantibodies against the CaSR. Unlike our previous patient with autoimmune hypocalciuric hypercalcemia, glucocorticoid therapy failed to normalize calcium and PTH levels.

Disclosures: JCP: Investigator, Solvay Pharmaceuticals, Inc. DMS: Speaker, Lilly USA, LLC, Novartis Pharmaceuticals, Sanofi-Aventis. EMB: Investigator, NPS.

Nothing to Disclose: EHK, CB, APW

P3-182

Bone Mineral Apparent Density and Vitamin D Status in Pediatric Neurofibromatosis Type I Patients.

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Background

Several studies have documented a high prevalence of short stature and low bone mineral density (BMD) in Neurofibromatosis type 1 (NF-1) patients; however, BMD calculations are inherently underestimated in short patients. In order to adjust for differences in height, we normalized BMD to a derived reference volume by correcting BMD values via the use of bone mineral apparent density (BMAD), an estimation of volumetric bone mass g/cm³, and whole body bone mineral content/height (BMC).

Objective

To study BMAD and BMC in children with NF-1 while accounting for gender, age, ethnicity, and stature, and to examine the prevalence of vitamin D deficiency in this group.

Methods

Hologic dual energy x-ray absorptiometry scans (DEXA) from 34 children and adolescents with NF-1 were analyzed (11 (32%) females; mean age 14.7 ±3.5). BMAD of the lumbar spine (LS) 2-4, femoral neck (FN), and total body BMC/ht were measured and Z-scores were calculated using a pediatric reference database (<http://www-stat-class.stanford.edu/pediatric-bones>). Osteopenia was defined as a BMAD score <-2. Serum 25-hydroxyvitamin D2 and D3 levels were measured using high performance liquid chromatography with UV detection. Vitamin D levels were defined as severely deficient (<10 ng/mL), mild to moderately deficient (10-24 ng/mL), and normal (25-80 ng/mL). Data were analyzed using paired t-tests and Pearson correlation coefficients; data are reported as mean ±SD where appropriate.

Results

Our cohort of patients included those with short stature; mean height standard deviation score was -1.2 ±1.0. Forty-eight percent of patients exhibited osteopenia at any bone site, with 40.6% at the LS, 18.2% at the FN, and 21.2% at total body BMC/ht. BMAD Z-scores for the LS (-1.70 ±1.4) were more impaired when compared to the FN (-0.58 ±1.4; p=0.0006) and the whole body BMC/ht (-1.12 ±0.9; p=0.03). Two-thirds of these patients were severely (6.5%) or mild to moderately (58.1%) vitamin D deficient, with 35.5% having normal vitamin D levels. Vitamin D levels did not correlate with BMAD of the LS, FN, or whole body BMC/ht, regardless of vitamin D deficiency status.

Conclusions:

In children with NF-1, vertebral BMAD were more severely affected than FN BMAD or whole body BMC. Vitamin D deficiency is prevalent in these patients but does not appear to correlate with BMAD or BMC scores.

Nothing to Disclose: MBL, EB, NS, AK, AG, AB, JCR, CAS, BW

P3-183

Temporal Assessment of Cranioplasty Resorption Following Decompressive Craniectomy.

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When traumatic brain injury (TBI) is accompanied by increases in intracranial pressure, measures are taken to improve the cerebral perfusion pressure. If conservative strategies fail, decompressive craniectomy is performed with cranioplasty several weeks to months later if there is survival. Due to advantages in economic and biological parameters, autograft cranioplasty is frequently performed. One complication is resorption of the autologous bone flap with rates of 50-100% being reported. When resorption occurs, sinking skin flap syndrome can compromise neurological outcome. Little is known about the timeline or mechanism underlying this complication. A retrospective chart review was carried out to identify patients with autologous cranioplasty for decompressive craniectomy. Information on clinical parameters and radiologic images were obtained. Utilizing the software Analysis of Functional Neuroimages (AFNI), we developed a method to calculate the density and volume loss in the bone flaps over time. CT studies were converted to volume format with the images de-obliqued, aligned to a standard scan and assigned thresholds. Each study was subjected to a mathematical operation of consecutive erosion and dilation in order to isolate the cranium and remove islands of confounding signal representing bone screws, cerebrospinal fluid shunts and medical apparatus present during image acquisition. This resulted in a volumetric measure of the amount of bone representing the cranium. This bone area measure was then used to create a percentage reflecting the amount of bone in each CT relative to the amount of bone in the first post-cranioplasty study. Patients with TBI who underwent autologous cranioplasty displayed a critical window of 30-50 days post-operatively in which the group that had critical resorption separated from the group without critical resorption with the outcome associated with age, especially those younger than 21, and the size of the initial cranial defect. The novel use of AFNI allowed us to map the resorption of patients and determine that there is a critical period for resorption, and by 50 days it is possible to predict which course a patient will take with 95 percent accuracy. It is hoped that this data can serve as the initial step in the development of a clinical prediction tool for identifying those at risk for this devastating complication and will help establish a potential window for therapeutic intervention in future patients.

Nothing to Disclose: MDR, JWG, TME

P3-184

The Renin-Angiotensin System, Blood Pressure and Heart Structure in Patients with Vitamin D Dependent Rickets Type II.

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Context: Studies in vitamin D receptor (VDR) knockout (VDR-KO) mice revealed an overstimulated renin-angiotensin system (RAS), high blood pressure and cardiac hypertrophy. VDR-KO mice correspond phenotypically and metabolically to humans with VDR loss-of-function mutations - vitamin D dependent rickets type II (VDDR II).

Objective: We examined the RAS, blood pressure levels, and cardiac structures in 17 (9 males and 8 females, aged 6 to 35 years) VDDR II patients with three mutations of the ligand- or DNA-binding domains on calcium therapy and a healthy control group.

Results: Serum calcium was 9.3 ± 0.9 g/dL in VDDRII Patients. The levels of $1,25(\text{OH})_2\text{D}_3$, the hallmark of VDDR II, were elevated in all patients - mean \pm SD of 177 ± 81 pg/ml in males and 220 ± 89 pg/ml in females. PTH levels were elevated at 130 ± 25 pg/L in males 59 ± 50 and 130 ± 25 pg/L in females with VDDR II. PRA was 1.89 ± 1.81 ng/ml/h in the patient group and 1.51 ± 1.51 ng/ml/h in the control group ($p = 0.570$), aldosterone levels were 63.4 ± 42.1 and 43.8 ± 32 pg/ml respectively ($p = 0.19$). None of the patients or the control subjects had aldosterone levels higher than the upper limit of normal (130 pg/ml). ACE activity was 34 ± 10 U/L in patients and 34.3 ± 14 U/L in controls, ($p = 0.95$).

Angiotensin (AT) II levels were 132 ± 97 pg/ml in the patients and 136 ± 96 pg/ml in controls ($p = 0.950$). Serum and urine electrolytes were within the normal range and not different between patients and controls. Neither systolic nor diastolic hypertension was detected in any patient during 24 hours of continuous blood pressure recording. Left ventricular mass (LVM) in males was normal at 110 ± 41 gr, ranging from 72 gr in the prepubertal patients to 170 gr in the adults. In females, LVM was normal at 77 ± 29 gr (ranged from 47 gr to 131 gr). LVM Z score was -2.2 ± 1.7 (range -3.9 to 0.2) in males and -1.6 ± 1.4 (range -3.1 to 1.2) in females. None of the adult patients exceeded the reference levels that indicate left ventricular hypertrophy (LVH), that is 117 gr/m^2 for males and 104 gr/m^2 for females.

Conclusions: Although there are many phenotypic and metabolic similarities between humans with VDDR II and VDR-KO mice, this cohort of patients with VDDR II did not exhibit disorders of the RAS, hypertension or gross heart abnormalities, such as reduced contractility or hypertrophy. Humans seem to have a redundancy mechanism to overcome VDR heart effects.

Nothing to Disclose: DT, YW, YS, YB, AH, VG, AL, ZH

P3-185

Clinical Spectrum of Hypophosphatasia Diagnosed in Adults.

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Introduction: Adult hypophosphatasia (HPP) is a rare disorder characterized by low serum alkaline phosphatase due to defective tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP). Limited data is available regarding the clinical presentation of hypophosphatasia diagnosed in adults.

Aim: To describe the clinical features, laboratory data, and radiologic data of a heterogenous group of adult subjects diagnosed with hypophosphatasia.

Methods: All Mayo Clinic patients diagnosed with HPP as adults from 1976-2009 were identified. Patients were included if they met the following criteria: age >18 years at the time of diagnosis, low serum alkaline phosphatase in the absence of bisphosphonate therapy, and one additional supporting element including elevated pyridoxal 5'-phosphate (PLP) or urine phosphoethanolamine (PEA), evidence of osteomalacia, such as pseudofracture or stress fracture, family history, or genetic test positive for HPP.

Results: Over the 33 year period, 22 patients were identified with HPP as adults. The mean age at diagnosis was 50.2 years, and the majority of patients were female (69% female). Primary complaints at presentation were musculoskeletal pain (60%) or fracture (26%). Overall, 54% of patients had a history of fracture, with multiple fractures occurring in 9. The most common sites of fracture included spine (9%, n=2), hip/femoral neck (23%, n=5), wrist (18%, n=4), and feet (23%, n=5). Other significant clinical features included radiologic chondrocalcinosis (27%) and documented calcium pyrophosphate crystal disease (14%). Serum alkaline phosphatase levels were on average 54% below the lower limit of normal (range 2-85% below). Urine PEA values were elevated in most but not all subjects in whom measurements were obtained (n= 15 of 17). PLP averaged 91.6 ug/L (normal, 5-50 ug/L) with all measured patients (n = 8) above the normal range. Parathyroid hormone was normal in all subjects tested (n = 10), however, hypercalcemia and hyperphosphatemia were noted in 3 (14%) and 4 (18%) patients respectively.

Conclusion: HPP in adults has a wide spectrum of nonspecific clinical manifestations, most commonly including musculoskeletal pain and fractures. In adult patients with complaints of unexplained musculoskeletal pain and low alkaline phosphatase, the absence of classical stress fractures and pseudofractures, should not exclude this diagnosis from the clinician's differential.

Nothing to Disclose: KEB, PJT, RAW

P3-186

Elevated Serum Serotonin Levels in Patients with Osteoporosis-Pseudoglioma Syndrome.

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Background: Osteoporosis-pseudoglioma syndrome (OPPG) is a rare autosomal recessive disorder characterized by congenital blindness and childhood osteoporosis, due to mutations in *LRP5*. Approximately 40 children with OPPG have been reported, 9 by our group⁽¹⁾. Studies in the mouse model of OPPG revealed elevated serum levels of gut-derived serotonin, normally inhibited by *LRP5*. Normalization of serum serotonin corrected the bone phenotype. Serum serotonin levels have been reported in only 4 humans with OPPG (one age 52 years⁽²⁾, and three ages 23, 13 and 11 years⁽³⁾). The purpose of this study was to measure serum serotonin in patients with OPPG and to evaluate for correlations between age, bone turnover and BMD Z-score.

Study Design: Fasting serum serotonin was measured in 6 OPPG patients, ranging in age from 29 days to 17 years, by Nichols (HPLC) in 2, and by Mayo Lab (LC/MS/MS) in 4. These patients are Old Order Mennonites, members of 2 related nuclear families. All OPPG patients were off bisphosphonates at the time of the study for 4-11 months; four of them had previously been treated with bisphosphonates, and one also with teriparatide. Urine N-telopeptide (NTX) was measured on a morning void.

Results: Serum serotonin levels are shown for 6 OPPG patients bellow. There was no correlation between serotonin and NTX ($p=0.23$), or with Z-scores at the total hip or lumbar spine ($p=0.89$, $p=0.95$). There was a significant correlation between serotonin and age ($p=0.03$).

| | Age | serotonin in mg/ml | Patient Serotonin/lab upper level of normal |
|---|----------|--------------------|---|
| 1 | 29 days | 316 | 1.37 |
| 2 | 2 years | 248 | 0.71 |
| 3 | 6 years | 353 | 1.01 |
| 4 | 7 years | 377 | 1.64 |
| 5 | 13 years | 473 | 2.05 |
| 6 | 17 years | 566 | 2.46 |

Discussion: We found mild elevations in serum serotonin in 6 patients with OPPG, including 4 patients under age 10, the youngest levels reported to date. We found a positive correlation between age and serotonin levels, as found in the mouse model of OPPG. The degree of elevation that we found (1.01-2.46) was lower than that seen by Yadav (4.0-5.0)⁽³⁾ and similar to Saarinem (2.27)⁽²⁾. However, we did not have internal controls and used upper level of normal as a reference, which could account for this difference. We found no correlation between serotonin and NTX or DXA Z-scores; however, 4 of 6 patients had been treated with bisphosphonates, which lowered NTX and improved Z-scores. Studies are needed in OPPG to determine whether normalization of serum serotonin in humans would correct bone phenotype.

(1) Streeten EA et al., Bone 2008;43:584-590

(2) Saarinem A et al., Clinical Endocrinology 2009. E pub

(3) Yadav VK et al., Cell 2008;135:825-837

Nothing to Disclose: SPRR, DHM, SAGM, EAS

P3-187

The Association of Coronary Calcium Scoring with Nonalcoholic Fatty Liver Disease in Apparently Healthy Korean Adults.

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Background: Nonalcoholic fatty liver disease (NAFLD) is a common pathologic condition that carries increased risk for various metabolic diseases. Recent studies raise the concern that patients with NAFLD could be at greater risk for atherosclerosis. We analyzed the association of coronary calcium scoring measured by multi-detector computed tomography (MDCT) in apparently healthy Korean adults.

Method: Among the participants in health check-up program from 2007 to 2009 in a health promotion center, 877 apparently healthy Korean adults (mean age 49 years, 65.8% male) in whom coronary calcium scoring detected by MDCT were available, were selected for the analyses. The presence of NAFLD was assessed by abdominal ultrasound examination. Subjects were divided into 3 groups according to the coronary calcium score (CCS)(CCS=0, 0<CCS≤100, CCS>100), and fasting glucose levels {normal, impaired fasting glucose (IFG) and diabetes mellitus (DM)}.

Results: Among the subjects, 365 subjects (41.5%) had NAFLD, and mean CCS increased as the age group increased from 30's to 70's (p<0.01). When mean CCS was compared according to the glycemic status by fasting glucose, mean CCS increased across the 3 groups from normal, IFG and DM (p=0.014). Subjects with NAFLD showed higher mean CCS, without statistical significance (19.7±76.6 vs. 12.8±74.3, p=0.182), and the proportion of subjects with coronary calcification was higher in subjects with NAFLD compared with non-NAFLD group (54.7 vs. 43.7%, p<0.01). The proportion of subjects with NAFLD increased as the severity of coronary calcification increased from 0 to higher than 100 (39%, 52.5% and 61.1%, p=0.002). When logistic regression analyses were performed with coronary calcification as the dependent variable, the presence of NAFLD persisted as the independent determinant for coronary calcification after adjustment for age, liver enzymes, systolic blood pressure, BMI, fasting glucose and total cholesterol levels, although the significance disappeared when gender was included in the model (OR for coronary calcification 1.593 with p=0.031, p=0.546 after inclusion of gender).

Conclusions: The presence of NAFLD could be considered as one of the risk factors affecting subclinical atherosclerosis in Korean adults.

Nothing to Disclose: EJR, SEP, CYP, WYL, KWO, SWP, SWK

P3-188

Avascular Necrosis (AVN) after Allogeneic Hematopoietic Stem Cell Transplantation (allo-HSCT): Implications for Pre-Emptive Therapy in High-Risk Populations.

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Background: AVN is a debilitating bone disorder with unclear pathophysiology. Patients (pts) receiving allo-HSCT are at greater risk for developing AVN. We sought to identify risk factors for AVN after allo-HSCT using a retrospective case-control study. **Methods:** Pts undergoing allo-HSCT at our institution from 1999-2008 without a prior diagnosis (dx) of AVN were eligible for inclusion. Demographic, transplant (tx), exam and laboratory data were extracted. AVN dx was by imaging. Controls were randomly selected for follow-up of >300 days. **Results:** Fifty cases and 156 controls were included. Median time from tx to AVN dx was 1.2 years (yrs; range 0.12-5.2). Median follow-up was 3.7 yrs (range 0.2-8.6) for AVN pts vs 1.9 yrs (range 0.3-9.0) for controls (P<0.001). Pts with AVN were significantly younger than controls at time of tx (median age 37 vs 48 yrs, P<0.001) and were more likely to have a history of tobacco use (68% vs 43%, P=0.012). The following tx variables were associated with increased risk of AVN: unrelated donor allo-HSCT (48% of AVN pts vs 30% of controls, P=0.043), total body irradiation (TBI) in preparation for tx (72% vs 46%, P=0.001), and systemic steroid (SS) treatment for graft-versus-host disease (GVHD) (98% vs 84%, P=0.009). TBI dose (myeloablative vs reduced intensity) was not associated with AVN. Mean peak dose of SS was higher in the AVN pts vs controls (1.6 vs 1.4 mg/kg/day prednisone equivalent, P=0.014). The median duration of SS treatment prior to AVN dx was 0.65 yrs (range 0-5.1), and 60% of these pts were receiving SS at the time of AVN dx. There were no significant differences in ethnicity, gender, baseline dx, BMI, bone density, incidence or severity of GVHD, or survival between the AVN and control pts. In multivariate analyses, age (≤ 40 yrs; OR=2.55, P=0.008), TBI (OR=2.29, P=0.027), and SS (≥ 1 mg/kg/day; OR=3.48, P=0.052) were independent predictors of AVN. **Conclusions:** The finding of a high rate of AVN in long term young survivors after allo-HSCT who are mostly cured of their underlying disease and have few comorbidities is of concern. The mechanism by which age influences the incidence of AVN after allo-HSCT remains unknown and may involve hormonal status, bone physiology, or yet unidentified genetic susceptibilities. Our data emphasize the need for early therapeutic interventions to prevent disabling long term complications and close follow-up in high risk populations for AVN in an effort to improve quality of life.

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

P3-189

Ghrelin Stimulates Proliferation and Differentiation of Neural Progenitors from the Subventricular Zone in Adult Mice.

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It is widely accepted that there are at least two neurogenic sites in the adult mammalian brain: the subventricular zone (SVZ) of lateral ventricles and the subgranular zone of the hippocampus dentate gyrus. Here we examined the possible influence of ghrelin, a peptide hormone secreted from the stomach, on cellular proliferation and neuroblast differentiation of progenitor cells in the SVZ. Double immunohistochemical staining revealed that ghrelin receptors were expressed in Ki-67-positive adult neural progenitor cells. In mice treated with ghrelin (80 mg/kg, i.p.) for 8 days, bromodeoxyuridine (BrdU) incorporation and doublecortin (DCX)-positive neuroblasts were significantly increased in the SVZ. We also found that the numbers of BrdU- and DCX-immunoreactive cells were significantly reduced after anti-ghrelin antibody (10 mg/kg, i.p.) treatment for 8 days. To study the function of endogenous ghrelin in adult neural progenitor cells, we counted the number of cycling cells in the SVZ by using proliferating cell nuclear antigen, a proliferation marker, and found significantly fewer in the ghrelin knockout (KO) mice than in their wild-type littermates. BrdU-, Ki-67- and DCX-immunoreactive cells were also significantly reduced in ghrelin KO mice than in wild-type animals. Furthermore, administration of ghrelin into the ghrelin KO mice significantly increased the number of newly generated cells in the SVZ. Finally, ghrelin treatment also increased BrdU- and DCX-immunoreactive cells in growth hormone- and insulin-like growth factor-1-deficient spontaneous dwarf rats. Therefore, our results indicate that ghrelin directly induces proliferation and differentiation of adult neural progenitor cells in the SVZ, suggesting an involvement of ghrelin in adult neurogenesis. Our data suggest that stimulating endogenous SVZ neural stem cells by ghrelin may be of potential therapeutic values in cell replacement based therapies of neurodegenerative diseases.

Nothing to Disclose: SP, JL, EL, EL, YK

P3-190

Corticotropin-Releasing Factor Induces Proliferation and Survival of Neural Progenitor Cells.

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Corticotropin-Releasing Factor (CRF), initially characterized as the main hypothalamic coordinator of stress response, is broadly expressed within the nervous system exerting modulatory effects via its two receptors CRFR1 and CRFR2. In the developing and adult brain CRF is expressed in areas where active proliferation and programmed cell death processes operate in parallel for the generation of appropriate number of neuronal populations. As recent studies suggest, exposure to stressful stimuli can interfere with the processes of neurogenesis and neuronal survival. Here, we demonstrate the presence of CRF receptors (CRFRs) in actively proliferating neural progenitor cells both in embryonic and adult brain by immunofluorescence using specific antibodies. We also show the expression of CRFRs in neural stem/progenitor cells (NS/PCs) isolated from fetal (embryonic day E14) mouse brain. In order to elucidate the role of CRF in neural proliferation and apoptosis we cultured primary NS/PCs and the human neuroblastoma cell line SH-SY5Y in the presence or absence of CRF (10⁻⁷M) for different time periods. The proliferative activity of CRF-treated cells was evaluated by BrdU labeling for 3 hours. Our results showed that CRF induces the number of total and BrdU- positive cells counted in a growth factor-independent manner. Stimulation with CRF also results in protection from apoptosis as shown by TUNEL experiments. Both CRF proliferative and neuroprotective action was achieved via CRFR1 as revealed by use of specific CRFR1 and CRFR2 antagonists. We further demonstrate using specific blockers that the proliferative effect of CRF is mediated by activation of the MAPK signaling cascade, more specifically erk1/2 activation, whereas its anti-apoptotic action is achieved via the PI3K/Akt pathway. The CRFR1-mediated effects of CRF in NS/PCs indicate the putative importance of this peptide in brain development and in neuronal survival during stress.

Nothing to Disclose: YK, ME, ER, KPK

P3-191

Expression of Ghrelin and Neurotensin Systems in the Temporal Lobe of Alzheimer Disease Patients.

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Ghrelin and neurotensin (NTS) are two neuroendocrine peptides with opposite roles in food intake and energy homeostasis. However, both peptides exert similar actions improving memory and learning processes. Biological actions of ghrelin are mediated by a G protein-coupled receptor (GPCRs), GHSR1a, which has a spliced variant, GHSR1b, of poorly known function. Neurotensin acts via two GPCRs (NTSR1 and 2) and one non-GPCR (NTSR3). Of note, GHSRs and NTSRs display a high evolutionary identity and comprise the so-called ghrelin receptor-like family. Since, ghrelin and NTS systems are clearly involved in energy balance regulation and cognitive processes, both have been proposed to be altered in Alzheimer disease (AD), a dementia syndrome strongly influenced by the metabolic status. Although it has been demonstrated that ghrelin and NTS can attenuate AD-related cognitive impairment, a comprehensive analysis of both systems in AD has not yet been undertaken. Here, we analyzed by quantitative-real time PCR the ghrelin/NTS axis in one of the most affected cortical regions in AD, the temporal gyrus. Our results demonstrate that both, ghrelin and NTS systems, are deeply altered in this region. Specifically, we have observed a marked reduction in mRNA levels of ghrelin (49%) and In2-ghrelin variant (41%; a recently discovered ghrelin isoform highly expressed in normal brain) in the temporal gyrus of AD patients. In contrast, mRNA levels of GOAT (the enzyme that acylates ghrelin and, likely, In2-ghrelin variant) did not vary in the whole temporal lobe of AD patients. Noticeably, whereas expression level of the biologically active ghrelin receptor, GHSR1a, was reduced (54%), mRNA levels of the dominant-negative GHSR1b were dramatically increased (300%) in the brain of AD patients. Moreover, we found that mRNA levels of GHSR1b were significantly higher than those of GHSR1a in AD patients. Similarly, we observed a marked decrease in the expression of NTSR1 (82%) and NTSR2 (59%), and a slight reduction of NTS mRNA levels (17%) in the temporal lobe of AD patients compared with matched controls. In striking contrast, mRNA levels of the non-GPCR NTSR3, a receptor involved in neuronal apoptosis, did not vary. Taken together, our results represent the first quantitative evidence of the modifications that occur in the ghrelin/NTS axis in the brain of AD patients, and could help to understand, at least in part, the severe cognitive deficit observed in this pathology.

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Nothing to Disclose: MDG, AR, JC-C, JA, RML, JPC

P3-192

Cognitive Dysfunction Due to the Neurotoxicity of Subchronic Exogenous Glucocorticoids Is Modulated but Not Reversed by Simultaneous Pharmacological Enhancement of Cholinergic Neurotransmission in Rats.

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The cholinergic neurotransmitter system and prolonged glucocorticoid-induced stress can affect cognitive functions in opposite ways. While pharmacological enhancement of cholinergic neurotransmission is known to induce neuroprotective effects, chronic glucocorticoids impair cognitive functions. Up to now, there is no consensus as to whether a subchronic stress period would affect cognitive function. Therefore, we sought to determine whether increase of cerebral acetylcholine (ACh) mediated by repeated physostigmine (acetylcholinesterase inhibitor) administration induces neuroprotective effects in a subchronic period and during exogenous glucocorticoid-induced stress. Male adult rats (n=40) were randomly divided into four groups with n=10 per group and treated for 4 days with: (I) placebo-, (II) corticosterone- (15 mg/day), (III) physostigmine- (0.014 mg/day), and (IV) physostigmine+corticosterone, respectively. Body weights and plasma corticosterone concentrations were measured. Psychometric investigations were conducted using a Morris water navigation task before and after a subchronic treatment. In cerebral tissue, ACh and acetylcholinesterase (AChE) content and ACh receptor density were determined. Tissue corticosterone concentration was measured in cerebral cortex, hippocampus, and adrenal glands. In corticosterone-treated rats, reduced spatial cognitive abilities were associated with a significant increase in plasma (+25%) and cerebral corticosterone levels (+350%) paralleled by a significant reduction in adrenal gland concentrations (-84%) as compared to placebo. Repeated physostigmine injections improved rats' spatial memory and increased cerebral ACh and AChE content (p<0.05), yet physostigmine administered together with corticosterone (group IV), despite similar quantitative increase of ACh and AChE, was not able to reverse the negative effect of corticosterone on rats' cognition. These results imply some interactions between corticosteroids and physostigmine, but their mechanism and convergent effects on cognition remain obscure and call for further investigations.

Nothing to Disclose: SL, KW, DO, JK, KP

P3-193

Arsenic Reduces Prolactin Secretion and Induces Cell Death in Anterior Pituitary Cells.

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Arsenic (As) is one of the most environmental toxic metals. Inorganic arsenic exposure via drinking water has been associated with cancer and serious damage to various internal organs. However, its effects on endocrine function have not been clearly established yet.

In the present study we have investigated the effect of As on anterior pituitary cell physiology. We examined the effect of As³⁺ (sodium arsenite) on prolactin (PRL) release (by radioimmunoassay) and on cellular viability (by MTT assay) in primary cultures of pituitary cells from adult male Wistar rats. Caspase-3 activation was also evaluated by a Ac-DEVD-pNA colorimetric assay.

Cells exposed to As³⁺ for 24 h showed a decrease of PRL release in a dose-dependent way ([PRL] as % of control, As 1 μM: 55.5±8.9*; As 10 μM: 17.2±3.9**; As 25 μM: 12.4±3.**; *p< 0.05, **p<0.01 vs. control). As³⁺, only at 25 μM decreased cellular activity after 24 h of exposure (Abs 600nm, as % of control; 1 μM: 111.5±5.5; 10 μM: 119.5±8.9; 25 μM: 63.2±8*; *p<0.05 vs. control). As³⁺ (25 μM) induced a decrease in PRL release (measured after 24 h of incubation) in a time-dependent manner although a slight increase was initially observed. In the same experiment, the cytotoxic effect of the metal was not evident at 1, 3 or 6 h of incubation but became irreversible at 9 h (Abs 600nm, as % of respective control; 9h: 73.9±4.1**, 12h: 71.6±2.9***, 24h: 54.8±4.6***; **p<0.01; ***p<0.001 vs. respective control).

Additionally, 25 μM As³⁺ induced caspase 3 activation (sample Abs 405nm x mg of protein x 100/control Abs 405nm x mg of protein, As 6 h: 110.9±9.3; As 9 h: 345±103*; *p<0.001 vs. control). The cytotoxic effect of the metal was confirmed by immunocytochemistry (acridine orange-ethidium bromide) assay in which an increased number of nuclei with necrotic and apoptotic morphology was observed.

Our results show, for the first time, that As³⁺ affects pituitary physiology by reducing both PRL release and cell viability *in vitro*. The decrease of hormone release could be an early event of the toxic effect of the metal.

Nothing to Disclose: SAR, FAQ, JPC, MEG, BHD

P3-194

Development of a New Model, the LoxTBM3R Mouse, To Investigate the Regulation of Circadian Rhythms by the Melanocortin-3 Receptor.

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CNS melanocortins maintain energy homeostasis, secreting neuropeptides that regulate appetite and, through autonomic and neuroendocrine pathways, regulate peripheral metabolic activity in response to peripheral signals of metabolic status. Our laboratory has reported a novel role of the melanocortin 3 receptor (Mc3r) in energy homeostasis. Mc3r are required for entrainment of food anticipatory activity (FAA) and maintenance of metabolic homeostasis during periods of restricted feeding (RF - 60% of normal calories provided at 1300h in a 12h light:dark setting; lights on 0600-1800h) (1,2). Perhaps related to the failure to entrain FAA to RF, Mc3r are also essential for normal oscillations of clock genes in the brain and the liver and for maintaining metabolism homeostasis and insulin sensitivity during RF. To further investigate the role and significance of Mc3r in the entrainment of circadian oscillators in response to feeding cues, we have (1) examined FAA in WT and *Mc3r*^{-/-} mice housed in constant dark (DD), and (2) created LoxTBM3R mice carrying a loxP-modified null *Mc3r* allele that can be reactivated by Cre-recombinase. *Mc3r*^{-/-} mice housed in DD exhibited impaired clock function, with increased variability in period length suggesting reduced precision. *Mc3r*^{-/-} mice also exhibited impaired entrainment of activity and clock gene expression in the brain and liver to food presentation. Mc3r expression in the mediobasal hypothalamus (MBH) of homozygous LoxTBM3R mice was undetectable. Determination of body composition by NMR (Nuclear Magnetic Resonance) revealed a significant increase of fat mass and reduced lean mass in homozygous LoxTB-MC3R mice. Female LoxTB-MC3R fed with a high fat diet for 6 weeks exhibit an increased body weight and adiposity compared to wild type mice fed with the same diet. Assessment of FAA in wheel cages indicates a reduced FAA in male LoxTBM3R mice submitted to a RF. Evidence of reactivation of the Mc3r allele was observed in the MBH of Nes-cre and Eiiia-Cre/LoxTB-MC3R mice. IN SUMMARY, we have (1) shown that Mc3r are required for entrainment of the circadian clock to caloric intake, and (2) validated the LoxTB-MC3R mouse. The targeted reactivation of MC3R will permit us to identify the neuronal subpopulations of Mc3r involved in the maintenance of energy homeostasis and circadian rhythms.

(1) Sutton et al., J. Neurosci 48, 12946 (2008).

(2) Sutton et al., FASEB J, 2009.

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Nothing to Disclose: KB, GMS, JZ, RLM, AAB

P3-195

Hypothalamic Insulin Signaling Regulates Lipolysis in Adipose Tissue.

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Unrestrained lipolysis due to white adipose tissue (WAT) dysfunction results in increased fatty acid release, insulin resistance and lipotoxicity, and plays a key role in the pathogenesis of type 2 diabetes. Insulin is known to suppress lipolysis in WAT, which is thought to be chiefly mediated by its binding to the adipocyte insulin receptor. It is presently unknown whether insulin also regulates WAT lipolysis indirectly by modulating sympathetic nervous system (SNS) outflow and, if so, in which anatomical brain structure. To test this male Sprague Dawley (SD) rats received a 6 h insulin infusion either intracerebroventricular (ICV) or directly into the mediobasal hypothalamus (MBH), while circulating glucose and insulin levels were controlled by a euglycemic clamp. In vivo lipolysis was assessed by [2H-5]-glycerol tracer dilution technique. The rate of appearance of glycerol was markedly suppressed by MBH (4.41 ± 0.61 $\mu\text{mol/kg/min}$; $p=0.002$; $n=5$) or ICV (5.55 ± 1.63 $\mu\text{mol/kg/min}$; $p=0.024$; $n=3$) insulin infusion compared to vehicle infused rats (12.84 ± 1.02 $\mu\text{mol/kg/min}$; $n=7$) indicating a suppression of lipolysis. The anti-lipolytic effects of MBH insulin were reversible by co-infusing the mitogen-activated protein kinase inhibitor U0126. Western blot analyses of lipolytic protein expression in the epididymal fat pads of the MBH insulin infused rats revealed that activating phosphorylation of hormone sensitive lipase (Hsl) at Ser 563 and 660 were significantly lower in the MBH insulin infused rats as compared to vehicle infused rats. This suppression of WAT lipolysis by hypothalamic insulin is likely due to a restraint of SNS outflow as surgical denervation or pharmacological sympathectomy lead to a similar suppression of Hsl activation in epididymal fat pads. Consistent with these results, mice lacking the neuronal insulin receptor as well as SD rats infused with the competitive insulin receptor antagonist S961 exhibit increased lipolytic rates compared to control animals. Finally, brain insulin exerted systemic anti-inflammatory effects in LPS induced sepsis that may be due to restrained lipolysis and reduced lipotoxicity. These data demonstrate that insulin signaling in the MBH restrains WAT lipolysis, and that any alteration within this brain-WAT circuitry can contribute to WAT dysfunction and increased fatty acid release, which in turn can induce insulin resistance, lipotoxicity and a pro-inflammatory state.

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Nothing to Disclose: TS, JO, KDA, BC, CL, SD, TM, KS, MP, SJF, SFP, CB

P3-196

Effects of Intra-Uterine Undernutrition on Hypothalamic Kiss1 Expression and the Timing of Puberty in Female Rats.

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OBJECTIVES : Recent studies have suggested that intrauterine undernutrition is closely associated with the pathogenesis of diseases after birth. Perinatal undernutrition is known to disturb the development of reproductive function and delay the onset of puberty in some species. Using a rat model, we determined the effects of prenatal undernutrition on the development of the hypothalamic kisspeptin system and evaluated whether the alteration of the kisspeptin system contributes to the delayed onset of puberty induced by prenatal undernutrition. We also evaluated the effects of prenatal undernutrition on the developmental changes in serum leptin levels because leptin was a putative positive regulator of the hypothalamic kisspeptin system.

METHODS AND RESULTS : We compared the timing of vaginal opening (VO) and the developmental changes in body weight, hypothalamic Kiss1 mRNA levels, and serum leptin concentrations between offspring with prenatal undernutrition (UN offspring) and normal nutrition (NN offspring). After birth, the UN offspring showed rapid growth and had caught up to body weight of the NN offspring by postnatal day 12. After postnatal day 16, the UN offspring showed significantly low Kiss1 mRNA levels compared with the NN offspring, despite their significantly high serum leptin levels (at days 20 and 28). The timing of VO in the UN offspring was delayed compared with that in the NN offspring, and chronic central injection of kisspeptin normalized the timing of VO in the UN offspring.

CONCLUSION : These results suggest that decreased hypothalamic kisspeptin action contributes to the delayed onset of puberty in prenatal undernourished female rats. Increased leptin resistance in the kisspeptin system might be involved in these alterations.

Nothing to Disclose: TI, TM, SF, RK, GG, MI

P3-197

Endocrine-Metabolic Characterization of Cortistatin Knockout (CST-KO) Mice Unveils a Gender-Dependent Role of CST, Distinct from SST, in Regulating Growth Hormone (GH)- and Adrenocorticotrophic Hormone (ACTH)-Axis Function.

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Cortistatin (CST) shares high structural, pharmacological and functional homology with somatostatin (SST) and binds all SST receptors subtypes (sst1-5) with similar affinity. Although CST has been considered a natural analog of SST, these peptides might in fact exert unique roles since: 1) CST substantially reproduces, but not fully mimics the *in vitro* effects of SST on pituitary secretions of human and animal models, 2) CST, but not SST, modulates sleep 3) tissue expression patterns and regulation of both peptides do not overlap in human tissues, 4) CST can bind other receptors, different to ssts (e.g. ghrelin and MrgX2 receptors), and 5) newly identified truncated sst5 receptor variants in human and mice display differential ability to mediate CST- and SST-induced Ca²⁺ signaling. The use of KO mice models may help to clarify whether CST can be simply considered (or not) a natural endocrine analog for SST. Accordingly, in this study we have carried out the first endocrine-metabolic characterization of the hypothalamic-pituitary component of the GH and ACTH axes of CST-KO mice as compared with their wildtype-littermate controls. Our results show that circulating levels of GH were increased in male and female CST-KO mice, although these changes did not result in increased body weight, changes in IGF-I levels (circulating or hepatic mRNA) or changes in whole hypothalamic GHRH or SST expression. CST-KO mice of both sexes had low circulating levels of insulin and high levels of corticosterone, which may explain the high GH levels observed in CST-KO mice. Interestingly, circulating ACTH and pituitary ACTH and GH mRNA levels were increased in female, but not male CST-KO mice compared with controls. We hypothesized that these gender differences in GH and ACTH could be due to the elevated total and acylated ghrelin levels, which were observed only in female CST-KO mice. The *in vivo* effects of CST-KO on GH and ACTH synthesis and release compare well with our *in vitro* data showing that CST (100nM; 24h) inhibited GH synthesis and release in primary pituitary cell cultures of female mice and baboons (a nonhuman primate). Altogether, our results demonstrate the existence of a sexually dimorphic role of CST on the regulation of the GH and ACTH axes that differ from that previously observed in SST-KO mice (1,2), providing further evidence the CST and SST serve unique roles in regulating hypothalamic-pituitary-hormonal axes.

(1) Luque RM et al., 2006 Am J Physiol Endocrinol Metab 291(2):E395-403.

(2) Luque RM et al., 2007 Endocrinology 148(12):5998-6006

Sources of Research Support: FI06/00804 (to JCC), FPU-AP20052473 (to MDG); VA Merit Award and NIH DK03367 (to RDK); BFU2007-60180/BFI, BIO-0139 and CTS-01705 (to JPC); BFU2008-01136; RYC-2007-00186 (to RML).

Nothing to Disclose: JC-C, MDG, BC, AP-S, FG-N, AJM-F, LdL, RDK, JPC, RML

P3-198

Ghrelin Regulates Expression of the NR4A Family of Nuclear Receptors.

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The peptide hormone ghrelin has numerous central nervous system (CNS) effects, including increased food intake, enhanced learning and memory, and neuroprotection via growth hormone secretagogue receptor (GHSR) binding. The GHSR is a G protein-coupled receptor that signals through Gαq, PLCβ, and PKC; however, the downstream target genes mediating the neuroprotective effects of ghrelin are unknown. Here, we show that ghrelin induces expression of the NR4A family of nuclear receptors (NR4A1, NR4A2, and NR4A3). Like ghrelin, the NR4A family of receptors regulates a diverse group of biological processes, such as neuronal development and the inflammatory response. These receptors are ligand-independent, and thus are primarily regulated at the gene expression level. Ghrelin treatment increased *NR4A1*, *NR4A2*, and *NR4A3* expression in HEK cells stably expressing the GHSR (HEKGHSR), with mRNA levels peaking between 45-90 min of ghrelin and returning to baseline by 24h. Ghrelin also increased activation of a luciferase reporter gene driven by the monomeric NR4A response element (NBRE). Treatment with thapsigargin and PD98059 blocked ghrelin-stimulated luciferase activity, indicating a role for intracellular calcium and ERK1/2 in ghrelin-stimulated NR4A function. Induction of *NR4A1*, *NR4A2*, and *NR4A3* mRNA by ghrelin was also blocked by PD98059; thus, ghrelin-induced increases in *NR4A* gene expression account for at least part of the stimulation of the NBRE-luc reporter. Des-acyl ghrelin, which does not activate the GHSR, did not affect NR4A expression; furthermore, ghrelin did not regulate other genes such as *Nrf2*, *PGC1a*, and *UCP2* in HEKGHSR cells. To determine if ghrelin regulates the NR4A family in the CNS, we created a stable neuronal line expressing the GHSR from SH-SY5Y neuroblastoma cells (SY5YGHSR). SH-SY5Y cells express tyrosine hydroxylase, as do the dopaminergic neurons affected in Parkinson's Disease. SY5YGHSR cells responded to ghrelin with increased IP-1 accumulation, while the GHSR inverse agonist substance P decreased IP-1 levels. Importantly, ghrelin increased *NR4A1*, *NR4A2*, and *NR4A3* expression and NBRE-luc activity in this neuronal cell line, suggesting that ghrelin may increase NR4A family expression in the brain. In summary, we have identified *NR4A1*, *NR4A2*, and *NR4A3* as ghrelin-responsive target genes in both kidney (HEK) and neuronal (SY5Y) cells, and these receptors are poised to mediate ghrelin's effects in the brain and other tissues.

Sources of Research Support: NIH Grant R01AG19230 awarded to RGS.

Nothing to Disclose: HEW, MJM, RGS

P3-199

Plasma Levels of the Neurosteroid Androsterone Increase after DHEA Therapy and Are Associated with Changes in Mood and Sexual Functioning in a Sexually Dimorphic Manner.

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Background: Previously, we observed that the antidepressant and libido-enhancing effects of DHEA were not associated with changes in plasma levels of DHEA or its metabolites (1). In a separate study, we also observed that CSF levels of the neurosteroid metabolite of testosterone (T), androsterone (ADT), correlated with changes in libido in men during both hypogonadism and T replacement (2). To further investigate the mechanism underlying the antidepressant effects of DHEA, we examined plasma levels of four neurosteroids implicated in the regulation of mood and libido.

Methods: 13 men and 10 women (ages - mean (SD) = 52.3 (7.5) and 50.5 (4.4) years, respectively) with depression participated in a crossover study in which DHEA and placebo were administered for 6 weeks each. Outcome measures included the Center for Epidemiologic Studies Depression Scale (CES-D) and the modified Derogatis Inventory for Sexual Functioning (DISF). In both scales, a clinically significant response to DHEA (i.e., response) was defined as a $\geq 30\%$ improvement relative to baseline. Blood levels of allopregnanolone, ADT, pregnenolone, and pregnanolone were drawn at baseline and at week 6 of each treatment phase. Assays were performed with gas chromatography/mass spectrometry. Data were analyzed with ANOVA-R and bonferroni t tests. **Results:** Compared with both baseline and placebo, DHEA significantly increased plasma levels of ADT ($F_{2,42}=3.3$, $p<.05$) but not those of the other neurosteroids. ADT levels during DHEA (but not during either baseline or placebo) were significantly higher in women compared with men (1301.0 vs. 796.7 pg/mL, $t_{63}=2.9$, $p<.05$). No significant correlations were observed between levels of any neurosteroid and either DHEA levels or measures of mood and libido. ADT levels were significantly greater in men and significantly lower in women who met criteria for antidepressant response on either the CES-D or DISF fantasy subscale compared with men and women who did not meet response criteria ($F_{2,38}=6.7$, $p<.05$; $F_{2,32}=7.1$, $p<.05$ respectively). No significant interaction between antidepressant response, sex and hormone levels was observed with the other neurosteroids measured. **Conclusion:** These data suggest that ADT contributes to the antidepressant and libido-enhancing effects of DHEA (albeit in a sexually dimorphic fashion), consistent with our previous findings that identified a role for ADT in the regulation of libido.

(1) Schmidt PJ et al., Arch. Gen. Psychiatry 2005;62:154

(2) Bloch M et al., Arch. Gen. Psychiatry 2006;63:450

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Nothing to Disclose: CM, DRR, PJS

P3-200

Interleukin-6 Receptor α Is Co-Localised with Melanin Concentrating Hormone in the Lateral Hypothalamic Area of the Mouse and the Human.

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Interleukin-6 (IL-6) deficient mice develop mature onset obesity. Furthermore, intracerebroventricular administration of IL-6 increases energy expenditure suggesting that IL-6 centrally regulates energy homeostasis. To investigate whether there is a possibility that IL-6 directly could influence the energy homeostasis at the hypothalamic level in rodents and humans, we have mapped the distribution of the IL-6 receptor α (IL-6R α) in the rodent and human hypothalamus. In mice, scattered IL-6R α -immunoreactivity was seen in neuronal perikarya and fibres throughout the lateral hypothalamic area (LHA). In addition, a dense population of immunoreactive (IR) neurons was found in the caudal part of the hypothalamus lateral to the fornix. In humans, the IL-6R α -IR was seen in perikarya of neurons located posterior to the level of the Monroe foramen. In the most anterior part of the IL-6R α -IR cell group, only scattered IL-6R α -IR neurons were located ventro-laterally from the fornix in the LHA. In more caudal areas, the density of the IL-6R α -IR neurons gradually increased and these cells occupied the LHA, perifornical- and dorsal hypothalamic areas and the hypothalamic dorsomedial nucleus. In the most caudal part of the hypothalamus, scattered IL-6R α -IR neurons were found in the posterior hypothalamic area. Double-labelling immunofluorescent studies demonstrated a complete overlap of IL-6R α - and MCH-immunoreactivities in both species. In mice, in addition to the MCH neurons, IL-6R α -immunoreactivity was also found in a population of orexin-IR neurons.

Our data demonstrate that MCH neurons of the human, as well as the MCH and orexin neurons of the mouse hypothalamus contain IL-6R α , raising the possibility that effects by IL-6 on energy balance are exerted directly on MCH neurons in humans, and both MCH and orexin neurons in mice.

Nothing to Disclose: CF, ES, PE, TF, EK, MP, ZL, BG, LK-L, RS, J-OJ

P3-201

Involvement of Estrogen and Progesterone in the Reduction of Epileptic Seizure Intensity Induced by Pregnancy in Wistar Audiogenic Rats.

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Women with epilepsy present changes in seizures produced by gonadal steroids, such as catamenial epilepsy and changes in seizures related with endocrine status such as menarche, pregnancy, and menopause. Gonadal hormones may affect the seizure threshold by altering neuronal excitability. In general, estrogen enhances and progesterone diminishes neuronal excitability experimentally. Wistar Audiogenic Rat (WAR) is a genetic model of epilepsy in which seizures are induced by high intensity sound stimulation. We have previously observed decrease of seizure intensity after pregnancy in some of these animals but the mechanisms were not clarified. In the present work we sought to investigate the involvement of progesterone (P4) and estradiol (E2) on the seizure-reduction induced by pregnancy. WARs from our own inbred colony and Wistar rats were submitted to acoustic stimulation (120 dB SPL) and the behavioral evaluation of the seizure severity index (SI) was calculated as previously described (1). WARs displaying (SI \geq 0.85) and Resistant rats (SI = 0) were mated with Wistar male rats. Sound stimulation was applied again one day before the experiment. The animals were decapitated on 7th, 14th and 20th days of pregnancy. Plasma levels of E2 and P4 were measured by RIA. On the 20th day of pregnancy, the seizures remitted in 50% of WAR (SI=0.0 in 33%). Two sub-populations of animals could be characterized: one displaying reduced SI (RSI) and one with non-reduced SI (NRSI). Higher P4 and E2 levels were observed in RSI than in the NRSI group. Moreover, RSI group presented P/E ratio similar to Wistar rats while NRSI group presented lower P/E ratio. To better clarify the gonadal steroids participation in the seizure reduction, WARs and Wistar rats were ovariectomized and separated in groups that received daily injections of P4 (500 μ g, *im*) and/or E2 (1 μ g/100g bw, *sc*) on different treatment schedules. Reduction of seizure was observed in 33% of the rats that received P4 alone. Reduction of seizures was observed in 67% of the rats that received E2 administered together with P4, in two different schedules. In conclusion, the present data show that higher levels of gonadal hormones during pregnancy are associated with reduction of epileptic seizure intensity in WAR. Moreover, estradiol may play an important synergic role with progesterone in the reduction of epileptic seizure intensity.

(1) M.C. Doretto et al., *Epilepsy Research* 2003; 54: 109

Sources of Research Support: FAPEMIG, CNPQ and CAPES.

Nothing to Disclose: AMR, MPG, MCD

P3-202

Cyclic Versus Continuous Progesterone Exposure Differentially Regulate Gene Expression in the Hippocampus of Adult Female Rats: A Taqman Low-Density Array Study.

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The Progesterone in Brain Aging and Alzheimer's Disease Program Project is designed to determine the neurobiology of progesterone (P4) in brain regions involved in cognition and vulnerable to age-related cognitive decline and Alzheimer's disease (AD). Recent research suggests that in addition to the timing, the progestin component of estrogen-based hormone therapy (HT) can have a significant impact on the clinical effect of HT as well (1). We have previously demonstrated that cyclic versus continuous P4 differentially regulated indices of AD neuropathology in a 3xTg-AD mouse model (2). In this study, we used Taqman Low-Density Arrays (TLDA) and compared the gene expression profiles induced by a chronic treatment with cyclic versus continuous P4 in the hippocampus of adult female rats. Genes analyzed included those encoding hormone receptors, amyloid metabolism, mitochondrial bioenergetics, and cholesterol trafficking. Cyclic P4 exposure in ovariectomized females induced a gene expression profile that paralleled to a large degree to the expression profile of ovary intact female rats. In contrast, continuous exposure to P4 induced a unique gene expression profile that overall was of much smaller magnitude. These results suggest that a regimen of cyclic P4 that more closely mimics natural hormone pattern of exposure could be a more effective paradigm to prevent potential negative impact of P4 depletion during menopause on brain functions, and which could be mediated by the membrane progesterone receptor, PGRMC1.

(1) Brinton et al., *Front Neuroendocrinol* 2008; 29:313-39

(2) Pike et al., *SfN Abstract* 2009; 543.21/U12

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Nothing to Disclose: LZ, ZM, TEM, LS, EC, CEF, CJP, RDB

P3-203

Genetic Manipulation of Neurons Controlling Timing of Puberty Onset in the Mouse.

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Mice, like humans, undergo puberty – a transition period of sexual development that occurs between juvenile stages and adulthood. As in humans, the physiological and behavioral changes that occur at puberty require an “awakening” of the Hypothalamic-pituitary-gonadal (HPG) axis, and absolutely require the function of conserved neuropeptides for HPG axis function, including Luteinizing Hormone Releasing Hormone (LHRH). Although it has long been known that the animal's age, weight, and even social environment are all critical determinants for the initiation of sexual development, little is known about how these inputs are integrated, or of the molecular mechanisms that underlie the coordinated control of puberty onset. The identification of Kisspeptin, a conserved neuropeptide activator of LHRH neuronal activity and a critical determinant of puberty onset, has provided an entry towards understanding how the timing of puberty onset is controlled. We have generated various genetic and viral tools to manipulate the function and signaling of Kisspeptin-expressing neurons. We will present our recent findings on the contribution of these neurons to the timing of puberty onset and to adult reproductive function, and our efforts to understand the distinct modes by which this neuron population is regulated.

Nothing to Disclose: AL, CD

P3-204

Acute Endocrine Alterations in Male Hypermarathon Runners: A New Category of “Critical Illness”.

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Introduction: Reliable data suggest that acute and chronic stressful events like trauma, exercise, surgery, and burns activate autonomous and neuroendocrine system as also the inflammatory reaction to maintain human homeostasis. Hyper marathon running is a stressful event that can significantly affect virtually any of the physiologic system of athletes.

AIM To determine the influence of strenuous exercise on the hormonal homeostasis of male hyper marathon runners.

Methods Twenty (20) male runners (mean age 47.3±8.6 y) who participated in the hyper marathon Nemea-Olympia, Greece (distance 180 km, mean duration 26h) and 18 healthy controls (40.4±6.7 y) were recruited. Blood samples were taken 4 hours before the start and immediately after the completion of the race. Hormone determinations were performed with immunochemistry and biochemical with conventional methods.

RESULTS

Acute changes in reproductive axis in male hypermarathon runners

| | SHBG mU/ml | LH mU/ml | FSH mU/ml | TESTO mU/ml | Free adrogen index |
|--------------------|-------------|------------|-----------|--------------|--------------------|
| controls n=18 | 33.2±12.2 † | 5.3±1.8†# | 4.4± 1.8 | 517.9±201.1† | 0.54±0.1† |
| Athletes pre n=20 | 42.9±17.0* | 3.2 ± 1.3* | 5.1±2.2* | 643.1±172.0* | 0.55±0.18+ |
| Athletes post n=20 | 51.2±23.4 | 3.0± 2.0 | 4.3±1.8 | 128.5±71.7 | 0.09±0.04 |

Acute changes in thyroid and adrenal homeostasis in male hypermarathon runners

| | TSH microU/ml | T3 ng/ml | T4 microU/ml | CORT microg/dl |
|--------------------|---------------|----------|--------------|----------------|
| Controls n=18 | 1.4+0.5 † | 1.2±0.1† | 8.2± 1.0† | 16.5±7.0†# |
| Athletes pre n=20 | 2.02+1.3* | 1.1±0.1* | 9.3±1.9* | 28.7±6.0* |
| Athletes post n=20 | 2.8+2.2 | 1.0±0.2 | 10.6± 2.1 | 39.6±14.5 |

* p < 0.05 athletes pre vs athletes post

+ p<0.001 athletes pre vs athletes post

p < 0.05 athletes pre vs controls

† p<0.05 athletes post vs controls

Hematological and biochemical variables were also affected after the race and indicated acute inflammatory reaction : significant increases in white blood cells (granulocytes),urea, creatinine, liver function markers (ALT,AST,LDH,γ-GT, TBil),CPK. Cytokines IL-6 and IL-10 as well as CRP were also elevated.

Conclusion Male hypermarathon runners seem to have hormonal and immune response after the race similar to critical ill patients as they present hypercortisolaemia, low T3 and central hypogonadism.The clinical importance of our results requires further investigation.

Nothing to Disclose: LL, AT, AT, SM, EL, NR, ID, IM, AGV, NAG, KBM

P3-205

A Third Lamprey Novel GnRH Receptor Similar to Gnathostome Type-II GnRH Receptors.

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Gonadotropin-releasing hormone (GnRH) is a central regulator of reproduction in vertebrates. Its function is mediated through a GnRH receptor (GnRHR), a class A 7-transmembrane GPCR. Previously, we identified a unique lamprey GnRH receptor (lGnRH-1) that shares several characteristics of both type-I and type-II vertebrate GnRH receptors (1). Last year, we reported the identification of an additional novel GnRH receptor (lGnRHR-2) in the sea lamprey, a basal vertebrate via the trace files produced by the Washington University Genome Sequencing Center. We now have identified and performed functional studies on a third novel lamprey GnRH receptor (lGnRHR-3). We compared the functional responses of Inositol Phosphate (IP) of these two receptors in transiently transfected COS7 cells when treated with increasing doses of lamprey GnRH-I, -II or -III. The ligands, lamprey GnRH-II and -III, stimulated a significant IP response in lamprey GnRH Receptor-2 and -3, however there was no IP response in either receptor when treated with lGnRH-I. Lamprey GnRH-II is a significantly more potent activator of the lamprey GnRH receptor-3. The third novel GnRH receptor retains the conserved structural features and amino acid motifs of other known gnathostome type II-GnRH receptors. The phylogenetic placement, structural and functional features of the lamprey GnRH receptors support that these receptors have retained key ancestral residues and motifs. From our phylogenetic analysis, we hypothesize that lamprey GnRHR-1 evolved from a common ancestor of all vertebrate GnRH receptors. Gene duplication events then gave rise to type I and type II GnRH receptors. The gene duplication event that gave rise to the type I GnRH receptors took place within the Gnathostome lineage, after its divergence from the ancestral agnathans. Further comparative studies on the lamprey GnRHs and their cognate receptors will help provide clues on the evolution of reproductive mechanisms and insights into our understanding of gene duplication, structure-activity relations, and the molecular evolution and functional diversity of these systems.

(1) Silver MR et al., *Endocrinology* 2005;146:3351

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Nothing to Disclose: AA-B, CM, SIK, MF, SAS

P3-206

Identification of Interacting Partners for the Mammalian GnRH Receptor. Impact on Receptor Signaling.

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In mammals, reproductive function is under the control of the hypothalamic neurohormone GnRH that controls synthesis and secretion of the two gonadotropins, LH and FSH, through stimulation of a heptahelical transmembrane receptor in pituitary gonadotrope cells. Although the mammalian GnRH receptor (GnRH-R) shares most of the features exhibited by the G protein coupled receptor family members, it lacks the carboxy terminal tail. Consequently, it is not internalized upon agonist binding and is insensitive to the classical desensitization mechanisms known to be involved in GPCR regulation. The mechanisms that regulate the signal transfer between GnRH-R and heterotrimeric G proteins still remain unknown. Due to its atypical structure, interactions between the intracellular loops of the receptor and cellular proteins are likely crucial for the regulation of GnRH-R signaling efficacy and/or specificity. Our goal was to identify such proteins and to elucidate their roles in GnRH-R signaling. To do so, we generated GST proteins fused to each intracellular domains of the mouse GnRH-R (GST-ICL1, GST-ICL2 and GST-ICL3) and incubated each of these fusion proteins with pituitary extracts. Interacting proteins were isolated by affinity chromatography in GST pull down assays, separated on a SDS-PAGE gel electrophoresis and stained with coomassie blue. Proteins that interacted specifically with the ICLs domains and not with the GST control were selectively picked, trypsin digested and analyzed by mass spectrometry. Among the large number of proteins identified, we first focused our interest on candidates potentially involved in GnRH-R signaling and more specifically on a protein that we named hit 22 in our interaction screen. This protein has been shown to regulate protein phosphorylation, gene transcription and more recently to inhibit the signal transfer between GPCR and G protein. We first showed that hit 22 interacts directly with the first and third ICLs of the GnRH-R as demonstrated in GST pull down assays with the purified hit 22 protein recombinant. We delineated the binding sites of hit 22 to a sequence involving basic amino acids in ICL 1 and 3. Work is now in progress to determine the impact of hit 22 on GnRH-R signaling and gonadotropin secretion by either decreasing or increasing its expression in cells or by comparing GnRH signaling in cells expressing the wild type GnRH-R or a mutant receptor unable to bind hit 22.

Nothing to Disclose: VS, GG, CD, CA, RC, JC-T

P3-207

GnRH Pulse Frequency Regulates SF-1, DAX-1 and SRF Gene Expression In Vivo in the Rat.

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Gonadotropin subunit genes are regulated in a differential manner by pulsatile GnRH, with faster frequencies favoring LH beta and slower frequencies FSH beta. However, the mechanism(s) by which rapid frequency GnRH pulses selectively stimulate LH beta transcription remain to be fully characterized. The rat LH beta promoter contains two GnRH-responsive regions. The proximal region has binding elements for SF-1 and the distal site contains a CARG Box (Serum Response Element) that binds SRF. Also of interest, DAX-1 binds SF-1 and suppresses the LH beta transcriptional responses to GnRH. The present study determined whether GnRH stimulates pituitary SF-1, DAX-1 and SRF transcription in vivo, and if regulation is frequency-dependant. Studies were conducted using a GnRH-deficient male rat model (i.e. castrated, testosterone-replaced). 24h later, rats were pulsed with 25ng GnRH iv every 30min or 240min for 1-24h (vehicle to controls). Rats were euthanized 5 min after the last GnRH pulse and pituitaries collected and processed for RNA. LH beta primary transcript (PT) and SF-1, DAX-1 and SRF PTs and mRNAs were measured by real time PCR. Results showed that rapid frequency pulses of GnRH increased LH beta PT 7-17 fold between 1-24h, whereas slow frequency pulses only increased LH beta PT 3 fold ($p < 0.05$ vs vehicle-controls). 30 min pulses of GnRH increased SF-1 PT (3 fold; $p < 0.05$) within 1h, then declined after 6h. SF-1 mRNA also increased within 1h ($p < 0.05$; coincident with, but prior to the peak increase in LH β PT). Slow frequency pulses had no effect on either SF-1 PT or mRNA. 30' pulses of GnRH also stimulated a transient increase in DAX-1 PT (2 fold after 1h; $p < 0.05$) and mRNA (1.7 fold after 6h; $p < 0.05$), but slow frequency pulses were ineffective. Similarly, slow frequency pulses of GnRH had no effect on SRF PT or mRNA. In contrast, 30' pulses stimulated a 2 fold increase in SRF mRNA within 1h ($p < 0.05$), and was sustained for 6h while SRF PT was unchanged, suggesting a non-transcriptional mechanism.

These findings support a mechanistic link between SF-1 and SRF in the frequency regulation of LH β transcription by pulsatile GnRH. Further, since DAX-1 gene expression is also stimulated by rapid frequency pulses, this may limit the duration of SF-1 transcriptional responses to GnRH, suggesting a potential negative feedback regulatory action.

Sources of Research Support: HD033039.

Nothing to Disclose: DJH, LLB, JCM

P3-208

Pulse-Frequency Dependent Gonadotropin Gene Expression by Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) in Perfused Mouse Pituitary Gonadotroph LbetaT2 Cells.

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We examined how pulsatile stimulation with Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) affected gonadotrophs. In static culture, Gonadotropin-releasing hormone (GnRH) stimulated transcription of all of the gonadotropin subunits. In contrast, PACAP increased common alpha-glycoprotein subunit gene (*Cga*) promoter activity, but failed to increase luteinizing hormone beta (*Lhb*) and follicle-stimulating hormone beta (*Fshb*) promoters. Messenger RNAs for *Lhb* and *Fshb* were slightly but significantly increased by PACAP stimulation. Co-treatment of the cells with GnRH and PACAP was not different to the effects of GnRH alone on *Lhb* and *Fshb* transcriptional activities as well as mRNA expressions. To determine the effect of pulsatile PACAP stimulation on gonadotropin subunit gene expression, perfused LβT2 cells were stimulated at either high frequency (5 min PACAP pulse every 30 min) or low frequency (5 min PACAP pulse every 120 min). High frequency PACAP pulses preferentially increased *Lhb* gene expression up to 2.29 ± 0.15 -fold, and low frequency pulses resulted in a 1.55 ± 0.16 -fold increase. *Fshb* gene expression was increased 1.87 ± 0.3 -fold by high frequency PACAP pulses and 4.3 ± 0.29 -fold by low frequency pulses. These results were similar to the frequency specific effects of pulsatile GnRH. Follistatin (*Fst*) gene expression was specifically increased by high frequency GnRH pulses. High frequency PACAP pulses further increased *Fst* up to 4.7 ± 0.57 -fold than low frequency pulse (2.72 ± 1.09 -fold). PACAP receptor gene expression was increased significantly following pulsatile GnRH regardless of pulse frequency. Low frequency PACAP pulses, however, increased PACAP gene expression (16.49 ± 8.41) to a larger extent than did high frequency pulses. Additionally, high frequency PACAP pulses specifically increased *Gnrh receptor* gene expression by 4.38 ± 0.81 -fold; however, low frequency pulses did not result in an increase. These studies suggest PACAP, like GnRH, specifically regulates *Lhb* and *Fshb* subunit gene in a pulse-frequency specific manner. This regulation may involve alteration in numbers of GnRH and PACAP receptors as well as FST expression.

Nothing to Disclose: HK, AO, INP, KM

P3-209

GnRH-Mediated Activation of Protein Kinase D in Immortalized GnRH Neurons and Pituitary Gonadotrophs.

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GnRH receptors (GnRH-Rs) expressed in native and immortalized GnRH neurons (GT1-7) and pituitary gonadotrophs (α T3) activate diverse signaling pathways by coupling to at least three G proteins. Such coupling is time- and dose-dependent, and switches between G_q , G_s , and $G_{i/o}$ according to the agonist concentration. These findings suggest that an agonist concentration-dependent switch in coupling of the GnRH-R between specific G proteins modulates diverse signaling pathways in these cell types. Protein phosphorylation has emerged as one of the fundamental mechanisms of signal transduction in many cells. Activation of the PLC family, including β , γ , δ , and ϵ , produces two second messengers: inositol 1,4,5- P_3 ($InsP_3$) and diacylglycerol (DAG), which elicit cellular responses through a variety of effectors. Protein kinase D (PKD), a member of a novel family of serine/threonine protein kinases, has a unique position in the signal transduction pathways initiated by DAG and protein kinase C (PKC). PKD is not only a direct DAG target but is also downstream of PKCs in a novel signal transduction pathway implicated in the regulation of multiple fundamental biological processes such as Golgi structure and function, sorting of membrane proteins, and exocytosis. GnRH-induced activation of GnRH-R in both GT1-7 neurons and α T3 gonadotrophs caused stimulation of PKD. GnRH-induced PKD phosphorylated both Ser742/Ser744 and Ser 916 in a time- and dose-dependent manner. This activation is abolished by a GnRH-R antagonist, consistent with GnRH-R-mediated activation. The PKC inhibitor Gö 6983 abolishes phorbol 12-myristate-acetate (PMA)-induced PKD phosphorylation, but only partly inhibits GnRH-induced PKD Ser742/744 activation. In contrast, GnRH-induced PKD activation was unaffected by the PKC-specific antagonist Gö 6976. These findings indicate that in addition to PKC, the agonist-stimulated GnRH-R also activates PKD, providing a mechanism of signal integration and amplification. In conclusion, these data suggest that GnRH-GnRH-R-induced activation of PKD in immortalized GnRH neurons and pituitary gonadotrophs causes complex molecular interactions that maintain GnRH and LH secretion from the hypothalamo-pituitary axis.

Nothing to Disclose: HF, PKL, LZK, KJC

P3-210

Regulator of G Protein Signaling 2 (RGS2) Is Induced by Pulsatile GnRH and Alters GnRH-Induced Gene Expression in L β T2 Cells.

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GnRH is released from hypothalamic neurons in a pulsatile manner and acts on the anterior pituitary via the GnRH receptor, a G-protein coupled receptor. GnRH pulse-frequency and amplitude modulate signaling and gene expression in the pituitary. Previously we showed that pulsatile GnRH can differentially activate the Gs and Gq signaling pathways, and that the Gq pathway is sensitive to desensitization. However, the mechanisms of this differential activation are not understood. Others have demonstrated that the Regulator of G protein Signaling 2 (RGS2) can be induced by continuous GnRH in L β T2 cells. In this study we aimed to analyze RGS2 gene expression after pulsatile and tonic GnRH and also the participation of RGS2 on GnRH-induced gene expression in L β T2 cells. Firstly we studied RGS2 gene expression in response to pulsatile and tonic GnRH. Cells were plated in 6-well dishes and, 48 h later, stimulated with pulsatile (30- and 120- min intervals, 5 min pulse) or tonic GnRH (10 or 100 nM) in static culture for 4 h. RNA was isolated, reverse transcribed (2 μ g) and RGS2 gene expression analyzed by QPCR. Pulsatile GnRH induced RGS2 gene expression with the high-pulse frequencies (30-min intervals) inducing higher expression than the low-pulse frequencies (120-min intervals) ($p < 0.005$, $n=4$). High-dose GnRH (100 nM) induced higher expression than the low dose (10 nM) at 120-min pulse intervals ($p < 0.05$, $n=4$), but no difference was observed at 30-min intervals between doses. Tonic GnRH also induced RGS2 gene expression, and the low-dose induced higher expression than the high-dose ($p < 0.005$, $n=4$). We also investigated the role of RGS2 in GnRH-induced gene expression. We infected L β T2 cells for 1 h with recombinant adenovirus encoding wild type (WT) RGS2, or control adenovirus expressing eGFP. After 48 h in culture, cells were treated with GnRH (100 nM) for 0, 30, 60 and 120 min. Cells were harvested, RNA isolated and reverse transcribed and Egr-1 and c-fos expression analyzed by QPCR. Cells infected with the adenovirus encoding WT RGS2 expressed less Egr-1 and c-fos after GnRH stimulation than control cells (Two-way Anova: Interaction: ns, Main effect Virus: WT vs Control, $p < 0.04$, $n=4$). These results indicate that pulsatile GnRH induces a feedback loop that would likely attenuate GnRH receptor response to subsequent pulses of GnRH. They also suggest a role for RGS2 in modulating GnRH-induced gene expression in pituitary gonadotropes.

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Nothing to Disclose: MOF, NJGW

P3-211

Stress Levels of Glucocorticoids Inhibit LH-Beta Subunit Gene Expression in Gonadotrope Cells.

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Glucocorticoids have long been considered to be potential mediators of stress-induced suppression of ovarian cyclicity, however, the mechanisms involved are not well understood. Recent evidence in sheep indicates that psychosocial stress can inhibit GnRH and LH pulsatile secretion; however, glucocorticoid actions via the glucocorticoid receptor are only necessary to mediate the suppressive effects of stress at the pituitary level. Based on these observations, we sought to develop a cellular model to investigate the effects of stress levels of glucocorticoids on gonadotrope function. As a first step, we tested the hypothesis that stress levels of corticosterone inhibit LH synthesis and secretion from L β T2 cells, an immortalized gonadotrope cell model. L β T2 cells were serum starved overnight then treated with corticosterone (500 ng/ml) or vehicle (0.1% BSA) for 24 h. During the final 10 min of hormone treatment, cells were challenged with GnRH (10 nM) or vehicle. Thereafter, conditioned cell media was collected to measure LH secretion in response to GnRH, and cells were harvested for protein content to measure LH β synthesis. LH was assayed by mouse LH sandwich Elisa and corticosterone by RIA. Cells treated with GnRH released significantly more LH compared to those treated with vehicle (1.4 ± 0.3 vs. 0.3 ± 0.1 , respectively). Corticosterone reduced the release of LH in response to GnRH by 40% ($p < 0.05$). A similar reduction ($p < 0.05$) in LH β cellular content was observed in cells treated with corticosterone (3.8 ± 0.6 vs. 5.5 ± 0.9 , corticosterone vs. vehicle, respectively). Of interest, corticosterone suppressed LH β synthesis regardless of whether cells were treated with GnRH or vehicle. Additional experiments utilizing the proximal 1800 bp of the rat LH β 5'-regulatory region linked to a luciferase reporter gene transiently transfected into L β T2 cells confirm that corticosterone inhibits ($p < 0.05$) expression of the LH β subunit gene. Ongoing studies are examining the promoter elements involved in the suppression of LH β subunit gene expression. These data compliment *in vivo* evidence that glucocorticoids can inhibit pituitary responsiveness to a pulse of GnRH and they suggest this regulation can act, at least in part, directly upon gonadotrope cells. Furthermore, the effects of corticosterone may be mediated via alterations in the response to GnRH, as well as by inhibition of LH β synthesis.

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Nothing to Disclose: KMB, PLM

P3-212

Mutagenesis in Carboxyl Terminal Sequence of the LH β Subunit Defines Determinant for the Regulated Secretion of Lutropin from GH₃ Cells.

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The pituitary lutropin (LH) and follitropin (FSH) are members of glycoprotein hormone family, which also include thyrotropin. The coordinated secretion and activities of LH and FSH are essential for control of gonadal function. LH is released through the regulated pathway, i.e., it is released by secretagogues, while FSH is secreted constitutively. Defining the sorting signals governing the trafficking of LH and FSH is critical for understanding the link between their secretion and reproductive function. Since LH and FSH are synthesized in the same gonadotrope cell and share an identical α subunit, the β subunit contains the determinants for sorting LH dimer into the regulated pathway. One unique feature of the LH β subunit is a carboxyl terminal hydrophobic heptapeptide (114-Leu-Ser-Gly-¹¹⁸Leu-¹¹⁹Leu-Phe-Leu-121), not found in FSH β subunit, which influences the intracellular behavior of the LH dimer. Previously we demonstrated that deleting the heptapeptide from LH β diverted the truncated LH dimer to the constitutive pathway, resulting in a significant decrease in the accumulated intracellular pool in transfected rat somatotrope-derived GH₃ cells. To examine the structure-function of the heptapeptide, in particular its hydrophobicity, the Leu118 and Leu119 residues were substituted with Alanine. Leu118 was also substituted with Glycine. Stable GH₃ cell lines expressing dimers were labeled with [³⁵S]cysteine and proteins from lysates and media were immunoprecipitated with CG β antiserum. Absence of Leu118 in the heptapeptide decreased the intracellular pool of the mutant with a corresponding increased secretion compared to the LH dimer. In addition, preliminary immunolabeling shows that LH119Ala behaves more like LH118Ala than LH wild type. In contrast, the secretion pattern of LH118Gly resembles LH dimer suggesting that other factors besides hydrophobicity play role in the regulated secretion of LH. Immunofluorescent analysis revealed that in contrast to randomly distributed LH puncta, LH mutants had fewer puncta and often puncta were fused together. These results suggest that the leucine residues in the heptapeptide of the LH β subunit, particularly residue 118, are critical determinants for the regulated secretion of LH.

Sources of Research Support: NIH Grant HD061907 awarded to IB.

Nothing to Disclose: AJ-S, IB

P3-213

Regulation of Follicle-Stimulating Hormone β Subunit (Fshb) Transcription by Liver Receptor Homolog-1.

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The pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), are critical regulators of mammalian reproductive function. The two share a common α subunit (α GSU or CGA) but possess unique β subunits (Lhb and Fshb), which confer biologic specificity. Expression of the gonadotropin subunits is under the control of multiple regulatory signals. Hypothalamic gonadotropin-releasing hormone (GNRH1) stimulates both Lhb and Fshb transcription. Activins, members of the TGF β superfamily, potently stimulate Fshb transcription. Gonadotrope-specific and hormonally-regulated expression of Lhb and Fshb are dependent upon the coordinated actions of several transcription factors. Of particular importance is steroidogenic factor 1 (SF1; NR5A1), which is required for normal Lhb and Fshb expression in gonadotropes in vivo. Mechanisms through which SF1 regulates Lhb have been characterized in vitro. In contrast, it has been technically challenging to demonstrate whether Fshb is a direct target of SF1 in gonadotropes. In the context of our investigations in this area, we observed that liver receptor homolog-1 (LRH1; NR5A2), but not SF1 over-expression induced murine Fshb transcription in both homologous and heterologous cells. LRH1 and SF1 are structurally related, bind the same consensus DNA cis-element, and are both expressed in the pituitary gland and in immortalized gonadotropes (L β T2 cells). Previous data indicate that LRH1 may function similarly to SF1 in Lhb regulation, at least in vitro (Zheng et al., 2007, J Mol Endocrinol 38:207). Here, we explored mechanisms through which LRH1 might regulate murine Fshb. We first mapped the Lrh1 transcription start site in the adult murine pituitary gland by 5' rapid amplification of cDNA ends (5' RACE). The transcript is initiated in intron 2 and is predicted to encode a truncated form of the protein; 61 amino acids shorter at the N-terminus than the canonical liver isoform. siRNA-mediated knockdown of endogenous LRH1 in L β T2 cells decreased basal, GNRH1-, and activin A-stimulated activity of a murine Fshb promoter-reporter, suggesting a role for the endogenous protein. In promoter truncation analyses, LRH1 responsiveness mapped to a proximal promoter region, although the precise cis-elements mediating its actions are still under investigation. Together, our data suggest that LRH1 may play a previously unappreciated role in Fshb transcriptional regulation in immortalized gonadotropes.

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Nothing to Disclose: JF, PS, DB, DJB

P3-214

TGIF and SnoN Repress Follicle-Stimulating Hormone β Expression in Response to GnRH Pulses in L β T2 Pituitary Gonadotropes.

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In the pituitary gonadotropes, the synthesis and secretion of the FSH is regulated by GnRH, TGF β family members and steroidal hormones. Previously through our microarray data, we identified the corepressors, TGIF and SnoN, to be differentially regulated by fast and slow GnRH pulse frequencies in the murine L β T2 pituitary gonadotropes. We showed that these corepressors repress the induction of the murine FSH β promoter by continuous GnRH and/or activin or by overexpression of Activator Protein-1 (AP-1) and Smad proteins. We have since extended those studies to assess the role of TGIF and SnoN in the response to pulsatile GnRH. Upon treatment of L β T2 cells with GnRH at high pulse frequency of 30 min lead to expression of SnoN and TGIF proteins, but no protein expression was detected to the slower pulse frequency of 120 min. In contrast, c-fos and c-jun proteins were expressed at both high and low GnRH pulse frequencies. In a pulse perfusion culture, TGIF and SnoN selectively repressed FSH β promoter activity induced by slow GnRH pulse frequency as compared to fast GnRH pulses, likely due to the fact that the endogenous proteins are already induced at the high pulse frequency. Mutation of the C-terminal phosphorylation sites on TGIF (Thr235/239) created a dominant negative mutant that increased FSH β promoter activity. The Smad 2,3 and 4 binding sites on SnoN are important for their repression of FSH β promoter as substitution mutations in these regions led to loss of repression by these corepressors. Whether SnoN and TGIF-mediated repression of the FSH β promoter involves the direct interaction of co-repressors with transcription factors bound to the AP-1 and Smad Binding Element (SBE) sites on FSH β promoter is currently being determined. Therefore, we propose a potential mechanism by which the corepressors SnoN and TGIF repress the production of gonadotropin FSH β .

Sources of Research Support: NIH grants HD12303, HD047400, HD43758 and EB009380-01.

Nothing to Disclose: DSM, RT, SAC, MAL, NJGW

P3-215

Acute Regulation of Rap1b Expression in Single Rat Gonadotropes.

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As Ras superfamily members, Rap GTPases have been implicated in a range of cell processes, e.g. Rap1b is reported to have an essential role in potentiation of glucose-stimulated insulin secretion in β -cells. GnRH self-potentiation in gonadotropes has characteristics similar to this signal amplification device, however there is little evidence identifying transcriptional products mediating the augmented LH secretion. In previous studies of rat anterior pituitaries, we identified Rap1b as a GnRH-modulated early gene using high density oligonucleotide microarray analysis. To study the rapid genomic events following GnRH receptor activation in single cells, we established a quantitative RTPCR assay to measure Rap1b transcripts in single rat gonadotropes. *Methods:* After 3 days in culture in 0.2nM estradiol, female rat pituitary cells were loaded with fura-2, transferred to a microscope stage with continuous media flow, challenged with a 15-min pulse of vehicle or 10nM GnRH or 1mM 8-bromo-cAMP followed by 25-min control medium, and cell contents harvested by patch pipette containing reverse transcriptase cocktail. For vehicle- or cAMP-treated cells, GnRH was pulsed 1-2 min before harvest for gonadotrope verification (rise in $[Ca]_i$). As control short-term GnRH treatment, cells were exposed to GnRH 1-2 min, a rise in $[Ca]_i$ noted, and cell contents immediately harvested. Single cell reverse transcription product was divided for use for Rap1b and a reference gene, Ppia, in two-round hemi-nested quantitative PCR assays. *Results:* Data were normalized to Ppia. No significant differences in Rap1b were found between the 40 min vehicle control group and the control short-term (<5min) GnRH exposure; therefore, control data were pooled. For gonadotropes collected at 40 min after GnRH initiation, a 6x increase in normalized Rap1b transcripts was found (GnRH 24 ± 5 , n=11; control 4 ± 1 , n=10; $P < 0.02$). Rap1b transcript number doubled in gonadotropes in response to cAMP (8 ± 1 , n=4; $P < 0.02$ vs. control). *Summary:* We show that Rap1b, a small GTPase identified in our earlier microarray study as a GnRH-modulated early gene, responds to GnRH or cAMP analog stimulation in single gonadotropes with increased expression by 40 min. These results are consistent with the physiological signature of GnRH self potentiation, and we suggest that, similar to the putative role in the priming of insulin secretory granules, Rap1b is a component in the priming of LH secretory granules.

Sources of Research Support: NIH: HD12137, DK66606, DK46943.

Nothing to Disclose: S-GC, SCS, DWW, JLT

P3-216

GnRH-Activated Signaling Cascades within the Gonadotrope Differentially Modulate the Activation of CREB and ICER, Mediators of FSH β Transcription.

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Episodic follicle-stimulating hormone (FSH) synthesis and release, under the control of pulsatile gonadotropin-releasing hormone (GnRH), is essential for maintaining fertility. In contrast to other gonadotropin subunit genes, transcription of pituitary FSH β -subunit (FSH β) is preferentially stimulated at low rather than high frequencies of pulsatile GnRH. GnRH mediates its effects through its cognate G protein-coupled receptor, GnRHR, in the gonadotrope. Two transcription factors have been implicated in the regulation of rat FSH β gene expression, cAMP response element binding protein (CREB) and the inducible cAMP early repressor (ICER). We hypothesized that these two transcription factors would be activated by distinct GnRH-stimulated signal transduction pathways. Stimulation of the gonadotrope-derived L β T2 cell line with 10 nM GnRH for up to 30 min resulted in a time-dependent increase in phosphorylated (p) CREB levels compared to untreated control cells. H89, a selective PKA inhibitor, markedly reduced pCREB induction at the 10 and 30 min time points. In contrast, a PKC inhibitor, GF109203X, and U0126, a MAPK pathway inhibitor, had little effect on the ability of GnRH to phosphorylate CREB. These data suggest that PKA signaling pathways play a major role in mediating GnRH stimulation of CREB phosphorylation. To explore the contributions of these signaling pathways to GnRH stimulation of ICER, L β T2 cells were stimulated with 10 nM GnRH for 0, 12 and 24 h, in the presence and absence of the KN-93 (a selective calcium/calmodulin-dependent protein kinase II inhibitor), GF109203X, U0126 and H89 inhibitors described above, followed by RNA extraction and measurement of ICER expression by RT-PCR. GnRH induced ICER mRNA levels at 12 and 24 h. This induction was markedly inhibited by U0126 pretreatment, with KN-93 having more modest effects. These data suggest that MAPK signaling pathways play the major role in mediating GnRH induction of ICER, with a lesser contribution by CamKII pathways. Taken together, these findings indicate that the signaling pathways by which GnRH stimulates CREB phosphorylation and ICER expression are distinct, and may represent a mechanism by which varying GnRH pulse frequencies modulate differential FSH β expression in the gonadotrope.

Nothing to Disclose: IRT, NAC, SX, RSC, UBK

P3-217

Signalling Diversity and Ligand-Induced Selectivity at the Human GnRH Receptor.

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The gonadotropin-releasing hormone (GnRH) receptor is a member of the rhodopsin-like family of G protein-coupled receptors (GPCRs). There is one functional GnRH receptor type, but two endogenous ligands (GnRH I and GnRH II) in humans. This indicates that the single human GnRH receptor mediates all activities of both GnRH I and GnRH II, although they display differential physiological and pharmacological profiles which are proposed to be mediated by different receptor active conformations, induced by differential ligand-receptor interactions. We have termed this GnRH ligand-induced selective signalling (LiSS). GnRH II differs from GnRH I by three amino acids in which Tyr⁵, Leu⁷ and Arg⁸ of GnRH I are replaced by His⁵, Trp⁷ and Tyr⁸. Mutation of certain amino acids in 7-transmembrane domains can specifically increase receptor binding affinity for GnRH II, but not for GnRH I, indicating that GnRH I and GnRH II stabilise different receptor active conformations. Further studies indicate that the residues at position 8 of GnRH analogues play a major role in selecting receptor conformation states. A key question yet to be answered is whether the different receptor conformational states can activate distinct intracellular signalling pathways. The human GnRH receptor was previously proposed to couple to G_{q/11}, G_s and G_{i/o}, mediating diverse physiological and pharmacological activities. In order to study G_{q/11}-independent signalling pathways at the GnRH receptor, we stably transfected GnRH receptor into a G_{q/11}-knockout MEF cell line and found that the GnRH receptor does not directly couple to G_s or G_{i/o}. However, stimulation of the cells with GnRH I and GnRH II led to different morphological changes, indicating that the GnRH receptor can mediate diverse G_{q/11}-independent signalling pathways which can be differentially activated by two endogenous ligands. The molecular mechanisms of the differential signalling and their physiopathologic roles will be discussed.

Sources of Research Support: Medical Research Council UK.

Nothing to Disclose: ZLL

P3-218

Pulsatile GnRH Causes Frequency-Dependent CREB Phosphorylation through an Acetylation-Mediated Mechanism to Control Oscillatory FSH β Transcription.

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Pulsatile hormone synthesis and secretion are critical for physiological processes, with disruption of episodic hormone release often associated with clinical disorders. Oscillatory FSH and LH secretion are under the control of pulsatile GnRH, with FSH β transcription and FSH secretion preferentially stimulated at low rather than high GnRH pulse frequencies. GnRH can stimulate FSH β transcription through a half CRE/AP1 response element. FSH β transcription is induced by GnRH-stimulated phosphorylation of promoter-bound CREB (pCREB), leading to the recruitment of the co-activator CREB binding protein (CBP). We have recently shown that this GnRH-responsive site plays an important role in the GnRH pulse frequency-dependent differential stimulation of FSH β transcription. We hypothesized that phosphorylation of CREB may be differentially controlled by changes in GnRH pulse frequency. To test this hypothesis, we perfused L β T2 cells, a gonadotrope-derived cell line, with either low or high frequencies of pulsatile GnRH and measured pCREB levels. Interestingly, levels of pCREB were higher and more sustained at low, rather than high, GnRH pulse frequencies, suggesting that this differential activation of CREB phosphorylation could contribute to GnRH pulse frequency-dependent FSH β transcription. Histone deacetylase (HDAC)-protein phosphatase (PP)-1-containing complexes are important in attenuating CREB phosphorylation, so we hypothesized that GnRH-induced CREB phosphorylation may be attenuated at high GnRH pulse frequencies by an HDAC-PP1 complex in the gonadotrope. To explore this possibility, we treated L β T2 cells in the presence or absence of the HDAC inhibitor, TSA. Western blot analysis revealed that TSA treatment caused a sustained increase in both basal and GnRH-stimulated pCREB levels, associated with a significant increase in both FSH β transcription and mRNA levels. To investigate whether this TSA-induced increase in FSH β transcription is mediated by CREB, we cotransfected L β T2 cells with a FSH β Luc reporter and either a wild type (WT) or dominant negative (dn) CREB expression vector. FSH β promoter activity was augmented with GnRH and TSA treatment in cells transfected with WT CREB, whereas cells transfected with dnCREB exhibited attenuated TSA responses. In conclusion, GnRH pulse pattern causes differential CREB phosphorylation to mediate GnRH pulse frequency-dependent FSH β transcription, potentially through an acetylation-mediated mechanism.

Nothing to Disclose: NAC, SX, RSC, UBK

P3-219

Modelling the Anterior Pituitary Gland Luteinizing Hormone Response to Two Pulses of GnRH and with Augmentation by Oxytocin.

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The biochemical interactions in gonadotrophs that produce a pulse of LH at proestrus in a rat are not fully understood. We postulated that there are two intracellular pathways that underlie the observed secretory response. One elicits the sharp rise in LH after exposure to GnRH and was named pathway F (fast). It was postulated that another pathway has a lag phase and has a later influence on LH release and was named pathway S (slow). These pathways were conceptually derived from known characteristics of the Ca⁺⁺-mediated and the cAMP/protein kinase A (PKA)-mediated pathways. The lag phase was considered to correspond to the synthesis of protein that was subsequent to phosphorylation of CREB and gene activation.

Data was obtained from perfusion studies of divided preoestrous hemipituitaries, which provided time course data for two short (4-minute) pulses of GnRH 60 minutes apart. In pathway F kinetics were assumed to be dependent on one rate limiting step. The kinetics of pathway S, once initiated, were assumed to be similar to the first pathway. Pathway S was modelled such that it augmented pathway F; hence the response of the intermediate on pathway F occurred at a level proportional to the concentration of the synthesised protein on pathway S. An optimisation programme was run to fit the model parameters to the data. Then the model was adapted to include both pulses of GnRH. The fit was excellent ($R^2=0.982$) and included an increased level of LH produced by the second, primed pulse of GnRH compared to the first, unprimed pulse. The exposure of the pituitary tissue to oxytocin together with GnRH elicited higher levels of LH release. The model was modified by the inclusion of a further parameter. All parameters were reoptimised. The fit was again excellent ($R^2=0.975$). The model for with oxytocin exposure contained parameters with values that were not significantly different from those for without oxytocin, except for that parameter pertaining to the explicit presence of oxytocin. The model therefore agreed with the biological environment.

Thus the model integrates pathways conceptualised with characteristics of Ca⁺⁺-mediated and cAMP/PKA-mediated mechanisms. Thereby further information has been provided in regard to the uncertainties that surround the roles of cAMP in gonadotrophin regulation. The model further includes details of both GnRH self-priming and augmentation of GnRH-stimulated LH release by a second peptide.

Sources of Research Support: Canterbury Medical Research Foundation; NZ Lottery Grants Board; University of Otago Division of Health Sciences.

Nothing to Disclose: JJE, TW, DJNW

P3-220

Bone Morphogenetic Proteins Regulate GnRH-Induced FSH Transcription in Gonadotrope L β T2 Cells.

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Bone morphogenetic proteins (BMPs) regulate gonadotropin transcription and production by the pituitary gonadotrope. It has been reported that BMP-6, -7 and -15 stimulate follicle-stimulating hormone (FSH) transcription and secretion by primary pituitary cells or a gonadotrope cell line. However, the involvement of BMP system in gonadotropin-releasing hormone (GnRH)-induced FSH production remains uncertain. In the present study, we attempted to elucidate the functional link between BMP signals and FSH transcriptional activity induced by GnRH using mouse gonadotrope L β T2 cells. In L β T2 cells, BMP-6 and BMP-7 enhanced mouse FSH β -promoter (-1.5 kb) activity in a concentration-responsive manner, which was inhibited by treatments with extracellular domains of ActRII but not of BMPRII. These findings suggest that ActRII is a functional type-II receptor for BMP-induced FSH β transcription. Notably, BMP-6, but not BMP-7, augmented GnRH-induced FSH β -promoter activity in L β T2 cells. Since GnRH stimulated MAPK phosphorylation in L β T2 cells, a functional link between MAPK and FSH β transcription was examined. Inhibitions of ERK pathway, but not p38 or SAPK/JNK signaling, effectively suppressed GnRH-induced FSH β transcription, suggesting that ERK pathway is functionally involved in the GnRH-induced FSH transcription. It was of interest that pretreatment with BMP-7, but not with BMP-6, suppressed GnRH-induced ERK1/ERK2 phosphorylation in L β T2 cells. Thus, the difference between BMP-6 and BMP-7 in enhancing GnRH-induced FSH transcription could be due to the differential effects of BMP ligands on GnRH-induced ERK signaling. On the other hand, GnRH treatment downregulated the expression of BMP-7 and BMPRII mRNA in L β T2 cells with a reduction of BMP-induced Smad1/5/8 phosphorylation. Collectively, BMP-6 and GnRH mutually augment FSH β transcription in L β T2 cells, in which GnRH provides a feedback inhibition of BMP-Smad signaling. These findings imply the presence of functional link between GnRH action and BMP system in the pituitary gonadotrope for fine-tuning of the FSH secretion.

Nothing to Disclose: MT, FO, HT, KI, TM, NT, EN, MAL, HM

P3-221

One Hormone, Two Functions - The Chorionic Gonadotropin in the New World Monkey.

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Objective

The luteinizing hormone (LH) and chorionic gonadotropin (CG) are essential for reproduction and male sexual development. While LH induces testosterone production, CG is essential for the establishment of pregnancy. This two hormone system is specific to primates and humans. In the New World Monkeys (NWM) the initial duplication of the ancestral LH-beta subunit (LHB) gene, which gave rise to the novel CG-beta subunit (CGB) gene, was followed by a genomic rearrangement event, leading to CGB expression in pituitary and placenta, whereas LHB became a pseudogene.

Aim

The aim of this study was to analyse the regulatory mechanisms triggering pituitary and placenta specific CGB expression in the marmoset (*Callithrix jacchus*).

Methods

DNA-isolation, RT-PCR, DNA sequencing of the CGB gene, bisulfite analyses, Dual-Luciferase-Assays and transfection of BeWo cells.

Results

We identified two different promoters, joined to two different exon 1, which regulate the expression of the CGB gene. In the pituitary a conventional exon 1 (including the exons 2 and 3 of CGB) is driven by a pituitary-specific promoter, while the novel exon 1 (spliced to the exons 2+3) is the pre-dominant form in the placenta. The placental full length core promoter (600 bp) displays a very high luciferase activity when transfected into the trophoblastic BeWo cell line. No change can be observed in promoter activity in when the promoter was truncated up to 360 bp length. Additional truncation experiments removing several transcription binding sites, three CTCF/Boris domains, two EGR sites and one CGB specific element, decreases activity stepwise down to 2% compared to full length promoter activity. Furthermore the placental promoter contains a distinct CpG island, which is absent in the pituitary promoter. In vitro methylation of the luciferase promoter constructs decreased their expression down to 2-10% compared to unmodified constructs.

Conclusion

In the marmoset CGB expression is regulated by a two promoter-two exon 1 system which is triggering the pituitary or placental expression. This underlines the plasticity of the primate LH/CG hormonal system and represents an interesting trait in the evolution of hormones which also provides further information of our understanding for the hitherto unknown regulation of CGB genes in the human.

Sources of Research Support: Alexander-von-Humboldt Foundation.

Nothing to Disclose: CA, AH, JG

P3-222

The Transcriptional Repressor, ICER, Reduces GnRH-Stimulated FSH β Transcription through Both Direct Effects on the FSH β Gene Promoter and Activin Modulation.

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GnRH and activin are major stimulators of FSH β transcription and work in synergy to augment FSH β gene expression. We have recently shown that a GnRH-responsive FSH β CRE site plays an important role in the GnRH pulse frequency-dependent differential stimulation of FSH β through ICER induction at fast GnRH pulse frequencies, which binds to this site to repress CREB-mediated FSH β transcription. This FSH β CRE is adjacent to a Pitx binding site, which in turn is important for both gonadotrope-specific and GnRH-stimulated FSH β transcription. The proximity of these two responsive sites suggests that proteins bound to these sites may interact to stimulate FSH β transcription. Pitx proteins have been shown to interact with SMAD3 to mediate GnRH/activin synergy on the FSH β promoter, so we hypothesized that Pitx1 may confer FSH β transcriptional stimulation through interaction with activin-induced phosphorylated (p)SMAD3. Pitx-pSMAD3 interaction, together with GnRH-stimulated CREB phosphorylation, could augment the recruitment of the co-activator CBP to stimulate FSH β promoter activity. ICER may disrupt transcription at this proposed integrative site by direct effects on the FSH β CRE and indirectly by inhibiting activin A production. This hypothesis is supported by the presence of a CRE site in the inhibin β A promoter, which may modulate inhibin β A expression and hence activin A production. We transfected L β T2 cells with an FSH β LUC reporter harboring either a wild type (WT) or a mutated Pitx binding site; this mutation abrogated GnRH/activin synergy and disrupted preferential stimulation of FSH β transcription at low GnRH pulse frequency. Although not detected under basal conditions in L β T2 cells, we found that inhibin β A mRNA is induced by GnRH stimulation, and that inhibin β A induction was associated with increased FSH β mRNA levels in response to both continuous and pulsatile GnRH. We showed that ICER can bind to the CRE site in the inhibin β A gene promoter, suggesting that ICER may inhibit activin A production in the gonadotrope. In conclusion, the inhibin β A gene is inducible by GnRH in a pulse frequency-dependent manner, and this may contribute to increased activin A production. ICER may mediate GnRH pulse frequency-dependent FSH β stimulation through direct effects on the FSH β promoter and indirectly by regulation of activin, which in turn will modulate FSH β transcriptional activation via the CRE-Pitx-pSMAD3 integrated GnRH/activin response element.

Nothing to Disclose: NAC, SX, RSC, UBK

P3-223

GnRH and Insulin Cross-Receptor Signaling in Gonadotropes.

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Inappropriate gonadotropin secretion is associated with a variety of metabolic imbalances. We and others have shown that gonadotropin secretion is inversely proportional to both increased body mass index and hyperinsulinemia. We have further shown that experimentally induced hyperinsulinemia suppresses baseline LH secretion in both normal women and women with polycystic ovary syndrome. These previous studies have suggested that the pituitary is directly affected by insulin. We have examined the effect of GnRH and insulin in a cultured gonadotrope cell model and demonstrated that combined treatment of GnRH and insulin resulted in a potentiated activation of ERK, a decrease activation of Akt, and co-regulation of translation. This suggests that convergent signaling effects of GnRH and insulin occur in the gonadotrope. To further explore these combined effects, we are investigating the roles of both the MAPK and PI3K/Akt pathways in convergent GnRH receptor and insulin receptor signaling in gonadotropes. We have also noted activation of the translational regulator mTOR after treatment with GnRH or insulin, or both. As a follow-up to our published studies and to determine the exact point of GnRH and insulin receptor cross-talk, we are evaluating the effects of insulin on the regulation of GnRH-dependent genes using mouse primary pituitaries cells and a cell model of mature pituitary gonadotropes (LβT2 cells). Preliminary results indicate that we can detect changes in Egr-1 in mouse primary pituitaries similar to those seen in LβT2 cells where we see an increase in Egr-1 mRNA. We will expand on these studies to help to determine the interaction of insulin in the pituitary and its effects on GnRH receptor signaling.

Nothing to Disclose: ELR, SAC, MAL

P3-224

Regulation of DUSP1 Translation by GnRH in the L β T2 Pituitary Gonadotrope Cell Line.

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The hypothalamic-pituitary-gonadal axis is a principal regulator of reproduction in mammals. GnRH, a critical component of the axis, regulates synthesis and secretion of gonadotropins like LH and FSH by the gonadotropes of anterior pituitary. LH is regulated at two different levels, the transcriptional and the post-transcriptional or translational level. The main focus of our study is at the level of translational control of GnRH over LH. Previous studies from our lab have shown that GnRH activates cap-dependent translation promoting translation of mRNAs. Recent data from our lab shows that translational regulation by GnRH is specific to some mRNAs and occurs independently of transcription. Evaluation of polyribosome profiles in GnRH-stimulated L β T2 gonadotrope cells showed Lh β mRNA redistributes to the transiently paused pool of mRNA as part of the unfolded protein response. In contrast, the mRNA encoding DUSP1, a protein phosphatase critical in resolving activation of MAP kinases, redistributes to actively translating polyribosomes. This has drawn our interest into looking for GnRH control over translation of Dusp1. Dusp1 mRNA encodes a binding site for the ARE (AU Rich Element)-binding protein HuR (Human Antigen R, or ELAV1 in mice) at its 3' UTR. Under chemical insult, HuR interaction stabilizes Dusp1 mRNA and promotes its translation. We looked for coincident redistribution of HuR in polysome profiles and also examined cytoplasmic and nuclear translocation of HuR via immunohistochemistry in response to GnRH treatment. Our data suggests that, in contrast to the response to other inducers of the unfolded protein response like hydrogen peroxide, the redistribution of HuR in response to GnRH treatment is opposite to Dusp1 redistribution in polyribosome profiles and HuR translocates in to nucleus rather than to the cytoplasm. We also found that HuR translocation is ERK-dependent and transient in response to GnRH stimulation. Future studies include evaluation of HuR-Dusp1 mRNA interaction and the potential role of other factors in mediating Dusp1 mRNA redistribution to polyribosomes in response to GnRH.

Nothing to Disclose: HCU, MAL, BG

P3-225

Obesity-Linked Factors Activate the Unfolded Protein Response in the Gonadotrope.

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As obesity rates continue to rise in the United States it is critical to investigate the mechanisms in which increased excess energy sources disrupt reproduction. There is a well-documented correlation of an increased body mass index (BMI) with decreased luteinizing hormone (LH) levels, which contributes to dysregulation of reproduction. It is not clear what contributes to this relationship. Obesity-linked factors, such as free fatty acids, lipopolysaccharides, and inflammatory cytokines, released from adipocytes activate the unfolded protein response (UPR) in some cells. The UPR is a regulatory process that, when activated, rapidly attenuates translation and posttranslational protein processing in the endoplasmic reticulum (ER) in response to accumulation of unfolded or misfolded proteins. The UPR is mediated by three major ER-resident signaling proteins: EIF2AK3, ERN1, and ATF6. In pancreatic β cells, chronic high glucose results in an increase of ERN1 activation resulting degradation of insulin mRNA. We have shown that there is a physiological role for the UPR signaling proteins in the gonadotrope. Therefore, high levels of obesity-linked factors may contribute to the BMI effect on decreased LH levels via the UPR in the gonadotrope. In order to investigate our hypothesis, we determined the effects of metabolic factors such as free fatty acids and lipopolysaccharide on ERN1 activation in the mouse gonadotrope and a gonadotrope cell model, L β T2 cells. Results show that free fatty acids increase ERN1 activation in the gonadotrope, likely resulting in decreased LH production. This suggests a mechanism by which increased BMI induces dysregulation of reproduction.

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Nothing to Disclose: DTM, LAL

P3-226

Oct1 Has a Role in Androgen Repression of Gonadotropin Releasing Hormone Transcription.

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The steroid hormone feedback loop, whereby gonadal steroids regulate hypothalamic control of reproduction, is a fundamental concept in the hypothalamic-pituitary-gonadal axis. The ultimate regulator of this axis is gonadotropin-releasing hormone (GnRH), secreted by hypothalamic GnRH neurons. Recently, it has become clear that GnRH neurons express steroid hormone receptors both *in vivo* and *in vitro*.

The androgen receptor (AR) mediates the effects of androgens in a wide array of reproductive processes, including sexual differentiation, puberty, gonadotropin regulation, and spermatogenesis. Alterations in androgen levels lead to reproductive defects in both males and females, including hypogonadotropic hypogonadism, anovulation, and infertility. Previous reports showed that androgens repress GnRH gene expression via AR, but the mechanism has yet to be determined.

In this study, we utilize a GnRH-expressing neuronal cell line, GT1-7, to show that androgen repression of GnRH transcription is dependent on the AR DNA-binding domain. Repression was mapped to two regions of the GnRH regulatory sequence, the promoter (P) and enhancer 1 (E1), by transient transfection assay. AR interacts with both of these regions, as determined by chromatin immunoprecipitation assay (ChIP), although neither region contains a canonical AR binding site.

Truncation analysis of P mapped the androgen response to the sequence between -126 and -86 bp (relative to the transcriptional start site); although required, this region was not sufficient for repression on a heterologous promoter. AR is known to interact with Oct1 in prostate cells. Mutation of known transcription factor (TF) binding sites indicated that multiple sites are required for androgen-mediated repression of P, including several Oct1 binding sites. Androgen repression was partially lost upon 3' truncation of E1 from -1571 to -1715, and a multimer of the E1 sequence between -1800 and -1766 also was repressed by androgen, suggesting that, like P, E1 contains numerous sites that contribute to repression by AR. The -1800/-1766 region includes an Oct1 binding site and the -1715/-1571 region contains two Oct1 binding sites, in addition to other known TF binding sites. Oct1 interaction with its binding sites in the GnRH P and E1 regions is required for tissue-specific GnRH expression, and Oct1 mRNA was repressed by R1881 treatment in GT1-7 cells, indicating a potential mechanism for androgen repression of GnRH transcription.

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Nothing to Disclose: MJB, PAP, PLM

P3-227

Regulation of the Subcellular Localization of the Adapter Protein and Candidate Human Obesity Gene SH2B1beta and Its Enhancement of GH-Dependent Macrophage Motility.

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GH acts as a chemoattractant for monocyte migration. However, how GH stimulates monocyte/macrophage migration is not well understood. We have previously demonstrated that the adapter protein SH2B1beta is recruited to the GH receptor-associated tyrosine kinase JAK2 in response to GH binding and promotes GH-dependent changes in the actin cytoskeleton in 3T3-F442A fibroblasts. Here we examine the role of SH2B1beta in GH-stimulated motility of macrophages. In transwell motility assays, SH2B1beta overexpression enhanced GH-dependent motility of RAW264.7 cells while SH2B1 knockdown using shRNA to SH2B1 inhibited it. SH2B1beta is known to localize and function at the plasma membrane. However, we have identified a nuclear localization signal (NLS) within SH2B1beta and shown that SH2B1beta cycles through the nucleus. Surprisingly, we found that deletion of the NLS not only prevents nuclear translocation of SH2B1beta, but it also prevents binding of SH2B1beta to the plasma membrane. To gain insight into how one motif (NLS) can serve as both a plasma membrane binding and nuclear localization motif, we examined whether phosphorylation might influence the subcellular location of SH2B1beta. Multiple (13) Ser/Thr flank the NLS. Mutational analysis implicated phosphorylation of Ser 161/165 in the release of SH2B1beta from the plasma membrane. These Ser lie in predicted protein kinase C (PKC) substrate motifs. Consistent with phosphorylation of Ser 161 and/or 165 promoting release of SH2B1beta from the plasma membrane, activation of PKC by treatment with the phorbol ester PMA stimulated release of GFP-SH2B1beta from the plasma membrane and accumulation in the nucleus within 5 min. Mass spectrometry of flag-SH2B1beta from PMA-treated cells revealed Ser161 to be phosphorylated. Overexpressing SH2B1beta lacking Ser161/165 in RAW cells was found to impair GH-induced cell motility, whereas overexpressing SH2B1beta S165E (to mimic phosphorylation of Ser165) mimicked GH-induced motility. Together, these results suggest that phosphorylation of Ser 161/165 regulates the distribution of SH2B1beta between the plasma membrane and the nucleus as well as the ability of GH to stimulate macrophage motility. Because SH2B1beta is recruited to multiple activated ligand-receptor complexes at the plasma membrane and is a candidate human obesity gene, this ability of the cell to regulate the subcellular localization of SH2B1beta is likely to affect additional cellular functions.

Nothing to Disclose: HWS, TJM, CC-S

P3-228

Characterization of STAT5 and Cux2 as Transcriptional Regulators of Sex-Specific Genes in Mouse Liver.

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Adenoviral vectors have a strong tropism for hepatocytes and can be used to infect liver cells in vivo with high efficiency. This technique was used to investigate the roles of two transcription factors in the expression of sex-dependent genes in mouse liver: STAT5, a GH-activated transcription factor, and Cux2, a putative female-specific transcriptional repressor (F/M~100 fold) [1]. Adenovirus expressing a dominant negative (DN) form of STAT5a was injected into 6-week male mice and gene expression evaluated in comparison to Adeno-GFP-infected controls. Female-specific genes *Abcd2*, *Acot3*, *Cux2*, *Cyp2b9*, *Cyp4a10* and *Nnmt* were substantially de-repressed 3 and 5 days post-injection, while several male-specific mRNAs and their hnRNAs were largely unchanged (*Cyp7b1*, *GSTπ*, *Slp*). Female-specific genes that respond slowly to continuous GH treatment of male mice [2] were only partially de-repressed by Adeno-DN-STAT5, even after 5 days (*Cyp2b13*, *Cyp3a16*, *Hao3*, *Sult3a1*). Liver *Igf1* mRNA levels were unchanged, suggesting that incomplete inhibition of endogenous liver STAT5 could explain the lack of effect of Adeno-DN-STAT5 on male gene expression.

Adenovirus encoding *Cux2* injected into 6-week male mice induced female-like levels of *Cux2* mRNA that persisted at least 7 days. Three female-specific genes were activated in the Adeno-*Cux2*-treated livers (*Acot3*, *Cyp2b9*, *Cyp4a10*), albeit variably, while several male-specific genes were unchanged. In an alternative approach, ChIP-seq analysis was used to identify potential targets of *Cux2* in female mouse liver chromatin. In an initial study, 171 *Cux2*-bound ChIP-seq peaks were detected, 40 of which contained a *Cux*-like DNA binding site, corresponding to a 19-23-fold enrichment over control sequences (computational analysis by Dr. Yijing Zhang of this laboratory). Notably, 151 of the 171 peaks coincided with genomic sites that show DNase hypersensitivity in mouse liver. Unexpectedly, 38 of these 151 sites showed increased DNase hypersensitivity in male compared to female mouse liver, corresponding to a 6.5-fold enrichment compared to all liver DNase hypersensitivity sites, while only one *Cux2* site showed female-enriched DNase hypersensitivity. These findings suggest as a working hypothesis that *Cux2* in female liver blocks the repressive activity of genomic regions that are preferentially open in male liver, where they target female-specific genes for repression, as suggested by the de-repression that follows Adeno-*Cux2* infection.

[1] Laz EV, Holloway MG, Chen CS, Waxman DJ. Characterization of Three Growth Hormone-Responsive Transcription Factors Preferentially Expressed in Adult Female Liver. (2007) *Endocrinology* 148: 3327-3337.

[2] Holloway MG, Laz EV, Waxman DJ. Co-dependence of growth hormone-responsive, sexually dimorphic hepatic gene expression on signal transducer and activator of transcription 5b and hepatic nuclear factor 4alpha. (2006) *Mol Endocrinol* 20: 647-660.

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Nothing to Disclose: TLP, DJW

P3-229

The Adapter Protein SH2B1 and Growth Hormone-Induced Gene Expression.

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Metabolic effects of growth hormone (GH) in adipose tissue have been shown to be mediated, at least in part, by changes in gene expression. The adapter protein SH2B1 is a signaling molecule for many cytokines and growth factors, including GH. SH2B1 is recruited to the GH-activated form of the GH receptor-associated tyrosine kinase JAK2, resulting in its phosphorylation by JAK2 on tyrosines and on serine/threonines by other kinases. SH2B1 has been implicated in GH regulation of the actin cytoskeleton. Recently, our laboratory has identified both a nuclear localization signal (NLS) and nuclear export sequence (NES) in SH2B1. SH2B1 constitutively shuttles in and out of the nucleus, even though its steady-state sub-cellular localization is mostly in the plasma membrane and cytosol. In PC12 neuronal cells stimulated with nerve growth factor (NGF), over-expression of wild-type SH2B1, but not SH2B1 that is incapable of entering the nucleus (SH2B1 mNLS), enhances the expression of a sub-set of NGF-induced genes. Together, these data led us to hypothesize that SH2B1 regulates GH-induced gene expression in adipocytes. To test this, cultured 3T3-F442A adipocytes with endogenous SH2B1 knocked-down by RNAi and control cells were stimulated with GH for 1 h and a sub-set of genes that depend on SH2B1 for their full GH-induced expression was identified by microarray analysis. Of the 16,361 total genes on the array, 611 were up-regulated by GH at least 60%; of those genes, 247 appeared to depend on SH2B1 for their full GH-induced expression. GH-dependent expression of *apelin*, *cish* and *arid5a* genes and their decreased GH-induced expression in cells with decreased levels of SH2B1 was confirmed by QPCR. The re-introduction of SH2B1 in 3T3-F442A pre-adipocytes in which endogenous SH2B1 is knocked-down is able to rescue GH-induced expression of *cish* and *arid5a*. Over-expression of SH2B1, but not SH2B1 mNLS, enhances GH-induced expression of these genes. These results are consistent with SH2B1 being required for maximal expression of a sub-set of GH-dependent genes. Further, nuclear localization of SH2B1 may be required for SH2B1 enhancement of expression of a subset of GH-dependent genes.

Nothing to Disclose: MD, HJ, CC-S

P3-230

Evaluation of GH Receptor Dimerization Interface Mutants.

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Growth hormone (GH) binding to the GH receptor (GHR) activates signaling by the JAK2-STAT5, Akt, and ERK pathways. Although GHR is believed to exist at the cell surface as a preformed dimer, GH engagement resulting in attainment of the active GHR signaling conformation is accompanied by covalent disulfide linkage between GHRs that is mediated by cysteine-241 in the receptor extracellular domain stem region and detected by anti-GHR immunoblotting of cell extracts resolved by SDS-PAGE under nonreducing conditions. We previously demonstrated that GH promotes GHR downregulation and that this requires the receptor's association with and activation of JAK2 (1). In this study, we compared structural and functional aspects of GHRs mutated in the extracellular domain dimerization interface, as expressed in a GHR- and JAK2-deficient human fibrosarcoma cell reconstitution system. GHR_{H150D} is a rabbit GHR with His-150 converted to Asp and was previously shown to predimerize ineffectively (2). GHR_{H150Q} is a human GHR with His-150 converted to Gln and corresponds to a receptor mutant identified previously in a compound heterozygote patient with GH insensitivity syndrome (3). Both receptor mutants exhibited similar half-lives as did wild-type rabbit GHR when expressed in the presence of JAK2. Likewise, all three GHRs were dramatically more short-lived (and to similar degrees) when expressed in the absence of JAK2. Both wild-type GHR and GHR_{H150Q} underwent GH-induced receptor disulfide linkage and allowed GH-induced STAT5 activation; in contrast, GHR_{H150D} was incapable of these responses. Furthermore, GH induced marked downregulation of wild-type GHR and GHR_{H150Q} if coexpressed with JAK2; GHR_{H150D}, however, manifested no GH-induced downregulation. Finally, GHR_{H150Q}, like wild-type GHR, preferentially localized to the lipid raft fraction of the plasma membrane. In contrast, GHR_{H150D} was relatively excluded from this fraction. We conclude that the impact of a point mutation of His-150 in the GHR dimerization interface on receptor signaling, trafficking, and structure, may critically depend on the specific nature of the mutation. Further studies to evaluate the determinants of the differences between GHR_{H150D} and GHR_{H150Q} are underway.

- (1) Deng L, et al., Mol Endocrinol 2007 21:1537
- (2) Fang P, et al., J Clin Endocrinol Metab 2007 92:2223
- (3) Yang N, et al., Mol Endocrinol 2007 21:1642

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Nothing to Disclose: LD, JJ, NY, MJW, VH, SJF

P3-231

Biochemical Characterization of Growth Hormone-Regulated and Stat5b-Binding Transcriptional Enhancers in the Rat IGF-I Locus.

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Growth hormone (GH) action leads to rapid activation of the insulin-like growth factor-I (IGF-I) gene via Stat5b, a latent cytoplasmic transcription factor that is activated by the GH receptor-associated tyrosine kinase Jak2 before translocating to the nucleus and binding to target DNA. Recent studies by our laboratory have identified 7 distinct GH-stimulated Stat5b binding domains in chromatin within the rat *igf1* locus with characteristics of hormone-activated transcriptional enhancers (1). Here we dissect binding properties and functional aspects of these 7 GH-regulated and Stat5b-mediated response elements, which each contain 1 - 3 sites matching a consensus Stat5b recognition sequence (5'-TTCNNGAA-3', where N = G, A, T, or C). When assessed by electrophoretic gel-mobility shift assay (EMSA), each site was able to bind Stat5b, with dissociation constants (Kd) that varied from ~0.8 to >50 nM. DNA substitution experiments indicated that binding affinity was a function of both the variable 3 central nucleotides (nt) and of DNA immediately flanking the 9 nt Stat5 recognition sequence. The HS7 domain, located in IGF-I intron 2, contains two Stat5b binding sites (R34 and R35, Kd 18 and >50 nM, respectively) separated by 60 nt of intervening DNA. Fusion of HS7 to IGF-I promoter 2 (P2) increased basal activity 5-fold, which rose to ~25-fold in the presence of GH-activated Stat5b in transfected cells. Mutations of R34 and R35 that prevented Stat5b binding abrogated basal and hormone-stimulated promoter activity. Substitution of R35 with the higher affinity R34 in HS7 doubled basal promoter function and led to a 50% rise in response to GH, and increased Stat5b binding affinity in vitro. Other DNA substitutions in R35 that enhanced Stat5b binding by EMSA also increased IGF-I P2 promoter activity. Conversely, replacement of R34 with the lower affinity R35 in HS7 caused a 50% decline in promoter function, along with a decrease in Stat5b binding by EMSA. Similar results were observed with substitutions as small as a single base change in R34 (CTG of the central core to CTA of R60). Taken together, our observations suggest that binding affinity for Stat5b is a key functional determinant for GH-activated enhancers that promote IGF-I gene transcription.

(1) Chia et al, manuscript submitted

Sources of Research Support: NIH Grant GBMO0115D.

Nothing to Disclose: BDV-M, KLM, DJC, PSR

P3-232

Growth Hormone Induces Hepatic Stat5 Phosphorylation in Early Postnatal Life in Mice.

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Growth hormone (GH) concentrations are high at birth and decrease slowly but it is not clear when and where GH signaling takes place or what the consequence of GH signaling is. We recently showed direct action of GH on several tissues by visualizing Stat5 phosphorylation (pY-Stat5) in response to a pulse of GH. We have now assessed response to GH in normal (WT) and transgenic GRF-M2 pups that are GH-deficient (GHD) (0-10 days (d) of age), to either a single injection of GH or to 5 d GH treatment.

GHD mice had smaller body weights than WT mice from birth, reaching significance at 7d of age. At 8 d of age, plasma IGF1-concentration was reduced in GHD mice ($p=0.0004$). 3d-old and 10d-old GHD and WT mice were treated with bovine GH (bGH, 10 μ g/g bw sc bd) or vehicle for 5 d ($n=5-8$ /group). 10d-old GHD mice, but not WT mice, responded by increasing weight gain ($p=0.01$), tibial length ($p=0.02$) and plasma IGF1 ($p<0.05$). In contrast, these parameters were not affected in response to bGH in 3d-old GHD mice ($p>0.01$). A single mouse GH (mGH) injection (1 μ g/g bw ip) at 6-7 d of age in GHD and WT mice, resulted in Stat5 signaling in liver, and growth plate, 25 minutes later, as detected by immunohistochemistry. In the next experiment, 7d-old GHD and WT mice were killed at random times. GHD mice ($n=12$) had undetectable plasma GH-concentration and no detectable pY-Stat5 in the liver. In 21 WT mice, GH-concentrations ranged from 4.1-55.2 ng/ml. Hepatic pY-Stat5 was detected in 8 mice, and these mice had significantly higher plasma GH concentrations (24.4 \pm 5.0 vs 9.1 \pm 1.0 ng/ml, $p=0.001$), and pY-Stat5 staining was most intense in the mouse with the highest GH-concentration (55 ng/ml). When GHD pups were given 1 μ g/g bw mGH on the day of birth, pY-Stat5 was, like in the 7d-old mice, detected in the liver.

In conclusion, 10d-old but not 3d-old GHD mice respond to 5 d bGH by increasing body weight and bone growth. In contrast, both 0d and 7d-old mice are able to respond to a single GH pulse by phosphorylating Stat5 in the liver. This suggests that hepatic GHR activation and GH signaling takes place in mice from birth, but that in the youngest mice this GHR activation does not result in growth acceleration. The presence of hepatic pY-Stat5 in only the mice that have high GH-concentrations suggests that hepatic Stat5 phosphorylation follows on an endogenous GH pulse like in older mice, and suggests that GH secretion may be pulsatile in mice as young as 7 d of age.

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Nothing to Disclose: EFG, MTD

P3-233

Deletion of IGF-1 Receptor in a Human Prostate Cancer Cell Line Reduces GH-Induced JAK2 and STAT5 Phosphorylation.

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Growth hormone (GH) signals somatogenic and metabolic effects by interacting with GH receptor (GHR) to activate downstream signaling molecules, including JAK2 and STAT5. Insulin-like growth factor-1 (IGF-1) is an important target gene induced by GH in a STAT5-dependent fashion. IGF-1 signaling is mediated by IGF-1 receptor (IGF-1R) and regulates cell proliferation and apoptosis. IGF-1R is a heterotetramer consisting 2 α -subunits and 2 β -subunits, with intrinsic kinase activity in its β -subunit cytoplasmic domains. We previously described a GH-induced GHR-JAK2-IGF-1R complex, suggesting IGF-1R participates in GH signaling (1). Recently, we demonstrated in primary mouse osteoblasts that IGF-1R may directly facilitate acute GH signaling (2). We now explore the contribution of IGF-1R to acute GH signaling in LNCaP, a human prostate cancer cell line. We first examined GH induced complex formation. We observed that acute treatment with GH, but not IGF-1, promoted coimmunoprecipitation of a complex including IGF-1R and tyrosine phosphorylated GHR and JAK2. To achieve a reduction of IGF-1R in LNCaP cells, we prepared a plasmid (pRNAU6.1/Neo-shIGF-1R) encoding an shRNA that targets human IGF-1R at a 19bp sequence beginning at nt3425 of its mRNA. LNCaP cells were transfected with pRNAU6.1/Neo-shIGF-1R or the empty vector as a control and transfected pools (vector vs. shIGF-1R) were selected. Cell extracts of LNCaP parental, vector pool, and shIGF-1R pool cells were analyzed. Substantial knockdown of IGF-1R in the shIGF-1R pool vs. vector pool cells was achieved, while GHR and JAK2 levels were unchanged. Acute (250 ng/ml; 15 min) GH signaling was examined in serum-starved shIGF-1R pool vs. vector pool cells. We observed markedly decreased GH-induced tyrosine phosphorylation of STAT5 in shIGF-1R pool cells vs. vector pool cells, similar to our previous findings in primary mouse osteoblasts. Interestingly, we also observed decreased GH-induced tyrosine phosphorylation of JAK2 in shIGF-1R pool cells (55% and 45% reductions in JAK2 and STAT5 tyrosine phosphorylation, respectively, when normalized for total JAK2 and STAT5 abundance). Thus, reduction of IGF-1R in LNCaP resulted in a marked decrease in acute GH signaling. Our data suggest that IGF-1R exerts a role at a very proximal level in GH signal transduction.

(1) Huang Y, et al., Mol Endocrinol 2004;18:1471

(2) Gan Y, et al., Mol Endocrinol 2010 in press

Sources of Research Support: NIH R01 DK 46395.

Nothing to Disclose: YZ, YG, YH, JJ, SJF

P3-234

Pharmacodynamic Responses of C-Type Natriuretic Peptide to GH in Growth Hormone Deficient Rats: Correlation with Linear Growth.

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C-type natriuretic peptide (CNP), a paracrine growth factor essential for endochondral bone growth in rodents and humans, is a member of a family of structurally related peptides best known for their cardiovascular actions. Whereas the CNP concentration in blood is low and close to assay detection limits, a stable product of the CNP gene (amino terminal proCNP, NTproCNP) is readily measurable in plasma and can be used to study the regulation of CNP secretion in vivo. Recent studies in lambs [1] and children [2] show that the plasma concentration of NTproCNP is strongly correlated with skeletal growth velocity raising the possibility that its concentration in plasma reflects the effects of GH/IGF-1 within growth plate tissues. In order to examine the dynamic response in CNP to changes in skeletal growth we have studied the tissue and plasma NTproCNP responses to GH in rats with GH deficiency. We generated a specific antiserum in sheep to rat proCNP(38-50) and used it to confirm the presence of the putative 5kDa protein (rat NTproCNP) by RIA-HPLC. Groups of 4 week-old GH deficient Sprague Dawley male rats (n=6 per group) received daily injections of GH (3mg/kg) or vehicle control for 1, 3 or 7 days. After 7 days of treatment, percentage increase in linear growth (nose-tail length) and body weight were both significantly increased by GH (54 and 26% respectively) compared to vehicle control (p<0.001). Plasma NTproCNP (174 ± 9 pmol/l v 101 ± 4 in controls) was increased by 73% (p<0.001) and in individual rats was significantly correlated with linear growth velocity (r²=0.80). At 24 h after starting treatment, plasma NTproCNP was increased by 39% in GH treated rats (116 ± 5 pmol/l v 83 ± 2 in controls, p<0.001) despite the lack of any measurable increase in linear growth - suggesting that the response in plasma concentration of NTproCNP may predict growth response at the level of the growth plate. These findings, the first to describe the dynamic response of CNP production to GH in growth hormone deficient states, provide further support for the use of plasma NTproCNP as a unique marker of growth plate responsiveness. Changes in plasma NTproCNP may have application in assessing efficacy of growth stimulants in children with growth disorders.

[1] Prickett TCR et al., *Ped Res* 2005;58:334

[2] Prickett TC et al., *J Clin Endocrinol Metab* 2008;93:225

Sources of Research Support: Canterbury Medical Research Foundation, New Zealand.

Nothing to Disclose: TCRP, JCB, TGY, AMR, EAE

P3-235

C/EBP beta Mediates Growth Hormone-Regulated Expression of Multiple Target Genes: Involvement of C/EBP beta Phosphorylation and Co-Activator Recruitment.

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Regulation of gene transcription by growth hormone (GH) can be mediated through phosphorylation and activation of C/EBP beta, as exemplified by induction of c-fos. For insight into whether other genes also depend on C/EBP beta during induction by GH, we examined a panel of genes which, like c-fos, were rapidly and transiently induced by GH, suggesting similar transcriptional regulatory mechanisms. The genes include btg2, zfp36, cyr61, and socs3 among others. The panel of GH-responsive genes was identified in a gene expression profile and verified by stimulation of mRNA expression by GH in 3T3-F442A adipocytes and preadipocytes. Sequence analysis predicted conserved C/EBP sites in promoters of many of these early response genes. Consistent with dependence on endogenous C/EBP beta, deficiency of C/EBP beta induced with shRNA significantly impaired responsiveness to GH. In parallel, addition of the MEK inhibitor U0126 strongly inhibited induction by GH of most of these C/EBP beta-dependent genes. Since phosphorylation of murine C/EBP beta at T188, an ERK substrate site, mediates induction of c-fos by GH, these findings together suggest that phosphorylation of C/EBP beta by the ERKs might play a role in the response of multiple genes to GH. For insight into how C/EBP beta phosphorylation contributes to GH-induced gene expression, we examined the importance of C/EBP beta phosphorylation for co-activator recruitment. In C/EBP beta-deficient cells, rescue with wild type C/EBP beta led to recruitment of the co-activator p300 to the c-fos promoter in a GH-dependent manner. In contrast, rescue with C/EBP beta T188A mutated at the ERK substrate site failed to induce recruitment of p300, indicating that the GH-induced phosphorylation of C/EBP beta at T188 is required for recruitment of the co-activator as well as induction of c-fos expression. Together these findings indicate that GH-stimulated expression of multiple early response genes is mediated by C/EBP beta and involves GH-stimulated, ERK 1/2 dependent events. At least one mechanism involves GH-induced phosphorylation of C/EBP beta leading to recruitment of the co-activator p300. These studies suggest that C/EBP beta, like the Stat proteins, mediates regulation of multiple genes in response to GH.

Nothing to Disclose: TXC, CCS, GL, NJL, HJ, CC-S, ZSQ, JS

P3-236

Analysis of GHRH-GH-IGF-1 Axis in Intrauterine Growth Retardation Rats.

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BACKGROUND/OBJECTIVE: Maternal calorie restriction is one of causes for intrauterine growth retardation (IUGR). In humans, postnatal growth failure is caused by IUGR. To reveal the mechanism underlying growth failure caused by IUGR, we analyzed growth hormone-releasing hormone (GHRH)/(GH)/ insulin-like growth factor (IGF)-1 axis in a rat model in which maternal food intake was restricted throughout the pregnancy.

METHODS: Nine-week old normal Wistar female rats were mated with normal male rats, and dams were divided into two groups, control dams which were allowed free access to food and water and food-restriction dams which were exposed to food restriction by 40% throughout the pregnancy period. After giving birth to pups, the food-restriction dams were released from food-restriction. The body weight and length of offsprings were measured at day 21, and they were divided into the 3 groups: control, offsprings bred from control dams; IUGR-N, offsprings bred from food-restriction dams with body weight above the means-2SD of controls; and IUGR-S, offsprings bred from food-restriction dams with body weight lower than the mean-2SD.

RESULTS: Plasma GH and GH mRNA expression in the pituitary were significantly lower in female IUGR-S, but not in female IUGR-N than in female controls. Similarly, GH mRNA expression in the pituitary was significantly lower in male IUGR-S but not in male IUGR-N than in male controls. GHRH and somatostatin mRNA expression in the hypothalamus and their receptor mRNA expression in the pituitary did not show significant changes in male or female IUGR-S. Hepatic GH receptor and IGF-1 mRNA expression were significantly lower in male and female IUGR-S, but not in male or female IUGR-N than in controls.

CONCLUSIONS: It is reported that epigenetic mutation occurs in hepatic IGF-1 gene which is thought to reduce gene expression and secretion of IGF-1 in IUGR rats (1). The results of the present study have shown that in addition to decreases in mRNA expression and secretion of IGF-1, decreases in GH secretion and pituitary GH and hepatic GH receptor mRNA expression may be involved in the mechanism underlying the growth failure in IUGR-S rats.

(1) Fu Q et al. FASEB J 2009; 23:2438

Nothing to Disclose: TN, TS

P3-237

Accelerating and Inhibitory Effects of a Chimeric Peptide Consisting of Somatostatin and a GH Fragment.

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The biological effects of a synthetic chimeric peptide (P7) consisting of the 104-113 amino acid rat growth hormone sequence linked to a non-cyclized 1-14 somatostatin were studied in vivo in female rats and in primary neonatal mouse mandibular chondrocyte cultures. One subcutaneous (s.c.) injection of 1 mg as well thirteen s.c. injections of 100 µg of P7 to adult rats resulted in similar weight gains of 25% more than the controls (p<0.001) and in a significant rise in serum IGF-I (p<0.001). On the other hand, in young rats injection of 1 mg of P7 resulted in loss of weight (p<0.01) and no change in plasma IGF-I in comparison to control rats.

In in vitro studies P7 induced a biphasic effect. Whereas 10⁻⁷M stimulated, a 10⁻⁶M concentration suppressed the expression of both IGF-I and IGF-IR. Furthermore, P7 in a dose dependent manner increased cell proliferation assayed by [³H]Thymidine uptake into DNA. During the in vivo experiments P7 did not induce antibodies.

In conclusion: The application of the biphasic activity of the P7 chimeric peptide is both of academic interest but may also be of clinical and therapeutic importance. Being a small peptide its actions by intranasal delivery are planned. Finally, an entire human analog consisting of the 104-113 amino acid human growth hormone sequence linked to the non-cyclized 1-14 somatostatin is also planned.

Nothing to Disclose: CC, GM, ZL

P3-238

β Cell Specific Disruption of the Growth Hormone Receptor Does Not Affect β Cell Mass but May Affect Its Function.

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The growth hormone (GH) -Insulin-like growth factor-1 (IGF1) axis and insulin signalling pathways are known to play important roles in the regulation of β-cell growth and function. However, the mechanisms of GH action in the β-cell remain largely unknown. To directly explore the role of GH receptor (GHR) signaling in the growth and function of pancreatic β cells, we used Cre-loxP system to generate a mouse model with the growth hormone receptor specifically disrupted in β cells (βGHRKO) by breeding mice carrying a GHR flox allele (LL) with mice expressing Cre driven by the rat insulin II promoter (RIP-cre).

Both PCR analysis using Laser Capture Microdissection of the islets and immunohistochemistry confirmed specific deletion of GHR in islet tissues. Histological assessment of β-cell mass revealed no significant changes between controls and βGHRKO mice. Despite the absence of GHR in islets of βGHRKO mice, serum IGF1 levels measured at 8 and 16 weeks of age, were not changed. Body weight and body length of βGHRKO mice were similar to controls (LL, RIP-Cre).

Assessment of glucose homeostasis demonstrated that the blood glucose and serum insulin levels were not different between control and βGHRKO mice in fed or fasting conditions. Additionally, glucose tolerance and insulin sensitivity were not altered in βGHRKO mice as compared to control mice. When acute insulin release in response to glucose load was measured, βGHRKO mice exhibited a trend towards decrease in insulin secretion 2 and 5 min following i.p. glucose injection, indicating β cell secretory function in vivo was mildly affected.

Taken together, our data suggest that GH does not affect β-cell mass however, it may play a role in the first phase insulin secretion following glucose load.

Nothing to Disclose: YW, HS, AV, SY, CL, SY, DL

P3-239

Induction of SIP1 by Growth Hormone (GH) in the Glomerular Podocyte: A Novel Action of GH with Implications for the Pathogenesis of Diabetic Nephropathy (DN).

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Background: Pituitary GH is implicated in the pathogenesis of microangiopathic complications of type I diabetes mellitus (T1DM) including DN. However, the precise cellular mechanism(s) for GH's role in the development of DN are unknown. Previous studies from our laboratory demonstrated that functional GH receptors (GHR) are expressed in glomerular podocytes. Microarray analysis identified ZFH1B (Zinc finger homeobox 1b or Smad-interacting protein 1 [SIP1]) to be upregulated in a GH-dependent manner in immortalized human podocytes. Downstream targets of SIP1 such as E-cadherin are implicated in the epithelial-myofibroblast transdifferentiation of podocytes observed in DN. The current studies were undertaken to elucidate the biological effects of GH-dependent modulation of SIP1 expression in the glomerular podocyte. **Hypothesis:** GH-dependent increase in SIP1 expression results in biological effects in the podocyte via inhibition of E-cadherin expression. **Results:** Exposure of podocytes to GH resulted in upregulation of SIP1 and downregulation of E-cadherin expression. Luciferase reporter assays revealed that GH inhibited E-cadherin promoter activity. Furthermore, mutation of putative SIP1 binding sites on the E-cadherin promoter abrogated the effect of GH on the E-cadherin promoter. SIP1 levels in the cell are also controlled by a SIP1 natural antisense transcript (NAT) and previous studies had established that increase in NAT results in increased levels of SIP1 protein¹. We used real time RT-PCR analysis to demonstrate GH-dependent increase in expression of SIP1 NAT in podocytes. A paracellular albumin influx assay was used to demonstrate GH-dependent increase in podocyte permeability to albumin. However, in podocytes with knockdown of SIP1 expression induced by lentiviral-mediated shRNA, GH failed to alter albumin permeability, thus confirming a direct link between GH's effects on SIP1 expression and GH's effect on podocyte permeability. **Conclusions:** GH increases expression of ZEB2/SIP1 in part by increasing expression of a SIP1 NAT. GH-dependent increase in SIP1 expression results in loss of E-cadherin in podocytes and increased podocyte permeability to albumin. We speculate that GH's effects on SIP1 and E-cadherin expression play a role in the pathogenesis of microalbuminuria and nephropathy in DM.

1. Beltran M, Puig I, Peña C, García JM, Alvarez AB, Peña R, Bonilla F, de Herreros AG. A natural antisense transcript regulates Zeb2/Sip1 gene expression during Snail1-induced epithelial-mesenchymal transition. *Genes Dev.* 2008 Mar 15;22(6):756-69.

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Nothing to Disclose: AKP, KK, PD, CL, MSB, RKM

P3-240**Use of the Cre-loxP System To Dissect out the Individual Roles IGF-I Receptors (IGFIR) and Insulin Receptors (INSR) Play in Mediating Somatotrope Function, *In Vitro* and *In Vivo*.**

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Growth hormone (GH), from pituitary somatotropes, stimulates peripheral tissues to produce IGF-I, which in turn feedbacks to directly inhibit GH synthesis and release. Insulin also directly inhibits somatotrope function, which could account for the negative association of GH and insulin in obesity and diabetes. Differentiating between the actions of IGF-I and insulin is confounded by the fact that IGFIR and INSR are both expressed in the pituitary, are activated by high doses of each other's ligand, and may interact as hybrid receptors or via crosstalk of downstream signaling pathways. To circumvent these problems the Cre/loxP system was used to inactivate Igflr and Insr in somatotropes, *in vitro* and *in vivo*. Treatment of pituitary cell cultures from Igflr^{fl/fl} mice (1) with a Cre recombinase adenovirus (Cre-Ad) reduced Igflr mRNA to <20% of Cre-Null-Ad controls and eliminated the inhibitory effect of IGF-I on GH release and synthesis, without altering the response to insulin. Conversely, Cre-Ad treatment of Insr^{fl/fl} (2) cultures eliminated the inhibitory effect of insulin, without altering the response to IGF-I. These results suggest IGF-I/IGFIR and insulin/INSR work independently to inhibit somatotrope function. To determine if the same is true *in vivo*, Igfl^{fl/fl} and Insr^{fl/fl} mice were crossbred to rat GH-promoter,Cre (rGHpCre) mice (3) generating somatotrope-specific knockouts of Igflr (Igflr^{rGHpCre}), Insr (Insr^{rGHpCre}) or both receptors (Igflr/Insr^{rGHpCre}). Knockout of either Igflr or Insr alone did not alter pituitary morphology or cell composition, while Igflr/Insr^{rGHpCre} pituitaries were smaller than homozygous floxed, Cre negative controls. Igflr^{rGHpCre} and Insr^{rGHpCre} mice showed modest elevations in GH, that were most pronounced in Igflr^{rGHpCre} mice, leading to increases in IGF-I only in females. Body weight of Igflr^{rGHpCre} and Insr^{rGHpCre} mice did not differ from controls up to 7 months, however male Igflr^{rGHpCre} mice had reduced fat mass, as recently reported by our group (4). Consistent with the independent actions of Insulin/Insr and IGF-I/Igflr observed *in vitro*, knocking out both receptors had an additive stimulatory effect on GH sufficient to elevate IGF-I in both sexes. Male, but not female, Igflr/Insr^{rGHpCre} mice were larger than controls starting at 6 weeks, without changes in fat mass (at 3 months). Studies are ongoing to further explore the differential impact of these somatotrope-specific changes on GH-axis and metabolic function.

- (1) Kloting N et al., Diabetes 2008; 57:2074
- (2) Bruning JC et al., Molecular Cell 1998; 2:559
- (3) Luque RM et al., Endocrinology 2007; 148:1946
- (4) Romero CJ et al., P1-244, 91st Annual Meeting, ENDO 09

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Nothing to Disclose: RML, JC-C, QL, CRK, LK, JCB, RDK

P3-241

Enhanced GH Gene Expression by Estrogen Receptor Activation.

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Introduction: Sex steroids have been shown to increase GH secretion *in vivo* and *in vitro*. In humans, serum GH levels correlate with changes in serum estrogen levels during the menstrual cycle. In ewes, an increase in serum GH levels occurs at the time of the spontaneous or estradiol (E₂)-induced LH-surge. In ovariectomized (OVX) or intact primate monkeys, E₂ also provokes an increase in GH and IGF-I. However, there is less evidence regarding the role of sex steroids on GH synthesis and whether the effects are mediated by estrogen receptors (ER).

Aim: The aim of this study was: 1) to evaluate the direct effects of estrogen on GH synthesis, and 2) to determine the role of ER α and ER β in mediating the effect.

Materials and Methods: Somatotroph-like GH₃ and MtT/S cells were used as *in vitro* model systems complemented by *in vivo* studies in wild-type OVX mice. mRNA levels were measured by qRT-PCR and serum GH and prolactin (PRL) levels were measured by a multiplex assay system.

Results: Both GH₃ and MtT/S cell lines were shown to express both ER α and ER β mRNA and protein. In GH₃ and MtT/S cells, an E₂ concentration-dependent increase in GH mRNA levels was observed between 10⁻¹⁰ and 10⁻⁷M, with a maximal effect at 1 nM E₂. In contrast, in the presence of the ER antagonist, ICI 182,780, GH mRNA stimulation by E₂ was completely abolished. Like E₂, both ER α and ER β specific agonists, PPT (propylpyrazole triol) and DPN (2,3-bis(4-hydroxyphenyl) propionitrile) respectively, increased GH and PRL mRNA levels in a concentration-dependent manner. In both cell lines, E₂ also increased the ER α /ER β mRNA level ratio. At these E₂ concentrations, mRNA levels for the Pit-1 transcription factor were also increased. In OVX wild-type mice, administration of either E₂ or PPT increased serum GH and PRL levels and GH and PRL mRNA levels to the same extent, suggesting an ER α mediated effect.

Conclusions: Thus, GH gene expression is increased by either ER isoform in cultured pituitary cell lines but *in vivo* the predominant increase is mediated by the ER α specific ligand. Regional tissue differences in ER isoform expression and not isoform-specific differences between ER α and ER β underlie the apparent ER α pituitary selectivity observed *in vivo*.

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Nothing to Disclose: DA, FW, SR

P3-242

Growth Hormone Deficiency in Obesity Is Associated to Cardiometabolic Risk Factors.

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In obesity there is a markedly decreased GH secretion. For both children and adults, the greater the body mass index (BMI), the lower the GH response to provocative stimuli, including the response to GH-Releasing Hormone (GHRH). The altered somatotroph function of obesity is not permanent; it can be reversed by a return to normal weight or by short term calorie restriction

The aim of the present study was to evaluate the relationship between GHRH-induced GH secretion in obese premenopausal women and cardiovascular risk markers or insulin resistance.

Forty eight premenopausal obese women, aged 35-52 yr, were studied. GH secretion, IGF-I, serum cardiovascular risk markers, insulin, leptin, mid-waist and hip circumference, total body fat and truncal fat were measured. None of the obese patients had diabetes mellitus or other medical problems nor were they taking any drugs. All subjects had regular menses.

After an overnight fast and while seated, GHRH was administered. We obtained blood samples for GH at baseline (0 min) and then at times 15, 30, 60, 90 and 120 minutes. Fasting blood was drawn for IGF-I, serum cardiovascular risk markers and hormonal determinations. Subjects were classified as meeting the criteria for GH deficiency (GHD) when peak GH after stimulation with GHRH was ≤ 3 mg/L.

Mean total and LDL cholesterol, fasting insulin and HOMA-IR were all higher, in subjects who would have been classified as GH-deficient compared with GH-sufficient. Peak GH secretion after stimulation was inversely associated with fasting insulin ($R=-0.650$, $P=0.012$), HOMA-IR ($R=-0.846$, $P=0.001$), total cholesterol ($R=-0.532$, $P=0.034$) and LDL cholesterol ($R=-0.692$, $P=0.006$), and positively associated with HDL cholesterol ($R=0.561$, $P=0.037$). There was a significant positive correlation between IGF-I and peak GH, and a negative correlation between IGF and HOMA-IR, but IGF-I levels were not decreased in the GHD group.

These data strongly suggest a role for insulin resistance in the decreased GH secretion of obesity and that the blunted GH secretion of central obesity could be the pituitary expression of the metabolic syndrome.

Nothing to Disclose: FP, JG-B, SS-A, TM, OV, FC

P3-243

Long-Term Reproducibility of Growth Hormone (GH) Secretion Pulsatile Characteristics.

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Therapeutic strategies to alter the GH hypothalamo-pituitary axis are based on baseline GH measurements. Therefore, the effectiveness of the treatment would depend on the precision of the measurements and the characteristics determined based on these measurements. In this study we studied the long-term (one year) reproducibility of the GH pulsatility parameters determined by deconvolution from a 24 h profile of GH secretion in normal older subjects. Seven men and 9 women on and 6 women off hormone replacement therapy (HRT), age 66.5 + 5.5 y, BMI (kg/m²) 26.2 + 3.9 were sampled from an indwelling iv cannula every 10 min for 24 h for measurement of serum GH. Standardized meals were served at breakfast, lunch and dinner. The sampling was performed at baseline, 6 and 12 months. The subjects were from the placebo arm of a recent randomized controlled study of the effects of the ghrelin mimetic MK-677 (1). GH was measured in duplicate by a chemiluminescence assay, Nichols (San Juan, Capistrano, CA). GH pulsatility was analyzed by Autodecon (2-3). The variation in the GH secretory parameters between placebo subjects was much greater than the intra-individual variation. In particular, the following parameters exhibit less variability within subjects than between subjects: basal secretion (p<0.001), pulse half duration (p<0.001), half-life (P<0.007), number of secretion events (P<0.015), total pulsatile GH production (P<0.001), total GH production (P<0.001), % pulsatile secretion (P<0.005), orderliness of the GH secretion (as measured by Approximate Entropy) (p<0.001), and phase of the 24 hour rhythm (p<0.001). Conclusion: a 24 h sampling for GH at 10 min intervals provides a good estimate of an individual's GH secretion characteristics over one year in older subjects.

(1) Nass et al, Ann Intern Med 2008;149:601

(2) Johnson et al, Analytical Biochemistry. 2008;381:8-17

(3) Johnson et al, Methods in Enzymology. 2009;454:367-404

Sources of Research Support: NIH grants HD28934, AG032555, RR-00847, RR019991, DK064122 and DK076037.

Nothing to Disclose: MLJ, LSF, RMN, MOT

P3-244

Growth Hormone Excess in McCune-Albright Syndrome: Emphasis on Diagnosis and Treatment in Children.

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Background: McCune-Albright Syndrome (MAS) is a mosaic disease caused by post-zygotic activating mutations of the *GNAS* gene product, $G_{\alpha s}$, and is characterized by fibrous dysplasia of bone (FD), café au lait spots and hyperfunctioning endocrinopathies, including growth hormone (GH) excess. We reviewed the NIH cohort of subjects with FD/MAS and identified all cases of GH excess to define diagnosis, prognosis and treatment, with special attention to children.

Patients/Methods: 162 subjects with FD/MAS were evaluated by a combination of clinical and laboratory testing. Most subjects who met criteria were treated pharmacologically (octreotide +/- pegvisomant) and/or with surgery or radiation. **Results:** Data are presented as range and median. Enrollment ages were: 3-35y, 13.2y. Length of follow up was: 1-23y, 5.9y. Head circumference was > 97% in 75% of subjects; all had craniofacial bone involvement including some degree of sphenoid sinus opacification. Optic neuropathy was found in 19% of subjects; 28% had hearing loss (4 mild, 3 moderate). **Laboratory findings:** random GH: 2.2-57, 10.2 ng/ml (normal 0-5); nadir GH values after oral glucose load: 1.0-60.2, 5.9 ng/ml (normal <1); nadir GH on frequent overnight sampling: 0.5-49.2, 4.9 ng/ml (normal <1); IGF-1 z-scores: -2.8-33.9, 3.0. All subjects had elevated serum prolactin. Based on a combination of clinical and biochemical data, 26 subjects (16%) were diagnosed with either GH excess (24/26) or GH autonomy (2/26). 18/26 were enrolled before the age of 18. In less obvious cases, diagnosis required interpretation of clinical findings and laboratory data. 21/26 were treated with long-acting octreotide; it was effective in 13/21. Six subjects who failed octreotide were treated with pegvisomant; it was effective in 5/6. The treatment goal in growing children was an IGF-1 z-score of 0. One subject was treated with focused irradiation, with some degree of efficacy. Two subjects had pituitary surgery, which was ineffective. No subjects treated during childhood have developed vision or hearing loss to date.

Discussion: These diagnosis and treatment of GH excess in MAS is important, and can be challenging in children. These data suggest early evaluation and intervention may prevent late morbidity. In addition to the management of MAS, these data have implications for other forms of gigantism and GH excess in children.

Nothing to Disclose: MG, MHK, BAB, JAB, EJF, CCB, CKZ, CMC, HJK, MTC

P3-245

Adolescent Acromegaly: Clinical Parameters and Treatment Outcome.

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Background: Patients with adolescent acromegaly present with tall stature, gigantism, tumor mass effects in the boys and menstrual irregularities in the girls. This study describes the clinical profile and treatment outcome.

Patients & Methods: Retrospective analysis of 34 consecutive patients (26 male) who presented to endocrinology department from January 1992 to December 2007 with onset of symptoms before 21 years of age. All patients were treated primarily with surgery and 7 (22%) were subjected postoperative radiotherapy.

Results: Commonest mode of detection was evaluation for features of acromegaly in 25 (75%), incidental 7 (20%) and apoplexy 2 (5%). Mean height at presentation was 174.6 + 13.7 (range 150-210) and 1/3rd had gigantism (> 97th percentile on growth chart). Growth hormone values were higher ($p = 0.04$) and hypogonadism was commoner. Hypertension 5 (14.7%), abnormalities of glucose homeostasis 8 (24%) and hyperprolactinemia 50% were seen.

Conclusion: Gigantism was uncommon, majority had macroadenoma, cure was achieved only in 1/3rd with surgery and radio therapy.

Nothing to Disclose: PD, AB, VU

P3-246

Conventional and Novel Biochemical Markers of Disease Status in Patients with Acromegaly during Long-Term Remission.

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Background: Tight control of disease activity in acromegaly has prognostic implications, but the biochemical definitions remain controversial. Discrepant values of IGF-I and GH are frequently reported. We therefore compared traditional and novel serum markers of GH secretion and IGF-I related variables and self reported health status in patients with acromegaly in long-term clinical remission after surgery alone versus SA treatment.

Methods: Sixty three patients (37 F/26 M, mean age: 59±1.6 yr) in long term remission based on normalized IGF-I levels after surgery (n = 36) or SA (n = 27) were studied in a cross-sectional manner. The two groups were comparable at the time of diagnosis as regards demographic variables, IGF-I and nadir GH levels. At the time of investigation each subject underwent 3 h of frequent serum sampling including a 2 h oral glucose tolerance test (OGTT). The following analyzes were measured: free and total GH, total and bioactive IGF-I, GHBP, IGFBP-1, 2, 3, glucose, and insulin. The self reported health status was performed by two questionnaires: EuroQoL and Acrostudy (PASQ).

Results: The two groups had similar levels of total and bioactive IGF-I [total IGF-I: (p=0.28); bioactive IGF-I: (p=0.70)]. Likewise levels of IGF-BPs and GHBP were identical in the two groups. Suppression of total and free GH (µg/l) during OGTT was blunted in the SA group (SA vs. Surgery) [total GH_{nadir}: 0.59 ±0.08 vs. 0.34 ±0.06 (p=0.01); free GH_{nadir}: 0.43±0.06 vs. 0.19±0.04 (p<0.01)], whereas the GH: IGF-I ratio was higher (P = 0.02). Females in both groups had significantly higher GH levels but similar IGF-I levels as compared to males [GH_{nadir}: P=0.01; IGF-I: P=0.57]. The insulin response (min) to oral glucose was delayed and the two hour glucose level (mmol/l) was increased in the SA group [T_{max}: 88 ±5.4 vs. 66 ±4.7 (P<0.01); 2 h glucose: 8.5 ±0.7 vs. 6.8 ±0.5 (p=0.02)]. The self reported health status was significantly better in patients treated with surgery compared to SA treatment assessed by the PASQ questionnaire (p=0.02).

Conclusions: In the presence of similar and normalized IGF-I levels SA treatment as compared to surgery alone is associated with: 1) Less suppressed total and free GH levels, 2) A reduced insulin response during the OGTT, 3) Less control of disease activity assessed by a disease-specific questionnaire. Our data support the current concept that biochemical assessment of disease activity in acromegaly should include both IGF-I and GH.

Nothing to Disclose: KZR, MM, SF, JF, JOLJ

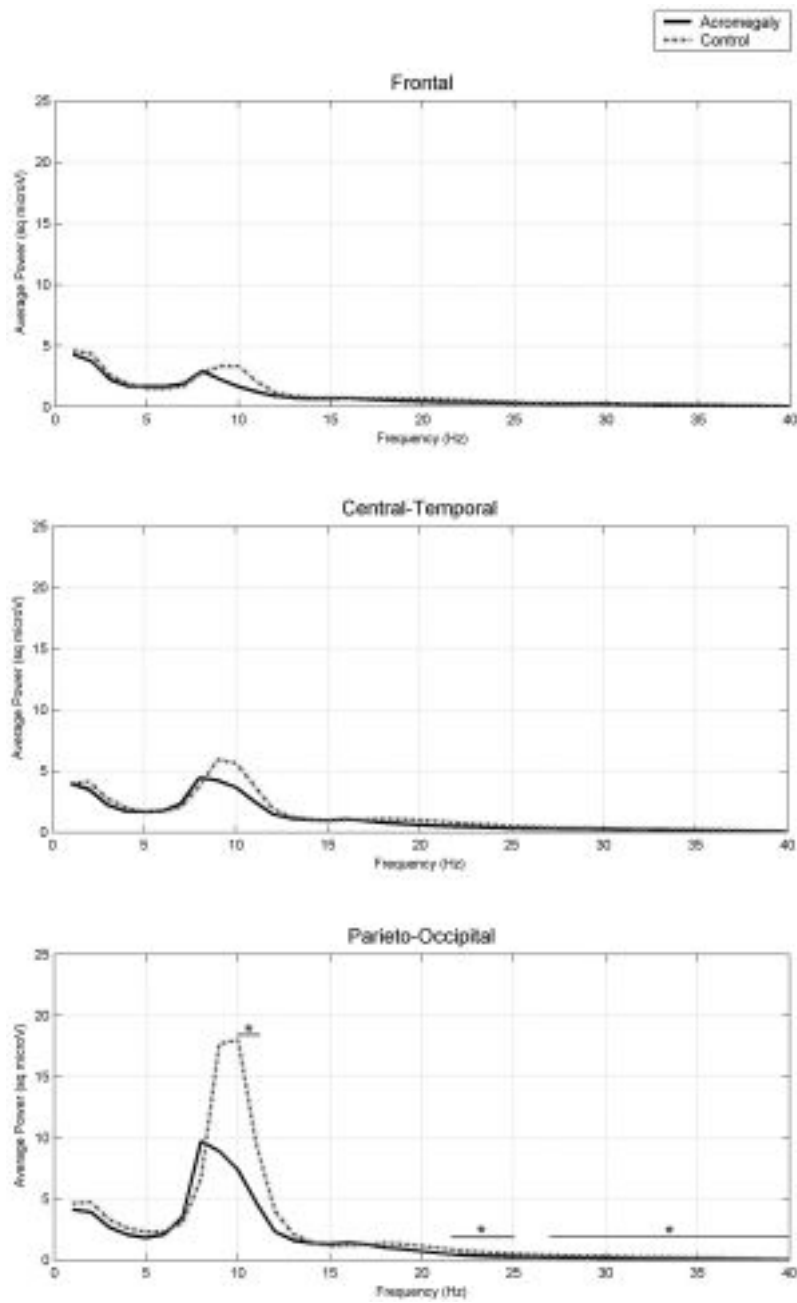
P3-247

Memory Problems and Brain Dysfunction in Patients with Active Non-Treated Acromegaly.

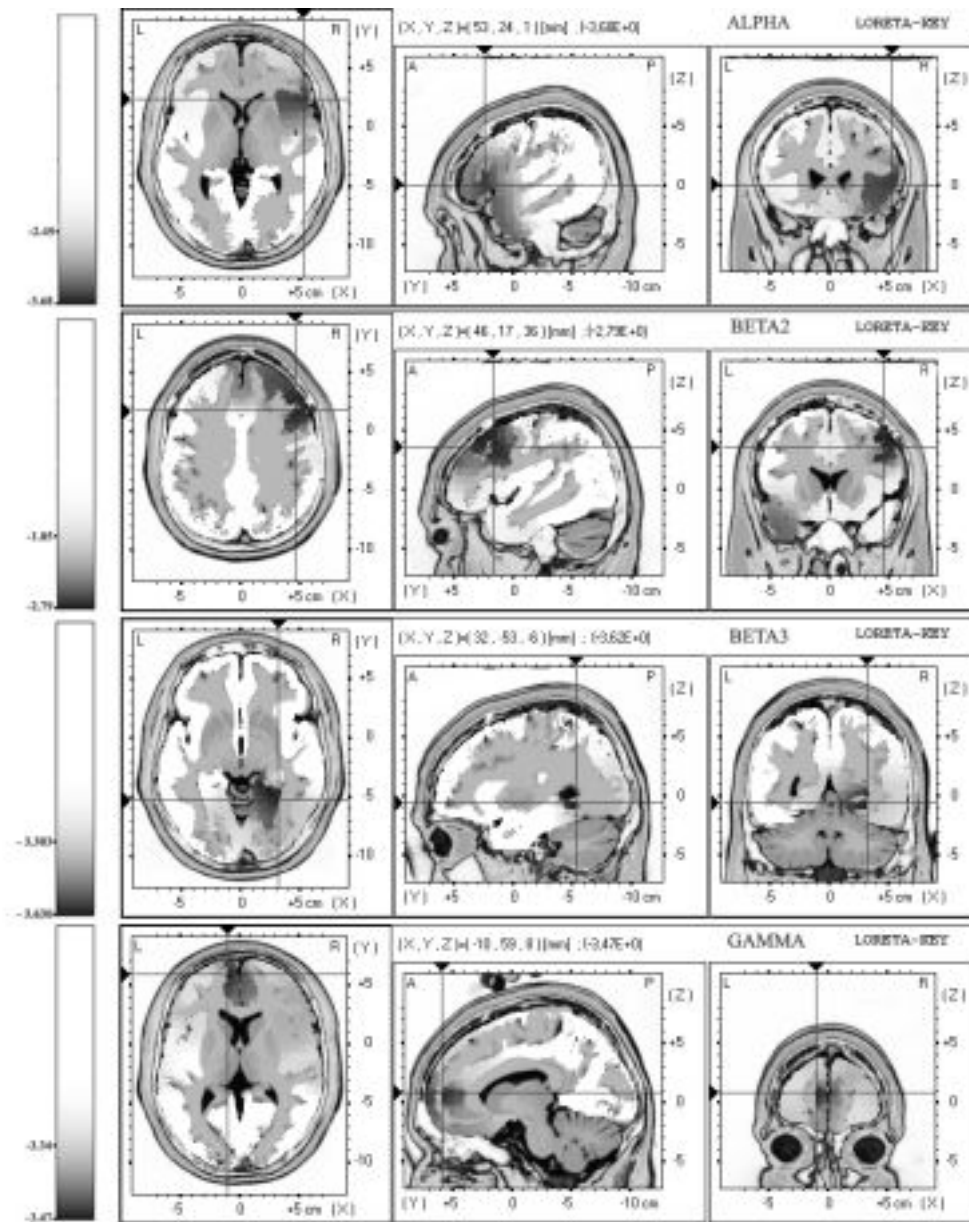
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Experimental and clinical data suggest that GH/IGF-I axis plays an important role in neurocognitive functions. Although research on GH-deficiency patients shows neurocognitive impairment, studies on acromegaly are quite scarce. This work focuses on describing cognitive and brain function in patients with naive acromegaly using neuropsychological and neurophysiological approaches. Sixteen naive acromegalic patients (12 females, median age [range] = 36 [24-59]) were assessed using a set of neuropsychological tasks. Attention, short and long-term memory, executive functions, visuospatial abilities, and verbal fluency were measured and patients' performance were compared with a sample of matched healthy subjects. A neurophysiological exam, which included resting state QEEG and LORETA EEG source reconstruction, were conducted in both groups. Hormonal data were collected as well as depression and quality of life (QoL) measures. Patients show cognitive deficits in tasks that assessed short and long-term memory. Patients show difficulties in working memory task and delayed visual and verbal recall (all $p < 0.005$). GH and IGF-I levels correlated significantly with a degree of neurocognitive impairment. Neurophysiological analysis showed fewer alpha and beta bands in patients' EEG.



LORETA analysis localized less alpha and beta EEG activity in prefrontal and middle temporal cortices.



This research is the first to show specific neurocognitive impairment in acromegalic patients. Our data identified deficits in short and long-term memory. Correlational data suggests that GH/IGF-I could be associated to these deficits. In addition, patients show less activity in brain areas that are functionally linked to memory processes. These areas also contain a relative high number of binding sites for GH and IGF-I.

Sources of Research Support: Novartis Oncology.

Nothing to Disclose: JFM-R, AM-A, AMS-M, EMV, ET-V, PB, FJT, MAG, JL-C, AL-C

P3-248

Analysis of Cardiovascular Risk Factors in a Group of 44 Acromegalic Patients.

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Acromegaly has several complications on the cardiovascular system, especially hypertension. **Objectives:** To evaluate clinical characteristics and laboratorial cardiovascular risk markers of a group of patients with acromegaly and to determine whether they are correlated with presence of hypertension and disease activity. **Study design:** Uncontrolled cross-sectional study. **Patients and Methods:** Forty-four patients with active or inactive acromegaly being followed at the Neuroendocrinology Clinic of the HCPA were submitted to clinical assessment, laboratory tests (biochemical parameters for acromegaly control, lipid profile, renin, aldosterone, 24-hour microalbuminuria, ultrasensitive C-reactive protein), and echocardiography. **Results:** The prevalence rates found in the sample were as follows: active acromegaly, 40.9%; hypertension, 56.8%; diabetes mellitus, 18.2%; obesity, 29.5%. Patients with active disease did not have the highest number of cardiovascular risk factors when compared with healed individuals. There were no correlations between disease activity and presence of hypertension, renin and aldosterone levels, or us-CRP. Patients with left ventricular hypertrophy had lower levels of GH and IGF-1 (nonsignificant p). There was correlation between acromegaly activity and microalbuminuria levels and HOMA index. **Conclusions:** There is no greater aggregation of cardiovascular risk factors in active acromegaly; there is correlation between disease activity and nontraditional cardiovascular risk parameters - microalbuminuria and insulin resistance.

Nothing to Disclose: MAC, DF

P3-249

Co-Treatment with Pegvisomant and a Somatostatin Analogue (SA) in Acromegalic Patients Who Are Well Controlled on SA Monotherapy: Preliminary Data from an Investigator-Initiated Study.

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Background: Co-treatment of acromegaly with a somatostatin analogue (SA) and the GH receptor antagonist Pegvisomant has proven feasible, but the regimen has mainly been tested in cases where disease control was not obtained with SA monotherapy.

Aim: To study if patients who are well controlled on SA monotherapy can be transferred to combination therapy with low dose Somavert and a reduced dose of SA.

Study end points: Serum IGF-I levels, glucose metabolism, and drug dosage.

Methods: 18 acromegalic patients well-controlled on SA mono therapy were included in an investigator-initiated, non-blinded, parallel study over 24 weeks, randomised to either: 1) unchanged SA monotherapy (n = 6), or 2) co-treatment (n=18) with Pegvisomant (15-30 mg twice a week) and SA (half the usual dosage). They were examined at baseline and after 24 weeks of treatment with a glucose clamp and an OGTT.

Results: IGF-I levels did not differ between co-treatment and SA either at baseline (204.0 ± 18.3 vs. 208.6 ± 18.5) ($p=0.88$) or after 24 weeks of treatment (189.0 ± 29.5 vs. 221.0 ± 16.6) ($p=0.35$). Insulin sensitivity as expressed by the glucose infusion rate during the clamp did not change either within (co-treatment: 4.2 ± 0.6 vs. 4.6 ± 0.6) ($p=0.85$); SA: 3.9 ± 0.6 vs. 4.7 ± 1.1) ($p=0.21$) [baseline vs. week 24] or between groups (co-treatment: 0.9 ± 0.6 vs. SA: 0.1 ± 0.4) ($p=0.30$) [week 24 - baseline]). The 2 h glucose value during the OGTT also did not change (co-treatment: 0.8 ± 0.4 vs. SA: 0.6 ± 1.4) ($p=0.9$) [week 24 - baseline]), despite the fact that insulin secretion became more pronounced in the co-treatment group (co-treatment: 59.6 ± 45.6 vs. SA: -132.2 ± 77.6) ($p=0.04$) [week 24 - baseline]). SA only received either Octreotid LAR 20 ± 4.5 mg/4 weeks or Lanreotid 80.0 mg/4 weeks (n=1) during the 24 weeks. The co-treatment group was reduced in Octreotid LAR from 20 ± 3.7 to 11.6 ± 2.2 mg/4 weeks (58 % reduction) or in Lanreotid from 71.7 ± 11.7 to 37.0 ± 5.7 mg/4 weeks (52 % reduction). The mean dosage of Somavert was 47.5 ± 4.1 mg/week.

Conclusions: 1) acromegalic patients well controlled on SA monotherapy can maintain safe IGF-I levels during 24 weeks co-treatment with low dose Pegvisomant and a reduced SA dose, 2) this regimen does not seem to provide a clinically significant improvement in insulin sensitivity or glucose tolerance, 3) our data do not challenge the concept of SA monotherapy, but combination treatment seems a feasible alternative in selected patients.

Sources of Research Support: Ipsen unrestricted grant. The Danish Council for Independent Research, Medical Sciences Grant.

Disclosures: JOJ: Research Funding, Ipsen, Novartis Pharmaceuticals, Pfizer, Inc.; Speaker, Ipsen, Novartis Pharmaceuticals, Pfizer, Inc.

Nothing to Disclose: MM, TK-H

P3-250

The Effect of Pegvisomant on Glucose Metabolism and Insulin Levels in Healthy Adults.

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Introduction: The sensitivity of the insulin and glucose metabolism to the growth hormone receptor antagonist Pegvisomant in healthy adults has rarely been examined. Aim of this study was an evaluation of serum concentrations of IGF-1, fasting plasma glucose (FPG) and insulin after a single injection of Pegvisomant, and a calculation of AUC of glucose and insulin and HOMA to assess insulin resistance.

Methods: Blood samples were taken from 10 healthy volunteers (CONTR) before and after injection of 1mg/kg body weight of pegvisomant. IGF-I, plasma glucose and serum insulin were measured daily for 10 days (baseline, d1-10). An oral glucose (75 g) tolerance test was performed at baseline, 5 and 9 days (d0, d5, d9) after injection of Pegvisomant in order to assess the state of glucose tolerance and to investigate insulin secretion. AUC glucose and insulin and HOMA values for insulin resistance (HOMA-IR) were calculated.

Results: Mean IGF-I in CONTR decreased significantly during day 4-7 from 207±57 ng/ml at baseline to a minimum of 150±58 ng/ml on d5 (p<0.01). Fasting plasma glucose (89±6 at baseline to 93±6 mg/dl on d5) did not change significantly. However, on day 6, insulin increased significantly by 73±4% (p<0.05), and HOMA-IR increased by 77±4% (p<0.05).

Regarding oGTT, basal insulin and basal HOMA-IR were significantly elevated on day 9 compared to baseline oGTT (10.2±5.2 vs. 4.1±4.2 mIU/l, p<0.001 and 2.2±1.1 vs. 0.9±0.3, p<0.001, respectively). 120min glucose values at baseline, d5 and d9 showed no significant differences (95±19 mg/dl at baseline, 96±18 mg/dl on d5 and 90±21 mg/dl on d9). 120min insulin levels were 35±25 mIU/l at baseline, 59±57 mIU/l on d5 (p=0.14) and 50±46 mIU/l (p=0.19) on d9, respectively. AUC insulin values during OGTT increased significantly from 6118±4282 to 9297±7157 at d5 (p<0.05) and to 9037±6453 (p<0.05) at day 9.

Conclusions: A single dose of Pegvisomant induced a transient increase in insulin levels in healthy adults, possibly due to a diminished effect of IGF-1 at the muscle IGF-1 receptor compensated by insulin, or an increase in endogenous GH.

Nothing to Disclose: CB, MB, KM, SP

P3-251

Harvey Cushing's Surgical Treatment of Acromegaly: 1896-1912.

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Background:

In 1912, Cushing published a monograph detailing his operative experience with acromegaly, reporting details of the patients' presentation and treatment, but did not offer data regarding mortality rates, outcomes, or follow-up. A review of Dr. Cushing's surgical cases at Johns Hopkins Hospital revealed new information about his early operative experience with acromegaly.

Methods:

The Johns Hopkins Hospital surgical records from 1896-1912 were reviewed. 34 patients who underwent surgery for pituitary disorders were identified, and 9 with symptoms of acromegaly were selected for further review.

Results:

9 patients presented with symptoms including coarsening of facial features, enlargement of hand and feet, changes in vision, and frontal headaches. 8 received surgical treatment; 1 underwent removal of a goiter, but was not surgically treated for acromegaly.

The mean age was 39 (range 23-54; median 37). The majority of patients (75.0%) were male. The average length of stay was 33.5 days, and the average follow-up time was 85 months (range 0.3-257).

Among all treated patients, there was 1 inpatient death, attributed to "glandular intoxication," and 7 patients whose discharge status was "improved". Of the 7 discharged patients, 1 committed suicide shortly afterwards, secondary to pre-existing mental health issues; 2 reported recurrence of headaches and vision changes; 4 remained improved for the duration of follow-up.

One patient underwent an "omega-incision" approach through a frontal craniotomy, and was improved upon discharge, but his headaches and vision changes returned over 167.3 months of follow-up.

7 patients underwent a naso-labial approach; 1 died during admission, and 6 had a discharge status of "improved." Of these, 1 had a return of his headaches over 8 months of follow-up; 4 remained improved documented over an average of 186.3 months of follow-up; 1 patient died of unrelated causes.

Conclusions:

This review analyzes the outcomes for 4 patients Cushing described in his monograph, 3 patients whose operations were mentioned only in brief, and 1 patient whose case has not been reported previously. We found a mortality rate of 12.5% for all patients operated upon. Additionally, this review reports the effect of operative treatment on the patients' long-term symptoms, which was not addressed in Cushing's own monograph.

Sources of Research Support: CP is supported by the Bean Student Research Award, American Osler Society.

Nothing to Disclose: CP, RS, GW, AQ-H

P3-252

Clinical Characteristics in Densely Granulated and Sparsely Granulated GH Cell Adenomas.

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PURPOSE: The aim of this study is to clarify the differences of clinical characteristics between densely granulated GH adenoma (DGA) and sparsely granulated GH adenoma (SGA). **MATERIALS AND METHODS:** Thirty-two DGAs and 30 SGAs were investigated in this study. GH cell adenomas with and without fibrous bodies on cytokeratin immunostaining in almost all cells examined were diagnosed as a DGA and SGA in this present study, respectively. Pathological diagnosis was made independently in all cases by two different pathologists and final morphological diagnosis of all cases used in this study corresponded with each other. In order to clarify the differences between both types of GH cell adenomas, following factors were investigated and compared statistically between both types; Age/gender, serum GH and IGF-1 levels, GH responses to octreotide (Oct) or Dopamine agonist (Dop) administration, cavernous sinus invasion of adenoma, tumor volume, intensity of T1WMRI and T2WMRI, and Knosp's grading. These data were available in all cases. **RESULTS:** SGAs were significantly more common than DGAs in younger (Mean age: SGA was 38.1 years old, and DGA was 48.8 years old. $p=0.001$) women ($p=0.008$). Greater response against Oct administration was more frequent in DGA than SGA ($p=0.001$). On MRI, SGAs showed high intensity of T2WMRI more frequently than DGA ($p=0.041$), and the frequency of higher Knosp's grade (grade 3 and 4) was more common in SGA than in DGA ($p=0.009$). In contrast no significant differences were found in serum GH or IGF-1 levels, GH response to Dop administration, frequency of cavernous sinus invasion, tumor volume, intensity of T1WMRI. **CONCLUSION:** It has been concluded that two different morphological types of pure GH cell adenomas have distinctive clinical, endocrinological, and neuroimaging characteristics and a thorough preoperative characterization of the clinical behavior of acromegaly can predict the morphological type of GH cell adenoma. Moreover, from a therapeutic standpoint, it should be taken into account that octreotide treatment can be expected ineffective more frequently in SGAs than DGAs.

Nothing to Disclose: NF, AS, KO, NI, AT, YT, TS, SY

P3-253

Demographic Data, Co-Morbidities, Malignancies and Mortality from the First Large-Scale Italian Study on Acromegaly.

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This report aims to present preliminary epidemiological data on the first large-scale retrospective Italian study on acromegaly. A total of 1512 patients attending 22 tertiary centres from 1980 to 2002 were included: 624 males (M) and 888 females (F). Mean age at diagnosis (\pm SD) was 45 ± 13 years. M patients were younger than F (43 vs 47 yrs, $P < 0.001$). Median estimated duration of disease was 6 years. Radiological evaluation revealed microadenomas in 30% of cases and macro in 70% (44% of which were intrasellar). At baseline, GH was 31.1 ± 36 $\mu\text{g/l}$. It was over 50 $\mu\text{g/l}$ in 15.7% of patients and under 2.5 $\mu\text{g/l}$ in 1%. Two patients had GH nadir during OGTT below 1 $\mu\text{g/l}$ but elevated IGF-I. Basal IGF-I levels, available in 66.4% of the patients, were 744 ± 318 ng/ml. Hyperprolactinemia was observed in 19% of patients (2/3 of whom F) and TSH hypersecretion in 0.7%. 26% of patients had one or more pituitary deficiencies: 4.1% hypoadrenalism, 8.1% hypothyroidism, 16.4% hypogonadism and 0.6% diabetes insipidus. First treatment was surgery in 53% of patients, medical therapy in 46% and radiotherapy/radiosurgery in 1%. 34.3% of subjects received just one treatment, 47.9% two, 16.4% three and 1.2% four treatments. At the end of the study, 62.6% of patients achieved remission, 44% of whom while on pharmacological treatment. The extrasellar extension of the tumor was the most important predictor of lack of remission (OR 1.96, CI 1.5-2.5, $P < 0.001$), followed by M gender and mean GH level at diagnosis. Diabetes mellitus was reported in 16.2% of cases, 17% of M and 15.6% of F ($P = \text{NS}$), while the figures for the Italian population are 4.4% in M and 5.2% in F. Hypertension was present in 33.7% of F and 30% of M in acromegalic patients, while in the general population the figures are 15.4% (F) and 11.8% (M). Both these complications had a much earlier age peak in acromegaly. During the follow-up, 122 malignancies (8.1%) were observed: 51 in M and 71 in F. The most frequent localization were urological (2.5%) in M (at variance with the general Italian population) and breast (2.5%) in F, followed by colorectal and thyroid in both gender. Sixty-one patients died during the follow-up: the main causes were vascular disease (37.7%), mostly cardiovascular, and malignancies (36.1%). As far as vascular diseases are concerned, F had a greater mortality for cerebral disease than M patients (14% vs 4%).

Nothing to Disclose: EM, MA, MT, AB, GR, BZ, AC

P3-254

Quebec Pituitary Tumor Registry: Treatment Outcome of 113 Patients with Acromegaly.

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Objective : To evaluate the quality of care of patients with acromegaly in two University hospitals in the province of Quebec, Canada.

Design and Patients: Retrospective analysis of clinical and treatment outcome data in the ongoing Quebec pituitary tumor registry. A total of 113 patients with acromegaly (49% females) followed at the University Hospitals of Montreal (CHUM) and Sherbrooke (CHUS) from 1974 to 2009 for a mean of 8.6 years (SD 8.5 years; range, 0.5 to 34).

Results : Age of patients at the time of diagnosis was 43 ± 13 years (mean \pm SD). Time delay from reported symptoms onset to diagnosis was significantly higher in men (7.3 ± 6.6 years) than in women (6.5 ± 5.2 years). The majority of patients had macroadenomas (81%) of which 34% were invasive, whereas 19% had microadenomas. Male patients had significantly higher random GH levels than females (40 ± 62 vs 25 ± 23 $\mu\text{g/L}$, $P < 0.0001$) however IGF-1 levels were not significantly different between men and women (982 ± 407 vs 827 ± 406 $\mu\text{g/L}$, respectively). At the time of diagnosis, diabetes or glucose intolerance were diagnosed in 45%, hypertension in 44%, sleep apnea in 59% and colon polyps in 51% of cases. Eighty-five percent of patients underwent surgery, 12% received radiotherapy and 68% were given medical treatment (somatostatin analogues(SSA) in 56%, cabergoline in 9% and pegvisomant alone or in association with SSA in 13%). Primary SSA therapy was given in 11% of patients. Remission after surgery or radiotherapy or control of disease during medical therapy, defined biochemically (IGF-1 normal for age and basal GH < 2 $\mu\text{g/L}$ or GH nadir post-OGTT < 1 $\mu\text{g/L}$), were obtained in 30% of patients following surgery alone, 27% after radiotherapy, 67% of patients with primary SSA, 50% of patients treated by SSA after failed surgery and in eight of ten patients treated with pegvisomant alone or in combination with SSA. Multimodal therapy achieved a global remission or disease control rate of 83.3% of patients. Hypopituitarism was present in 30% of patients

Conclusion : The Quebec tumor registry is a useful tool to analyse treatment modalities and outcome of acromegaly in Quebec. Our preliminary analysis of partial data reveals that a majority of patients received multimodal therapy resulting in adequate disease control.

Sources of Research Support: Novartis and Ipsen.

Nothing to Disclose: SV, CB, NA-J, HB, RC, GH, OS

P3-255

Twelve Years of the Spanish Acromegaly Registry (REA). Trends of Acromegaly Treatment and Outcomes in Spain.

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The Spanish Acromegaly Registry (REA) is an online epidemiological database created in 1997 to collect clinical and biochemical data of patients with acromegaly in Spain. **Aim:** to study trends in acromegaly treatment and outcomes in Spain. **Methods:** registered endocrinologists include data from acromegalic patients seen at their practices on this online registry. Investigators were urged by the neuroendocrine group of the Spanish Society of Endocrinology and Nutrition (SEEN) in 2009 to update new and prior patients. Variables collected included: demographics, estimated date of symptoms, date of diagnosis, pituitary imaging (tumor size and extension), tumor persistence after surgery, visual fields, GH (baseline and after OGTT) and IGF-1 concentrations. IGF-1 -reported as normal or abnormally high or low, according to local reference values-, was also recorded. Date of diagnosis and co-morbidities, medical, surgical and radiation treatments were included. All clinical variables were collected at diagnosis and at yearly - biyearly intervals. Descriptive statistics was used to analyze data only in updated patients. **Results:** As of 31st December 2009, 1570 patients with acromegaly were included in the database of which 475 had been updated and fulfilled sufficient data accuracy and completeness for analysis; 62% were women and 38% men; 77% had a macroadenoma and 23% a microadenoma. 93% of patients had surgery and 35 % radiation therapy. Medical treatment was used in 97%. When analyzing type of treatments performed by decades, we observed a clear decrease in radiation therapy, while surgery rates were maintained over time and use of medical therapy has increased. Over decades, use of dopamine agonists has decreased (40%, 21% and 9% for the 80s, 90s and 2000s, respectively), whereas use of long-acting somatostatin receptor ligands (37%, 57% and 73%) and Pegvisomant (0, 0 and 12%) has increased. At last data analysis 76% of patients had normal IGF-1 levels and were considered controlled whereas 24% were not, which reflects a marked improvement in comparison to the results reported in 2004 from the same database, when only 40% of patients were cured according to criteria of cure at that time (GH after OGTT <2 ng/ml). **Conclusion:** Treatment of acromegaly in Spain has changed over time. Surgery is still the mainstay of treatment and use of medical therapy has increased, whereas radiation treatment rates have decreased. Cure rates have clearly improved over time.

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Disclosures: SW: Speaker, Novartis Pharmaceuticals; Study Investigator, Novartis Pharmaceuticals.
Nothing to Disclose: GS, SG, AP, EV, ET, CF, CB, CDP

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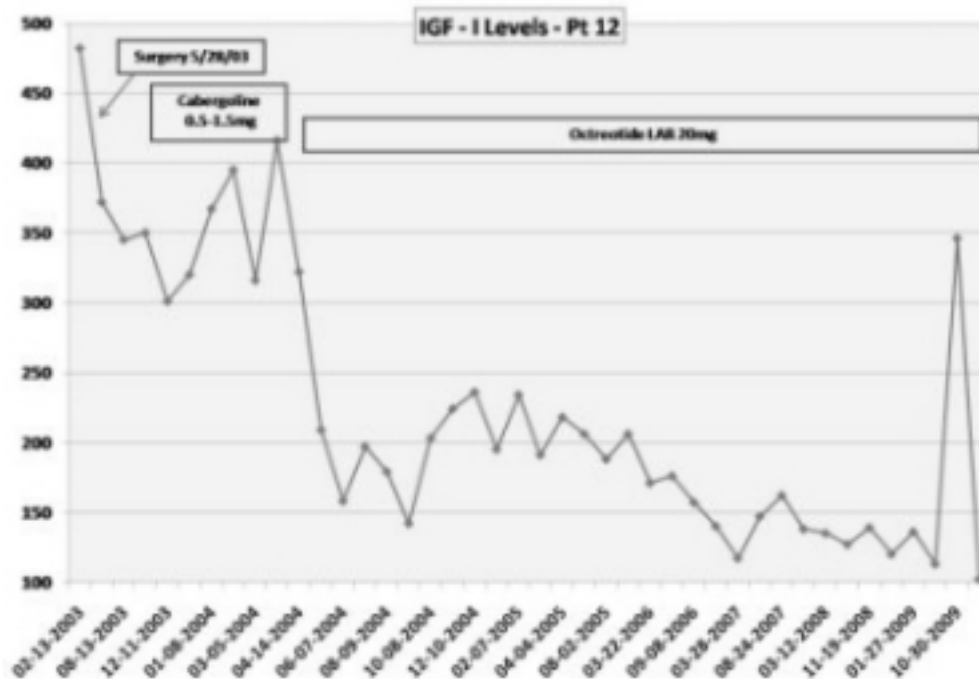
Development of an Acromegaly Registry To Follow Treatment.

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Existing acromegaly registries largely focus on clinical characteristics, outcome, morbidity and mortality and retrospective treatment outcomes. In order to further expand current understanding of benefits of various treatment modalities, we developed a registry focused on reliability of diagnosis and relative degree of treatment response to any combination of surgery, radiotherapy and medical treatment in 86 patients (data entered in 72). The data included were both retrospective and prospective. Diagnosis was considered probable or definite according to strict criteria. It was considered probable in 13/72 and definite in 59/72 (clinical signs, elevated IGF-I or elevated random GH level + a GH nadir of >0.3 ng/ml and tumor on imaging). At enrollment, 7/58 treated patients were considered cured based on hGH being reduced to <1, <0.4, or less than 0.03 ng/ml. Thirty of 58 were considered controlled (IGF-I normal for age and gender with hGH <2.5, if available). Twenty-one of 58 patients were not controlled (elevated IGF-I or discordant hGH/IGF-I levels).

So far, treatment data have been entered on 21 patients. Eighteen of 21 had surgery: 6 were cured (GH nadir <1 ng/ml and normal IGF-I), 5 of whom had a mean reduction in hGH of 92.66% while the mean reduction in hGH in the uncured patients was 62.9%. Fifteen of 21 were treated with medical therapy: 12 were treated with Octreotide LAR (6 also received short acting), 1 received SOM230, 7 received some form of somatuline, 4 were treated with dopamine agonists, and 5 received pegvisomant. Three patients received RT at the ages of 22, 26 and 40 and had post-surgical hGH reductions of 48.3%, 46% and 86%. Representative effects of treatment on serum IGF-I can be seen in one patient below. Note: normative levels have not yet been added to the graph.



Sources of Research Support: Novartis Pharmaceuticals Investigator-initiated grant.

Disclosures: DLK: Study Investigator, Novartis Pharmaceuticals, Pfizer, Inc., Ipsen.
Nothing to Disclose: MS, DB-M, DCH-C, NL, NRN, MLL, LBE, ASM, HBL

P3-257

Diagnosis of Acromegaly: Results from 100 Patients Followed in a Single Center.

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Acromegaly, a disease of an insidious nature, has a long delay from onset of symptoms to diagnosis. The clinical manifestations of acromegaly have been extensively reported; however, information concerning the type of medical professional who makes the initial diagnosis of acromegaly is limited. Our objective was to identify the medical professional who first suspected acromegaly in our country.

A total of 100 patients (54% male and 46% female) with acromegaly were included in the study, which was performed at a university endocrinology clinic. Data was collected by retrospective review. All patients had immunohistochemicals confirmation of a GH-secreting adenoma. Overall, 80% were macroadenomas, and 20% were microadenomas.

The mean age of the acromegalic patients was 48.9 ± 10.4 yr (range 21-72), while the delay in diagnosis was long with an average of 5.53 ± 3.75 years from symptom onset. Mean GH and IGF-I levels at diagnosis were 31.25 ± 20.92 $\mu\text{g/mL}$ and 1028.33 ± 275.24 ng/mL respectively.

The evaluation of acromegaly was initiated by an internist in 25% of the cases, an endocrinologist in 17%, neurosurgeon in 16%, neurologist in 7%, ear nose and throat specialist in 5%, rheumatologist in 5%, and primary care physician in 3%. An ophthalmologist, general surgeon, cardiologist, obstetrician/gynecologist, nurse, and a medical school student, each made the diagnosis in 2% of patients, while 1% was self-referred for diagnosis. A patient family member made the diagnosis in 3%, as an algologist, orthopedist, nephrologist, radiologist, psychiatrist, and pulmonologist each diagnosed 1% of patients. Acromegaly was diagnosed incidentally in five patients, where the patients were unaware of signs or symptoms of acromegaly before diagnosis. Among the five patients diagnosed incidentally, two were recognized at a diabetes mellitus evaluation with endocrinologist. Three were diagnosed as a result of a workup for thyroid nodules. It is critical that acromegaly diagnosis was most commonly suspected by the internist, endocrinologist, and neurosurgeon (58%) in our country. To this end, achieving a high rate of treatment success and avoiding long-term co morbidities will be accomplished through early recognition. Educational programs targeted at early recognition of acromegaly are essential. Healthcare professionals, especially primary care physicians, should have increased awareness of acromegaly in our country.

Nothing to Disclose: KA, NUC, SD, TE

P3-258

Acromegaly: Comparison of Two Immunoassays in the Determination of IGF-I Levels and Its Correlation with Oral Glucose Tolerance Test (OGTT).

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Introduction: IGF-I determination in serum or plasma is an essential tool in the diagnosis and follow-up of acromegaly. Hepatic production of IGF-I is regulated by GH and circulates bound to several IGF-I binding proteins which extends its half life. IGF-I is not liberated in a pulsatile way and has no significant variability in 24 hs.

Objective: To evaluate two different methodologies in IGF-I levels determination and its correlation with GH nadir in OGTT in acromegalic patients.

Material and methods: We analyzed 37 acromegalic patients, 20 women and 17 men, mean age was 45±12. IGF-I levels were assayed by Immulite 1000, DPC (IMM) and DSL-5600 ACTIVE[®] IGF-I Coated-Tube IRMA (DSL) and OGTT (basal and 30, 60, 90, 120 minutes) were performed by measuring plasma glucose and GH assay by quimioluminescent immunoassay (Access); we considered a nadir <1ng/ml as normal response. Nine patients were under medical treatment (cabergoline: 4, octeotride: 4, and cabergoline plus octeotride: 1) and 28 without treatment. Statistical analysis: Wilcoxon and, Bland and Altman tests. Differences were considered significant at p< 0.05.

Results: Basal glucose levels were 97.86±10.91 mg/dl and mean GH was 2.8 (1.59-14.4) ng/ml. Mean IGF-I levels performed by IMM were 602±318 ng/ml and 1006±596 ng/ml by DSL, there was a statistically significant difference between both methodologies (p<0.01). Bland and Altman test showed a bias of - 403.2 ng/ml with lower values by IMM. We observed elevated IGF-I levels in 84% by IMM and in 97% by DSL, and only one patient had normal levels with both methodologies. Elevated IGF-I levels and GH nadir >1ng/ml were observed in 70% of the patients, increased IGF-I with normal GH nadir in 13.5%, normal IGF-I with GH nadir >1ng/ml in 2.7% and normal IGF-I with normal GH nadir in 13.5%. Patients under treatment: 3 showed normal GH nadir with elevated IGF-I levels, in 2 of them by both methodologies, and in the other one it was normal by IMM and elevated by DSL; the other 6 showed GH nadir > 1ng/ml, 5 of them presented elevated IGF-I by both methodologies and the other one showed discrepancy in IGF-I levels. **Conclusions:** IGF-I levels determined by IMM and DSL were statistically significantly different. IGF-I levels showed a negative bias by IMM. Most of the results of GH nadir were consistent with IGF-I levels but we observed discrepancy in 30% of the patients, whether they were under treatment or not.

Nothing to Disclose: LB, SM-G, MM, GS, MG, AA, CB, AC, KD, NGB, MG, ML, JN, AR, MS, MV, DK, PFD

P3-259

Utility of IGF-1 in the Evaluation of Patients Suspected To Have Acromegaly in a Single Tertiary Center.

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Context: Serum IGF-1 is the first line screening test for the diagnosis of acromegaly. While the Oral Glucose Tolerance Test (OGTT) is considered the gold standard for the diagnosis of acromegaly, there is a debate concerning its precise role. Some consensus guidelines recommend OGTT to confirm the diagnosis in all patients, whereas others recommend it only in selected cases.

Objective: To report the utility of IGF-1 for establishing the diagnosis of acromegaly in a tertiary referral center.

Methods: We identified 60 patients with acromegaly (Gr 1), from a prospectively maintained pituitary database, who underwent pituitary surgery at the Cleveland Clinic between 1999 and 2009. All patients had clinical feature(s) suggestive of acromegaly. We selected a control group of 68 patients (Gr 2) with pituitary tumors who underwent surgery and had negative immunostaining for growth hormone (GH). Age- and sex- adjusted standard deviation scores (SDS) were calculated for individual IGF-1 levels. The -2 to + 2 SDS represents the lower and upper limit of the normal reference range. Results are expressed as median (range). IGF-1 levels were measured using either Nichols advantage, ARUP or Siemens Immulite 2500 chemiluminescence assays.

Results: The median (range) age and sex ratio (F/M) in Gr 1 and Gr 2 were 48.5 (20-69), 35/25 and 51.5 (23-73), 32/36 respectively. The SDS IGF-1 values prior to surgery or medical therapy in Gr 1 and Gr 2 were +8.8 (+2.8 to +39.2) and, -1.59 (-2.8 to +1.0) (P<0.0001), respectively. Random GH levels in Gr 1 and Gr 2 were 9.8 ng/mL (0.2->200) and 0.2 ng/mL(<0.1- 3.8), respectively. IGF-1 was elevated in all patients with acromegaly but in none of the controls.

OGTT was performed in 14 patients in Gr 1. The nadir GH level was 4.15 ng/mL (0.2 - 45.7). In addition, we identified 4 patients in the Pituitary Clinic with elevated SDS IGF-1 values of +2.5, +2.5, +2.7, and +5.4 (the latter a 16 year-old girl), who subsequently underwent OGTT and were found to have a nadir GH level <0.1 ng/mL.

Conclusion: In our experience, an elevated IGF-1 is sufficient for the diagnosis of acromegaly in clinically suspected cases. The OGTT can be used to exclude the diagnosis of acromegaly when there is modest elevation of IGF-1.

(1)Cordero RA et al.,Rev Endocr Metab Disord 2008;9:13

(2)Roberts B et al.,Pituitary 2007;10:205

(3)Ben-Shlomo A et al.,Endocrinol Metab Clin North Am 2008;37:101

(4)Cook DM et al.,Endocr Pract 2004;10:213

Nothing to Disclose: SKS, ALK, CF, BH, RP, RW, AHH

P3-260

Acromegaly Due to Ectopic GHRH Secretion: A Retrospective Series.

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Acromegaly is usually caused by somatotroph pituitary adenoma. However, an ectopic tumoral GHRH secretion can induce GH hypersecretion in less than 1% of acromegalic patients. This paraneoplastic acromegaly has been described in several case reports but series are rare. We report here the preliminary results in a study of the 22 patients recruited in France and diagnosed between 1995 and 2009. Blood samples were sent from 11 French Academic hospitals and plasma GHRH levels were determined by radioimmunoassay. The assay developed in our laboratory involves a rabbit polyclonal antibody raised against GHRH 1-44 and radiolabelled ¹²⁵I GHRH prepared with chloramine T method. Normal circulating GHRH concentrations are <30 ng/L. We identified 22 cases of GHRH-related acromegaly. The sex distribution showed a female predominance (15 females vs 7 males). Mean age at the first GHRH determination was 41 years (range 15-70 years). Plasma GHRH concentrations ranged from 247 to 9779 ng/L (median = 547 ng/L). GHRH determination was performed for the following reasons : association of acromegaly with a previously known endocrine tumour, acromegaly without evidence of pituitary adenoma (normal or hyperplastic pituitary), acromegaly in a familial context of MEN1, acromegaly not cured after pituitary surgery. GHRH-producing tumours were of pancreatic origin in 7 patients, bronchial in 10 and ileal (appendix) for 1. The tumour localisation was unknown in 4 patients. Among the 19 patients with follow-up information, 13 showed GHRH levels lower than 30 ng/L after tumour removal and/or somatostatin-analog treatment, 5 kept elevated levels corresponding to metastatic diseases and one died from cardiovascular disease. Therefore, this clinical study confirms that plasma GHRH is a useful parameter in identifying patients with ectopic acromegaly and is a good marker for the follow up.

Disclosures: PC: Advisory Group Member, Pfizer, Inc.

Nothing to Disclose: LG, PC, FC, AT, BC, VR, OC, GR, PB, AM, BD, FB, JLS, GS, FB-C

P3-261

Predictive Factors of Impaired Glucose Tolerance and Diabetes Mellitus in Newly Diagnosed Acromegalic Patients.

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Active acromegaly is frequently associated with alterations of glucose metabolism and growth hormone (GH)-induced insulin resistance is considered to be the major underlying mechanism. It is not known however which factors predispose acromegalic patients to develop impaired glucose tolerance or overt diabetes. To further clarify this issue, we analyzed data from 138 naive acromegalic patients (74 men/64 women; mean age \pm SD: 45 ± 14 years). All patients underwent an oral glucose tolerance test (OGTT), unless already treated for diabetes at the time of diagnosis (n=23). They were subdivided into 3 groups according to current criteria of the American Diabetes Association: (1) normal glucose tolerance (NGT): n= 66, 48%; (2) impaired fasting plasma glucose: 100 -125 mg/dl (IFG) or impaired glucose tolerance (post-OGTT 120 min-glucose: 140 -199 mg/dl; IGT): n=36, 26% and (3) diabetes mellitus (DM): n=36, 26%. The homeostasis model assessment (HOMA) was used to evaluate indexes of insulin sensitivity (HOMA-S) and β -cell function (HOMA- β). Patients with NGT were younger (mean age \pm SD: 40.3 ± 13.0 vs. 49.6 ± 14.5 and 49.8 ± 13.0 years in groups 2 and 3; $p < 0.001$), while diabetic patients had a higher BMI (mean \pm SD: 29.6 ± 4.0 vs. 27.2 ± 5.7 and 27.1 ± 4.0 Kg/m² in groups 1 and 2; $p < 0.05$). There was no difference among the 3 groups regarding sex ratio, pituitary adenoma size, symptom score and HOMA-S, while HOMA- β was reduced in group 2 (- 18%, $p < 0.05$) and group 3 (- 30%, $p < 0.001$), compared to group 1. Mean IGF-I z-score was higher in IFG/IGT (5.0 ± 1.9) and in DM patients (5.3 ± 1.7) than in NGT patients (4.4 ± 1.3 ; $p < 0.01$). In contrast, fasting GH and post-OGTT nadir GH levels were not different between groups. Significant correlations were observed between fasting glucose and age, BMI and IGF-I z-score ($p < 0.001$), between HOMA-S and IGF-I z-score ($p < 0.001$), but not between glucose metabolism parameters and fasting or nadir GH. In multivariate analyses, IGF-I was an independent predictor of insulin resistance and hyperglycaemia, while age was the only factor negatively affecting β -cell function. In conclusion, IFG/IGT or overt DM are observed in more than 50% of naive acromegalic patients. An older age, a higher BMI and a greater IGF-I z-score at diagnosis are all significant risk factors associated with impaired glucose homeostasis, whereas fasting or post-OGTT nadir GH levels are unable to predict glucose tolerance alterations.

Disclosures: PHC: Advisory Group Member, Pfizer, Inc.

Nothing to Disclose: OA, MB, PK, AB, DM

P3-262

Prostatic Disorders and Acromegaly: Results of a Brazilian Center.

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Background: Acromegalic patients have an increased prevalence of prostatic disorders. Increased size of the prostate together with an elevated incidence of other structural changes, such as nodules, cysts, and calcifications were reported.

Design and methods: Thirty-six men with active acromegaly aged 45.5±10.7 yr were submitted to clinical prostatic evaluation (digital rectal examination and International Prostate Symptoms Score- IPSS), transrectal ultrasound (TU) and laboratorial assessment (serum levels of GH, IGF-I, testosterone and total PSA). Ten patients were newly diagnosed without any previous treatment. Fifteen had already been submitted to only surgery and seven to surgery and radiotherapy. Eleven patients were using octreotide LAR, two cabergoline and nine both drugs. Mann-Whitney and Spearman correlation tests were used with significance set at p -value <0.05. This study was approved by the Institutional Review Board.

Results: The patients were evaluated 36 months (range 1-276 months) after the diagnosis of acromegaly. The median IPSS value was 4 (range 0-21). The median GH, IGF-I (% above the upper limit of reference value) and total PSA were, respectively, 5.1ng/mL (range 0.3-68.9), 164% (range -36 to 535) and 0.6 ng/mL (range 0.04-2.5). Eleven patients (31%) presented prostatic hyperplasia by TU (prostate volume >30g), calcifications were detected in 11 (31%), one of them under 40 yr, cystic dilatations in five (14%) and no nodules were found. The median total PSA levels were higher in the patients with prostate hyperplasia (1.13 ng/mL vs 0.45ng/mL, p = 0.008). Prostate hyperplasia was not found in patients less than 40 yr (n =12). Five out of eleven (45%) patients with prostate hyperplasia had between 40 and 50 yr. The median prostate volume was lower in the 17 (47%) hypogonadic without testosterone replacement vs eugonadic patients (20g vs 28g, p =0.018). There was correlation between IPSS value and prostate volume (p =0.044), but there was no correlation between GH and IGF-I levels and time of diagnosis with the prostate volume.

Conclusions: The frequency of prostatic hyperplasia and other abnormalities were high in patients with acromegaly but not correlated with GH and IGF-I levels and time of diagnosis. The correlation between the IPSS and prostate volume reinforces that acromegalic patients should be carefully evaluated for prostate disorders.

Nothing to Disclose: LLC, GABL, SAC, LCDM, MRG

P3-263

Ambulatory Arterial Stiffness Indexes in Acromegaly.

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Background: Previous studies have shown that large artery stiffening is increased in acromegaly, with a potential role in cardiovascular outcome. Ambulatory arterial stiffness index (AASI), a measure based on the relative behaviour of 24-hour systolic and diastolic blood pressure (BP), as well as the index derived from AASI symmetrical regression (Sym-AASI), have been suggested as markers of arterial stiffness and strong predictors of cardiovascular mortality.

Aim: To evaluate AASI and Sym-AASI indexes in 96 consecutive patients with active acromegaly and 69 age, sex and mean BP (MBP) matched controls (CTR). **Patients and Methods:** Diagnosis of acromegaly was based on clinical features, GH - IGF-I measurements and evidence of pituitary tumour at MRI. 24h-ABPM was carried out using a TAKEDA-2430 recorder (15-30 min intervals), and derived AASI and Sym-AASI were obtained. Patients were divided into two subgroups, normotensive (NT) and hypertensive (HYP). Hypertensive acromegalic patients included all those receiving anti-hypertensive therapy, while hypertensive controls were untreated. According to 2007 ESH-ESC guidelines, 34 acromegalic subjects were hypertensive (> 130/80 mmHg) at ABPM (M15/F19, MBP 101.1±7.2 mmHg, mean age 54.8±12.8 yr), and 62 were normotensive (M31/F31, MBP=85.2±5.6 mmHg, mean age=46.5±14.0 yr). In acromegalic patients, correlations of arterial stiffness indexes with anthropometric, biochemical (GH, IGF-1, metabolic profile), and echocardiographic parameters were assessed. **Results:** See Table. Inter-group differences were assessed by Student t-test.

| | NT-ACRO | NT-CTR | P | HYP-ACRO | HYP-CTR | P |
|----------|-----------|-----------|---------|-----------|-----------|-------|
| AASI | 0.52±0.17 | 0.36±0.14 | <0.0001 | 0.59±0.15 | 0.49±0.17 | 0.011 |
| Sym-AASI | 0.23±0.14 | 0.15±0.12 | 0.005 | 0.28±0.12 | 0.20±0.10 | 0.009 |

AASI and Sym-AASI were significantly higher in acromegalic patients than in controls, either for the NT or the HYP subset. Among different parameters, Sym-AASII was directly correlated with the age of patients only (r=0.27; P=0.009).

Conclusions: Our data show that the ambulatory arterial stiffness indexes are increased in acromegaly, independently of BP elevation. This may have important implications in predicting cardiovascular risk in this disease. The role of GH/IGF-I axis as functional component of arterial stiffening has to be further clarified.

Nothing to Disclose: FD, AG, RC, BF, LM, MB, CM, RV, NS, FF, PM

P3-264

Cardiovascular Complications of Acromegaly.

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Background. GH and IGF-I hypersecretion in acromegalic patients leads to cardiovascular complications. Normalization of GH and IGF-I blood levels can stop the progression of left ventricle hypertrophy (LVH), thus decreasing cardiovascular (CV) morbidity and mortality.

Objectives. Compare the echocardiographic (echo) parameters in 2 groups of acromegalic patients based on the level of GH and IGF-I at the time of the initial echo. Evaluate the influence of CV risk factors and the impact of the disease duration on the echo parameters.

Materials and methods. Out of 175 acromegalic patients followed at our center, 47 people, mean age 53.6, range 31-82 years, initial diagnosis after 1995, were divided in 2 groups: 19 (10 males) "Controlled Patients" (CP) with active acromegaly treated with Somatostatin Analogs (SA) or Pegvisomant (GH < 2.5 ng/ml and normal IGF-I); 28 (9 males) "Non Controlled Patients" (NCP) on medical therapy (GH > 2.5 ng/ml and/or abnormal IGF-I). Parameters evaluated: GH and IGF-I at diagnosis and at echo, presence of hypopituitarism, disease duration, echo parameters: left ventricular end-diastolic diameter (EDD), left ventricular end-systolic diameter (ESD), interventricular septal thickness (IVST), left ventricular posterior wall thickness (LVPW), LV mass (LVM), ejection fraction (EF), transmitral flow (E/A), CV risk factors (smoking, BMI, hypertension, lipids and diabetes mellitus).

Results. The thickness of the posterior wall is smaller in CP vs. NCP (0.9 ± 0.2 ; 1.0 ± 0.2 ; $p = 0.027$), as well as the LV mass (88.6 ± 19.0 ; 105.0 ± 26.7 ; $p = 0.033$); the ejection fraction is greater in CP than NCP ($68\% \pm 8.7$; $63.7\% \pm 8.2$; $p = 0.023$). There is a direct correlation between disease duration and thickness of the interventricular septum and of the LV posterior wall ($p < 0.05$ for both), and an inverse correlation with the EF ($p < 0.05$). In CP, IGF-I levels at echo correlated with LV mass. Only the BMI showed a significant negative correlation with EF ($p < 0.01$).

Conclusions. Results confirmed that IGF-I hypersecretion promotes LVH which compromises systolic function. Controlling the disease will halt LVH and reduce CV morbidity and mortality.

Nothing to Disclose: FG, SC, FC, MF, DM, MS, ET, AT, MV

P3-265

Cardiac Effects of Three Months Treatment of Acromegaly Evaluated by Magnetic Resonance Imaging and B-Type Natriuretic Peptides.

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Context Long-term treatment of acromegaly prevents aggravation and reverses associated heart disease. A previous study has shown a temporary increase in serum levels of the N-terminal fraction of pro B-type natriuretic peptide (NT-proBNP) suggesting an initial decline in cardiac function when treatment of acromegaly is initiated.

Objective To investigate short-term cardiac effects of treatment in acromegalic patients as evaluated by the gold standard method cardiac magnetic resonance imaging (CMRI) and circulating levels of B-type natriuretic peptides (BNP and NT-proBNP).

Subjects and methods Eight patients (5 males and 3 females, mean age 53 ± 12 years (range 30-70) and 8 healthy control subjects matched for age and gender were included. CMRI was performed at baseline and after three months of treatment. Levels of IGF-I, BNP and NT-proBNP were measured after 0,1,2 and 3 months.

Results Median IGF-I Z-score decreased from 4.5 (range 2.5 to 6.4) to 2.3 (-0.1-3.3). At baseline the patients had increased left ventricle mass index (LVMI) compared to control subjects (Δ LVMI 35 g/m^2 (95%CI, 8-63 g/m^2 , $P=0.016$).

After three months of treatment there was an increase in end-diastolic volume index EDVI (Δ EDVI 9 mL/m^2 (95%CI, 3-14), $P=0.007$) and an increase in levels of BNP (median (ranges) 7 (0.58-286) vs. 20 (1-489) pg/mL , $P=0.033$) and of NT-proBNP (63 (20-1004) vs. 80 (20-3391) pg/mL , $P=0.027$).

Conclusions Assessed by the highly sensitive and precise CMRI method, three months treatment of acromegaly resulted in an increase in EDVI, and increased levels of BNP and NT-proBNP suggesting an initial decrease in cardiac function.

Sources of Research Support: Unrestricted research grant from NovoNordisk Scandinavia.

Nothing to Disclose: MA, JF, AK, CLP, LOK

P3-266

Impaired Arterial Properties in Active Acromegaly Are Reversed by Effective Therapy.

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Objective

Active acromegaly is associated with increased cardiovascular morbidity and mortality. Normalization of GH and IFG-1 levels has been shown to improve some of the cardiac function abnormalities. The aim of the study was to evaluate parameters of arterial function in active and controlled disease.

Design/patients

The arterial properties of 18 subjects (7 males and 11 females) with acromegaly were studied by repeat non-invasive measurements of arterial properties assessing pulse wave velocity (PWV), central blood pressure, augmentation index (AIx), and large/small artery compliance (C1;C2) and by ultrasonography, common carotid intima-media thickness (IMT) and flow-mediated dilatation (FMD).

Nine subjects with active acromegaly (GH-7.76±12mU/L; IGF-1-469.9±246ng/ml) and nine subjects with controlled disease (GH-1.59±0.1mU/L; IGF-1-160±0.3ng/ml) were studied.

Results

Mean age was 48.3±19 years in subjects with active disease and 56.4±13 years in those with controlled acromegaly, p=NS). Weight, waist circumference, BMI, HDL- and LDL-cholesterol and triglycerides were similar in the two groups. Glucose tolerance abnormalities were found in 5 (50%) patients with active disease and in two (27%) cured subjects, with significantly higher fasting plasma insulin level in the active group (21.9±11.27 vs. 14.8±3.8mIU/mL; p=0.0075). Systolic blood pressure (SP) but not diastolic blood pressure (DP) was significantly higher in patients with active disease (128±17 vs. 113.1±12mmHg; p=0.005). Central blood pressure was higher in the active disease group (SP: 118±12 vs. 106±12mmHg; p=0.04; DP: 74±8 vs. 66±6mmHg; p=0.02) as was systemic arterial resistance (1427±323 vs. 1299±215dynes.sec.cm⁻⁵; p=0.006). Hypertension was found in three patients with active disease and two in cured subjects. Analysis of arterial function parameters were performed with adjustment for age, gender and SBP. Small artery elasticity index (C2) was lower in the active disease group (6.71±4.1 vs. 8.5±4.2ml/mmHg×100; p=0.03). There were no significant differences in other arterial stiffness parameters including PWV, AIx, C1, IMT and FMD.

Conclusions

Patients with active acromegaly have decreased small artery elasticity index, increased systemic systolic BP, increased central SP and DP as well as increased systemic arterial resistance. These vascular derangements could contribute to the increased propensity for cardiovascular disease in acromegaly but are apparently reversed by effective treatment.

Nothing to Disclose: MY, EI, JZ, IA, MI-S, SG, KMT, NS, YG

P3-267

Comparison of Endoscopic and Microscopic Endonasal Transsphenoidal Surgery Approaches in Acromegalic Patients.

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OBJECTIVE: Recently transsphenoidal (TS) surgery became the first treatment choice for pituitary tumors. Endoscopy is increasingly used in the therapy of pituitary lesion. In this retrospective study, we compared the efficacy of two TS surgery [endoscopic endonasal transsphenoidal (EET), and microscopic endonasal transsphenoidal (MET)] approaches in first time for the treatment of patients with acromegaly.

METHODS: Seventy-one patients who underwent primary TS surgery for acromegaly between 2004 and 2009 in Ankara Numune Research and Training Hospital were analysed retrospectively. Patients who underwent MET (n=40) and EET (n=31) were compared and groups were age and sex matced (mean age 40.3±10.8 vs 43.4±10.6 years, p=0.159; male/female 19/24 vs 17/14, p=0.366). Pituitary magnetic resonance imaging was performed before and 3 months after the treatment. Insulin-like growth factor 1 (IGF-I) levels in the normal boundaries according to age and gender and suppressed growth hormone (GH) level below 1 ng/ml as a response to OGTT-GH suppression test were considered as "biochemical cure".

RESULTS: Baseline GH (ng/ml), IGF-I levels (ng/ml) and adenoma volumes (cm³) were similar in MET and EET groups [(26.7±31.9 vs 23.2±19.3, p=0.588; 1084.3±372.5 vs 966.7±383.1, p=0.189; 3.9±3.3 vs 2.7±3.8, 0.198), respectively]. Postoperatively mean percentage of decrease in GH levels and tumor shrinkage were significantly higher in EET group compared to MET group but mean percentage of decrease in IGF-I levels were similar in both groups [(78.5±33.1 vs 54.5±33.8, p<0.001; 94.5±13.8 vs 67.6±39.8, p<0.001; 39.7±54.5 vs 25.6±41.3, p=0.05), respectively]. Percentage of complete tumor resection and biochemical cure achievement were significantly higher in EET group compared to MET group [(51.6% vs 10.0%, p<0.001; 60.6% vs 39.4%, p=0.003), respectively]. While we observed 2 diabetes insipidus cases (1 transient and 1 permanent) in MET group after surgery there was no diabetes insipidus in EET group.

CONCLUSION: The endoscopic endonasal transsphenoidal approach for acromegalic patients as first surgery had provided better disease control with higher rates of complete tumor resection and biochemical cure.

Nothing to Disclose: DB, MB, YT, YA, SI, UO, GA, SG

P3-268

Efficacy of Cabergoline in the Treatment of Acromegaly: A Meta-Analysis.

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Context: The dopamine agonist cabergoline is used since 1985 in the treatment acromegaly. However, clinical studies on cabergoline in acromegaly involved few patients, were not randomized or controlled and their results were very variable.

Objective: To obtain a more accurate picture of the efficacy of cabergoline in acromegaly via a metaanalysis.

Design: We systematically reviewed all studies assessing the effects of cabergoline in the treatment of acromegaly (cabergoline and acromegaly were the 2 key words) until 2009 in 4 databases (Pubmed, Pascal, Embase and Google scholar). Fifteen studies (11 prospective) were identified, including 237 patients; none were randomized, placebo-controlled. A meta-analysis was conducted on individual data obtained either directly from the articles when available or collected by asking to investigators if unavailable in the published papers (227 patients).

Results: In 9 studies (150 patients) where cabergoline was used alone, 32% of the patients achieved normal IGF-I levels. Mean (\pm SD) decrease in IGF-I levels was $33\pm 25\%$, mean maximal dose of cabergoline used was $2.5\pm 1,5$ mg/week, mean treatment duration was 10.8 ± 8.2 months. Mean IGF-I levels decrease from 620 ± 250 to 400 ± 207 ng/ml ($p < 0.001$). This change was related to baseline IGF-I ($\beta = -0.55$; $p < 0.001$), treatment duration ($\beta = -7.6$; $p < 0.001$) and age of the patients ($\beta = -2.8$; $p = 0.03$). A trend was observed for dose ($\beta = 22.9$; $p = 0.07$) and baseline PRL concentration ($\beta = 0.12$; $p = 0.07$).

In five studies (77 patients), cabergoline was used in association with a somatostatin analog (SA). At baseline, when using SA alone, 2.6 % of the patients had normal IGF-I levels. With the combined use of cabergoline and SA, 52% of the patients achieved normal IGF-I levels (mean decrease in IGF-I was $30\pm 32\%$). Mean IGF-I levels decreased from 542 ± 280 to 358 ± 201 ng/ml ($p < 0.001$). This change was related to baseline IGF-I ($\beta = -0.27$; $p = 0.001$).

Conclusion: As demonstrated by our meta-analysis, control of acromegaly, if assessed by normalization of IGF-I levels, can be achieved with cabergoline in almost a third of the patients. When patients are resistant to SA, adding cabergoline allows normalization of IGF-I levels in more than half of the patients.

Disclosures: PC: Advisory Group Member, Pfizer, Inc.

Nothing to Disclose: LS, PM

P3-269

Effectiveness of the Addition of Cabergoline to Acromegalic Patients Resistant to Longterm Therapy with Octreotide LAR.

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BACKGROUND

Long-acting somatostatin analogs (SAs) are the mainstay in the medical treatment of acromegaly. However, even at high doses, longterm treatment with these drugs fails to achieve adequate control of GH and IGF-I levels in approximately 35% of patients both as primary or adjunct therapy. Cabergoline, a potent dopamine agonist, is a cheaper alternative to SAs that allows control of GH and IGF-I in up one third of patients. Better results are found in patients with concomitant hyperprolactinemia.

OBJECTIVE

To evaluate the effectiveness of the addition of cabergoline to acromegalic patients resistant to longterm therapy with octreotide LAR.

PATIENTS AND METHODS

A total of 52 patients resistant to longterm therapy (at least, 12 months) to octreotide LAR (30-40 mg every 28 days IM) were given cabergoline (at the maximum dose of 3.5 mg/week). Data on acromegalic patients submitted to combined therapy were collected in 4 Brazilian endocrine centers.

RESULTS

Among the 52 patients evaluated, 54% were females. Their age ranged from 23 to 71 years (mean \pm SD, 48.4 \pm 14.5). Normalization of IGF-I levels were achieved in 40.4% of patients after the addition of cabergoline (at doses ranging from 1 mg to 3.5 mg/week). Compared to non-responsive subjects, responsive patients had higher mean % ULNR-IGF-I values (226.4 \pm 95.2 vs 171 \pm 88.1; $p = 0.01$) and higher mean prolactin (PRL) levels (93.2 \pm 76.5 vs 27.4 \pm 30.3 ng/mL; $p = 0.001$). However, among the responsive patients, the rate of hiperprolactinemia and normal PRL levels did not significantly differ (50% vs 43%; $p = 0.77$). Moreover, the rate of positive PRL immunohistochemical staining was similar in responsive and non-responsive patients.

CONCLUSION

The combination of octreotide LAR + cabergoline may be useful in acromegalic patients partially responsive to octreotide LAR, even in the absence of hyperprolactinemia or if immunohistochemistry is not positive for PRL.

Nothing to Disclose: LV, LAN, RMM, MFA, LAC, JLA, PF, RMM, LP, LM, GC, MSF

P3-270

McCune-Albright Syndrome and Acromegaly: Response to Therapy with Long-Acting Somatostatin Associated with Dopamine Agonist in Five Patients.

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Introduction: GH hypersecretion affects about 20% of the patients harboring McCune-Albright Syndrome (MAS). Surgical treatment is not always possible due to thickening of bone structures. Medical treatment is the available option followed or not by radiotherapy. Somatostatin analogs (SA) are the first line option, but an important amount of patients fail to achieve control. The association with dopamine agonists (DA) was shown to be beneficial for some cases, though the results of this association have demonstrated to be variable. The aim of this study is to retrospectively analyze and describe the clinical presentation and therapeutic response of five medically treated acromegalics with MAS. **Results:** Patients enrolled were between 25 and 38 years old (mean \pm sd: 33.0 \pm 5.4), 60% were male, the mean age at the onset of fibrous dysplasia was 5.2 years, and 25.8 years when acromegaly was diagnosed. At the initial MRI, patients presented with 3 macroadenomas, one hyperplasia and one without apparent lesion. Hyperprolactinemia was found in 80%. None of the patients were referred to surgery. Radiotherapy was performed in two patients. At the last visit, all patients were on octreotide LAR (20-30 mg every 28 days) and cabergoline (3.5 mg/week) with a mean follow-up time of 7.0 \pm 4.6 years. At the diagnosis, the mean hormonal levels were: random GH 140.7 ng/mL, IGF-1 724.2 ng/mL, prolactin 163 ng/mL. After treatment with SA and DA (mean treatment time 5.6 years), a marked reduction of the random GH and IGF-1 was obtained (60% and 42.7%, respectively) but only one achieved hormonal control. Prolactin was normalized in all subjects. Two patients underwent radiotherapy (follow-up time of 7.6 and 10.4 years), and further reduction on IGF-1 was observed. One patient treated with radiotherapy developed bone sarcomatous transformation (skull base) 10 years after irradiation. Treatment with SA and DA association was described in 12 patients in the literature, from whom only 25 % reached hormonal control. **Conclusion:** SA and DA association allowed a significant reduction of GH and IGF-1 levels and prolactin normalization in all patients. However, only one patient (20%) accomplished acromegaly control. Due to the radiotherapy risks, including secondary tumors, this approach should be indicated only in selected cases. Pegvisomant therapy remains the option for the uncontrolled patients.

Nothing to Disclose: BBA, FGD, LCSL, MCBVF, VNB, RSJ, MDB

P3-271

Pharmacokinetic and Pharmacodynamic Behaviour of Lanreotide Autogel® after Subcutaneous Administration of 120 mg Every 56 Days in Patients with Active Acromegaly.

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Objective:

To describe the pharmacokinetic (PK) profile of lanreotide Autogel® 120 mg (ATG-120, marketed as Somatuline Depot® in the US) administered by subcutaneous route (s.c.) every 56 days to patients with active acromegaly and to develop a population pharmacodynamic (PD) model describing the inhibition of growth hormone (GH) after ATG-120 administration.

Methods:

Two Phase III, multicentric, open studies were used: i) A-92-52030-046: 98 acromegaly patients received 3 or 5 s.c. doses of ATG-120 every 28, 42 or 56 days; ii) A-93-52030-077: 63 acromegaly patients received 2 injections of ATG-120 s.c. every 56 days, and then, based on GH level, ATG-120 s.c. every 56, 42 or 28 days. PK and PD analysis was performed in two steps: i) PK: comparison of PK behaviour between patients with acromegaly and healthy volunteers after administration of Autogel® (1) was performed using visual predictive check [VPC]; ii) PD: the model of GH inhibition induced by lanreotide after injection of ATG-120 every 56 days was determined.

Results:

After VPC application, similarity of PK profile between acromegalic and healthy volunteers has been demonstrated. The decrease of mean GH levels at increasing lanreotide concentrations was well described by a Simple Inhibitory E_{max} model. The population mean of E_{max} (maximum achievable inhibition) and EC_{50} (lanreotide concentration needed to reduce GH to 50% of E_{max}) was 86% and 0.433 ng/mL, respectively. The median lanreotide level needed to decrease GH to 2.5 ng/mL ($C_{2.5}$) was 1.05 ng/mL. Covariate analysis showed that baseline of GH (E_0) was negatively correlated with pre-treatment status, age and weight. Steady-state drug levels after administration of ATG-120 every 56 days were higher than $C_{2.5}$.

Conclusions:

ATG-120 administered every 56, 42 or 28 days to patients with acromegaly exhibits a similar PK behaviour to that seen in healthy volunteers. GH inhibition induced by lanreotide after administration of ATG-120 every 56 days was well described by a Simple Inhibitory E_{max} model. PD profile and PD parameters obtained after administration of ATG-120 every 56 days were similar to those estimated after injection of Autogel® 60, 90 and 120 mg every 28 days (2), indicating schedule independency. These results provide a PK and PD rationale to support the feasibility to extend the dosing interval for lanreotide ATG-120 to 56 days.

(1) Troconiz et al., Clin Pharmacokinet. 2009;48(1):51-62

(2) Cendros et al., Metabolism. 2005 Oct;54(10):1276-81

Disclosures: JR: Employee, Ipsen. JMC: Employee, Ipsen. CP: Employee, Ipsen. RO: Vice President, Ipsen. JF: Vice President, Ipsen.

P3-272**Somatuline Depot-Treated Patients with Acromegaly in the United States: Preliminary Results from the Somatuline® Depot [lanreotide] Injection for Acromegaly (SODA) Study.**M Gordon MD¹, M Molitch MD², J Grua MD³, VM Rios, Jr BS, MA⁴, S Chang PhD⁴ and S Blethen MD, PhD⁴.¹Allegheny Gen Hosp Pittsburgh, PA ; ²Northwestern Univ Chicago, IL ; ³Western Endocrine Salt Lake City, UT and ⁴Ipsen, US Brisbane, CA.**Introduction:** SODA is an ongoing, multi-center, observational study of lanreotide in patients with acromegaly.**Methods:** After obtaining informed consent, data existing in a patient's medical record as part of routine care were collected. Targeted adverse events (AEs) were AEs observed in previous studies with lanreotide or other somatostatin analogues (SSA).**Results:** As of November 2009, 54 patients (32 M, 22 F) were enrolled in SODA from 21 sites (see Table 1). Most patients were previously treated with long-acting SSAs (78% of patients). Most patients (69%) were on a 90 mg dose of lanreotide at enrollment, and the dose was subsequently increased in seven patients and decreased in one patient due to IGF-1 levels.

Table 1. Patient Characteristics

| Prior treatment | Mean age, yr | Mean time since diagnosis, yr | Radiotherapy, n (%) | Baseline mean IGF-1, ng/mL | Baseline normal IGF-1, n (%) | 6 month f/u patients, n |
|--------------------------------|--------------|-------------------------------|---------------------|----------------------------|------------------------------|-------------------------|
| None ¹ (n=7) | 46.4 | 2.7 | 2 (29%) | 444.5 | 3 (43%) | 3 |
| Long-acting SSA ± other (n=42) | 49.9 | 9.7 | 13 (31%) | 290.3 | 19 (45%) | 11 |
| Other ² (n=5) | 52.0 | 7.3 | 1 (20%) | 253.8 | 3 (60%) | 2 |

¹ Except surgery or radiotherapy; ² Includes pegvisomant and dopamine antagonists

Four patients had a history of cancer (breast, thyroid and lymphoma). Only 12 patients had an oral glucose tolerance test (OGTT). The two primary reasons that an OGTT was not performed were cited as physician choice and the procedure was not routinely performed for disease management. Diabetes was noted as the reason an OGTT was not performed in six patients. Sixteen patients had 6-month follow-up data; mean IGF-1 levels decreased from baseline to 6 months (275.1 ng/mL vs 232.8 ng/mL) and had normalized in 10 patients (63%) compared with 7 of these patients (44%) at baseline. The most commonly reported AEs were abdominal pain and headache (both 24%), followed by arthralgia (21%), constipation (15%), anxiety (15%) and diarrhea (12%). Serious AEs (unrelated to therapy) were reported in one patient: one report of sepsis and one report of stroke, metabolic acidosis and acute kidney failure.

Conclusions: These data suggest that lanreotide is generally well tolerated and associated with IGF-1 control in most patients with acromegaly. The adverse events reported in these patients are those expected for SSAs.

Sources of Research Support: Ipsen, US.

Disclosures: MG: Study Investigator, Ipsen; Medical Advisory Board Member, Ipsen; Investigator, Pfizer, Inc., Novartis Pharmaceuticals. MM: Study Investigator, Ipsen; Consultant, Ipsen, Pfizer, Inc. JG: Study Investigator, Ipsen; Speaker, Lilly USA, LLC; Investigator, Lilly USA, LLC; Speaker, Serono. VMR: Employee, Ipsen. SC: Employee, Ipsen. SB: Employee, Ipsen; Consultant, Ipsen.

P3-273

Somatostatin Responsiveness May Influence Glucose Metabolic Status of Acromegaly after Treatments.

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Acromegaly had insulin resistance with long standing stimulation of excess growth hormone (GH). GH would physiologically elevated blood sugar followed by excess insulin secretion. We usually use somatostatin (SMS) analog as a medical therapy. We reviewed seventeen Acromegalics (M:F=11:6) for the last five years, who had taken SMS suppression test before treatment, and oGTT (75 gram of glucose) with checking GH and insulin before and after treatment in Kyung Hee University Medical Center, Seoul, South Korea. Serum insulin had been checked at before and 30 min. after glucose loading. Average follow-up duration from trans-sphenoidal adenomectomy (TSA) and followed by medical therapy were 9.5 and 9.3 months. Responder of SMS suppression test was defined as GH was < 1 mg/L within four hours after 100 mg of octreotide injection. We found that baseline, nadir, Dpeak of GH by oGTT, and IGF-I were significantly decreased after treatment. (Table 1.) Metabolic parameters of glucose by oGTT were not different from after treatment (FBS, postprandial glucose, HOMA-IR, baseline and stimulated insulin, and c-peptide). With SMS suppression test, responders showed improved tendency of HOMA-IR with low fasting serum c-peptide. These meant that SMS played a major role for glucose and insulin resistance in Acromegalics.

Table 1. Metabolic parameters of Acromegalics after treatment.

| Parameters | No | before-Rx | after-Rx | p value |
|-------------------------------------|----|-------------|---------------|---------|
| peak Glucose-oGTT, mg/dL | 17 | 203 ± 78 | 186 ± 54 | 0.352 |
| glucose difference-oGTT, mg/dL | 17 | 87 ± 41 | 84 ± 37 | 0.799 |
| FBS, mg/dL | 17 | 117 ± 50 | 102 ± 26 | 0.257 |
| postprandial glucose 2 hr, mg/dL | 17 | 184 ± 87 | 160 ± 61 | 0.247 |
| Baseline GH, µg/L | 16 | 18.9 ± 21.5 | 8.1 ± 14.3 | 0.007* |
| Nadir GH by oGTT, µg/L | 16 | 15.2 ± 19.2 | 6.8 ± 12.5 | 0.007* |
| GH peak difference by oGTT, µg/L | 16 | 12.9 ± 18.4 | 5.0 ± 7.4 | 0.016* |
| serum IGF-I, µg/L | 16 | 922 ± 418 | 438 ± 307 | 0.001* |
| HOMA-IR | 17 | 2.8 ± 1.6 | 2.1 ± 2.0 | 0.102 |
| Fasting insulin, µU/mL | 17 | 10.0 ± 4.9 | 8.4 ± 8.6 | 0.495 |
| Stimulated insulin by oGTT, µU/mL | 17 | 57.3 ± 69.9 | 49.3 ± 47.2 | 0.694 |
| Fasting c-peptide, ng/mL | 17 | 2.0 ± 1.0 | 1.5 ± 0.6 | 0.103 |
| Stimulated c-peptide by oGTT, ng/mL | 17 | 5.7 ± 4.9 | 4.1 ± 2.7 | 0.212 |
| Tumor volume, mm ³ | 15 | 591 ± 699 | 4,333 ± 4,982 | 0.055 |

*; p<0.05

Nothing to Disclose: S-WK, JOM, JHK, SRK, CBL, DWB, SJO

P3-274

Immunoexpression of Proliferation and Angiogenesis Markers in Patients Treated with Long-Acting Somatostatin Analogues.

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Somatostatin analogues are the gold standard of medical treatment in acromegalic patients because they have potent to provide hormonal control and induce tumor shrinkage. Somatostatin analogues play antiproliferative role and inhibit angiogenesis therefore reduce tumor growth rate.

The aim of this study was to compare immunoexpression of proliferation marker Ki-67 and angiogenesis markers CD31 and vascular endothelial growth factor (VEGF) in somatotropinomas removed from acromegalics treated with long-acting somatostatin analogues and untreated patients. Sixteen patients who received long-acting somatostatin analogues therapy before surgery (median of treatment duration was 6 months) and thirty seven untreated patients matched for sex, age, tumor size and extension, pre-treatment hormonal activity were studied. We used immunohistochemical staining of removed adenomas for evaluation of proliferation marker Ki-67 and angiogenesis markers CD31 and VEGF. The number of vessels stained with antibody to CD31 was significant lower in patients who received somatostatin analogues therapy before surgery than in untreated acromegalics (Mann-Whitney test, $p=0,006$). We did not show any differences in VEGF and Ki-67 immunoexpression between these groups of patients. So our study demonstrates that long-acting somatostatin analogues therapy inhibit angiogenesis in somatotropinomas, but no data has been received, confirming antiproliferative effect.

Nothing to Disclose: EIM, EGP, AYA, AYG, NNM, LYR

P3-275

The Clinical Experience at a Single Centre with Pegvisomant Therapy in Acromegaly.

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Patients with acromegaly resistant to conventional drug treatment currently can advantage with GH-receptor antagonist pegvisomant. To date, at doses up to 40 mg/day, it is capable of normalizing circulating IGF-I in until 97% of patients. Here we present the long-term experience of our centre with pegvisomant as a therapy for acromegaly. This is a retrospective study including a total of 30 patients treated with pegvisomant (21 female and 9 male patients) for up to 7 years. Before starting pegvisomant, all patients had IGF-I values above the upper limit of age and sex-adjusted normal range. A total of 10 patients received combined treatment with pegvisomant and somatostatin analogs. Pegvisomant has been administered at a daily dose ranging from 10 to 40 mg.

Results: a total of 27 patients (90%) normalized the IGF-I value on pegvisomant therapy. A transitory increase of liver enzymes was observed in 4 patients (13%) and completely resolved after temporary stopping of pegvisomant. We did not observe adverse reaction at the injection site. No patient presented increase of the tumor at pituitary magnetic resonance imaging. In a 60 years old patient, control MRI documented a gradual decrease of pituitary adenoma which disappeared after 6 years of treatment with pegvisomant, without other pharmacological association. In 2 cases, the treatment with pegvisomant at 10 mg daily dose induced an excessive reduction of IGF-I: in one patient, after 4 months of treatment, we observed severe fatigue, diffuse myalgia, sleep disturbance. IGF-I levels fell down 50 mg/dl. In the second case, after a month of therapy, the patient showed osphyalgia with subsequent diagnosis of spinal canal stenosis and IGF-I levels were extremely low. Once GH receptor antagonist therapy has been stopped, despite the incomplete normalization of IGF-I levels in one case, the clinical symptoms suddenly and significantly improved in both patients.

Our experience indicates that pegvisomant is a safe and effective treatment in acromegaly and that it is well-tolerated. However, caution should be taken in starting pegvisomant therapy at the lowest dose and accurately monitoring IGF-I levels 4/6 weeks after the initiation of pegvisomant. Moreover, it is necessary to carefully consider all new symptoms manifested by patients treated with pegvisomant.

Nothing to Disclose: AF, AB, AG, LT, VC, FV, SP, AD, DI, ML, CA, GM, AP, LDM

P3-276

Efficacy and Safety of Co-Administration of Lanreotide Autogel 120 mg Monthly with Pegvisomant Weekly in Patients with Acromegaly Partially Controlled by Somatostatin Analogues.

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Recent studies have shown that the co-administration of lanreotide (lan) with pegvisomant (peg) was able to normalise IGF-1 secretion in patients with acromegaly who were partial responders to somatostatin analogues (SSAs). 57 subjects with acromegaly (mean age 51.6 ± 12.7 years) not previously controlled by SSAs (either treated by SSAs for at least 6 months with IGF-1 > ULN, or on peg for at least 3 months) with IGF-1 > 1.2 ULN (n=54) or with IGF-1 > ULN and GH nadir $\geq 1 \mu\text{g/L}$ (n=3) after a 4 month run-in period on lan 120 mg monthly entered into a co-administration period to evaluate the efficacy and safety of combining monthly injections of lan 120 mg with peg for 28 weeks. Peg starting dose of 60 mg once weekly could be progressively adapted every 8 weeks based on IGF-1 levels from 40 to 80 mg once weekly up to 60 mg twice a week. Serum IGF-1 was normalized at the end of the co-administration period in 33 (58%) subjects. The median weekly dose of peg taken by subjects who normalized IGF-1 level was 60 mg. The percentage of subjects normalized was significantly higher in non diabetics (24/38= 63% than in diabetic patients 9/19= 47%). Older subjects were more likely to achieve normalisation than younger ones (odd ratio: 3.395 (p=0.03)). Serum IGF-1 was normalised in 45 (79%) subjects at least once at any time during the co-administration period (p < 0.0001). Small mean improvements were seen in the quality of life scores, however these changes were associated with large data variability. Co-administration of lan and peg was well tolerated. Five subjects reported treatment emergent adverse events (TEAEs) leading to peg withdrawal: thrombocytopenia and urticaria were serious AEs considered drug-related while abdominal pain and vomiting was a serious AE considered non drug-related; the 2 other (non serious) TEAS were transaminases increase > 5x ULN which returned to normal after peg withdrawal. Altogether 6 (10.5%) subjects experienced transient increases of transaminases $\geq 2x$ ULN, not related to their diabetic status. Co administration of lan Autogel 120 mg monthly with peg weekly appears to be safe and to improve hormonal control in a majority of patients with acromegaly partially controlled by somatostatin analogues alone. When compared to the available data on peg monotherapy, co administration needs substantially less peg per week to achieve the same efficacy.

Sources of Research Support: Ipsen Group.

Disclosures: A-JvdL: Consultant, Ipsen, Novartis Pharmaceuticals, Pfizer Global R&D. IB: Speaker, Ipsen, Novartis Pharmaceuticals, Pfizer Global R&D. JC: Scientific Board Member, Ipsen; Speaker, Novo Nordisk. AC: Speaker, Ipsen, Novartis Pharmaceuticals, Pfizer Global R&D. CL: Employee, Ipsen. PB: Employee, Ipsen.

Nothing to Disclose: PC, JM, SJCMMN

P3-277

Pharmacokinetic (PK) Behaviour of BIM 23A760 after Subcutaneous Administration in Healthy Volunteers and Patients with Active Acromegaly.

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Objective:

To develop a population PK model that can describe the PK profile of BIM 23A760 after a single and repeated subcutaneous (s.c.) administrations at different dose levels in healthy volunteers and patients with active acromegaly.

Methods:

Two clinical studies were used to perform the population PK analysis: i) Clinical Study: 2-55-52060-001 was a randomised, double-blind, placebo controlled, sequential single dose and repeated doses escalating study in two parts (Parts A and B) to investigate and compare the safety and tolerability of single and multiple ascending doses of BIM 23A760 given by s.c. injection of a 5 mg/ml formulation concentration. In Part A, 30 healthy volunteers were randomized in five cohorts receiving single s.c. injection of BIM 23A760 at sequential dose levels of 0.25, 1, 4, 6 and 10 mg. In Part B, 18 healthy subjects received repeated s.c. injection of BIM 23A760 at one of the following dose regimens: 2 mg at Days 1, 4 and 7 for cohort 1; 2 mg at Day 1, 4 mg at Day 4, and 6 mg at Day 7 for cohort 2, and 6 mg at Day 1 and 6 mg at Day 4 for cohort 3; ii) Clinical Study: 2-55-52060-002 was an ascending dose, multicentre study to investigate the pharmacodynamics (PD) of BIM 23A760, administered by single s.c. injection to patients with acromegaly. Two cohorts of 5 and 6 patients for 1 and 4 mg, respectively were dosed on Day 1. The data were analysed by nonlinear mixed-effect modelling using the program NONMEM (ver. VI) applying different validation approaches such as visual predictive check.

Results:

The pharmacokinetic profile of BIM 23A760 was characterized by a first peak around 2 hours and a second peak between 2 and 4 days followed by decreased gradually decreasing concentrations. A one-compartment linear model with first-order elimination was constructed which consisted of two absorption processes: rate constants for immediate-release and sustained-release. The immediate-release rate was approximately 900 times higher than the sustained-release rate. The model fits accurately all assayed dose levels and schedules, suggesting comparable PK behaviour between patients with acromegaly and healthy volunteers.

Conclusions:

The pharmacokinetic profile of BIM 23A760 was effectively described by the derived population model for all dose levels and single and repeated administrations. Similarity of PK profile between patients with acromegaly and healthy volunteers has been demonstrated after single dose administration of BIM 23A760.

Disclosures: JR: Employee, Ipsen. MCL: Employee, Ipsen. JMC: Employee, Ipsen. SA: Employee, Ipsen. ES: Employee, Ipsen. CP: Employee, Ipsen. RO: Vice President, Ipsen. JF: Vice President, Ipsen.

P3-278

Gigantism Due to Somatotroph Hyperplasia in a 4 Year Old Boy: A Genetic Mystery.

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Background: Rare in early childhood, gigantism is usually due to a GH-secreting pituitary adenoma. We describe a 4-year-old boy with growth hormone and prolactin excess as a result of somatotroph and lactotroph hyperplasia.

Clinical case: From birth to 2 years of age, the boy grew along the 90th percentile. Then, his growth velocity increased gradually to 15 cm/year. At 4 y 8 m, his height reached 129.7 cm (+ 5.2 SD; height age, 8.5 y) and his weight, 35.5 kg (+ 4.0 SD). His family history was non contributory. On examination, he had coarse facial features, no cutaneous signs and Tanner 1 genitalia. Bone age was 5 years. The fasting serum GH level was high, > 35 ug/L [>35 ng/ml], and was not suppressed by an oral glucose load. Serum prolactin and IGF1 concentrations were elevated; 185.2 ug/L [185.2 ng/ml] (normal range, 0-18) and 74.5 nmol/L [569.2 ng/ml] (normal range, 6.4-36 [48.9-275.0]), respectively. A brain MRI showed a 1.5 X 1.8 X 1.3 cm intrasellar solid mass. The surgically-removed pituitary tissue revealed somatotroph and lactotroph hyperplasia, without adenomatous changes. Post operatively, GH and prolactin levels remain high and the patient requires medical management.

GH cell hyperplasia is rare in adults. In children, only four cases causing gigantism have been described, two were combined hyperplasia and adenoma and only two demonstrated pure hyperplasia¹. Etiologies for all age groups were attributed to McCune-Albright syndrome (MAS), GHRH-producing tumors, Carney complex and idiopathic congenital causes². Moreover, germline mutations in the aryl hydrocarbon receptor-interacting protein (AIP) gene have recently been associated with pituitary GH-secreting adenomas, but have never been related to somatotroph hyperplasia. We have excluded these diagnoses: negative GNAS1 (on hyperplastic pituitary DNA), and negative AIP gene mutations; normal GHRH level, 24-h 5HIAA urine collection and chromogranin A. We are not suspicious of Carney complex given his lack of pathognomonic physical features and his normal echocardiogram; nonetheless, his PRKAR1A mutation analysis is ongoing.

Conclusion: To date, the etiology of gigantism in our patient remains undetermined. Additional genetic studies including novel genes are underway to better understand molecular cause of somatotroph hyperplasia.

1. Scheithauer BW, et al. *Endocrine Path* 1995; 6:173.

2. Horvath E, et al. *Pituitary* 1999; 1: 169.

Nothing to Disclose: MM, CR

P3-279

Development of Acromegaly in a Patient with Well-Controlled Prolactinoma after 20 Years on Bromocriptine Treatment.

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Hyperprolactinemia is reported in up to 25% of acromegalic patients. However, the occurrence of acromegaly after a prolonged stable course in a well treated prolactinoma has rarely been described in the literature. We report a case of acromegaly diagnosed after 20 years of well controlled prolactinoma. A 29 year-old woman presented in 1988 with galactorrhea and oligomenorrhea. Hyperprolactinemia was diagnosed and head CT suggested empty sella together with a 2 mm lesion. Treatment with bromocriptine resolved her galactorrhea and normalized her prolactin levels and her menses. Imaging in 1994 showed no progression of her presumed adenoma. Over the years she developed obesity, hypertension, impaired glucose tolerance, carpal tunnel syndrome, knee arthritis and benign colonic polyps. Her initial IGF1 was within the normal range. This was repeated in recent years given her clinical course, but remained normal until 2007. She continued to have normal menstruation. When reevaluated in 2009, on exam she was an obese lady with broadening of her nose and coarsening of her facial features compared to photos from the 1980s, but otherwise she had no acromegalic features. Her prolactin level was normal (14.7 to 23.3 ng/dl, normal 2.8-29.2 pg/ml), while her IGF-1 increased from 283 (in the normal range in 2006) to 340 and 471 ng/dl (normal 94-252 ng/ml). 75 gm glucose suppression test showed a growth hormone level of 5.8ng/dl at 1 hr and 2.7ng/dl at 2 hours, confirming the diagnosis of acromegaly (normal < 1ng/ml). Pituitary MRI demonstrated partial empty sella with a 5 mm pituitary adenoma. She underwent trans-sphenoidal surgery and pathologic findings demonstrated a pituitary adenoma with growth hormone and prolactin expression and fibrotic changes, likely from bromocriptine treatment. At follow up 1 month later, her IGF-1 and prolactin levels and her other pituitary hormones are normal. This case illustrates how clinically significant acromegaly may develop in prolactinoma patients decades after their initial diagnosis even if their prolactin remains normal. While this patient's co morbidities were attributed to her obesity, treatment of her acromegaly may improve her clinical outlook and prevent further morbidity. Clinicians should be aware of the association of acromegaly in prolactinoma even after years of normal prolactin and should consider periodic IGF-1 levels indefinitely in prolactinoma patients.

Nothing to Disclose: IS, WTC, GAY, CH, VGD, GEV, CML

P3-280

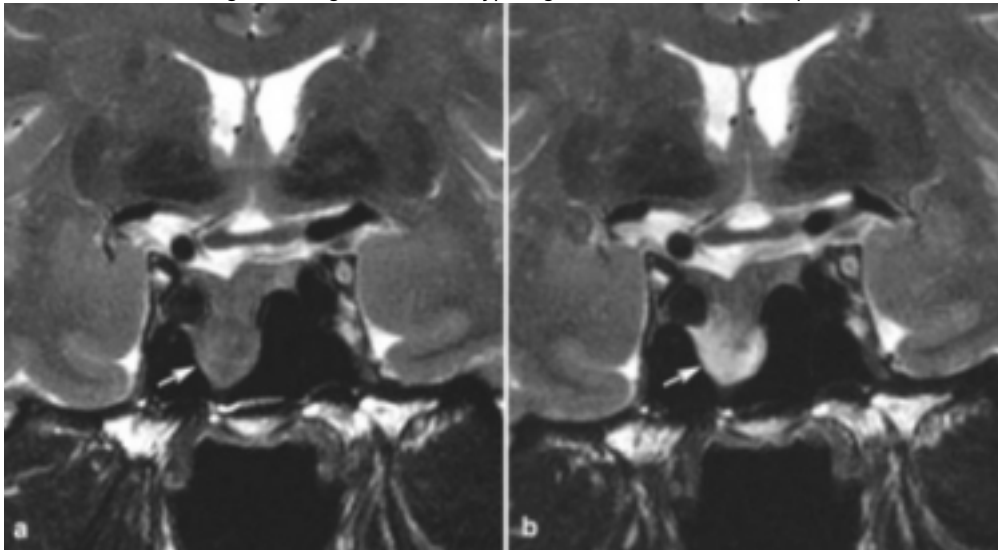
Signal Changes on T2-Weighted MRI Can Assess the Efficiency of Somatostatin Analogs with GH-Secreting Pituitary Adenomas When No Shrinkage Is Demonstrated.

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Background : The objective of treatment of acromegaly by somatostatin analogs (SA) are reversal of symptoms, restoration of normal GH and IGF 1 levels and reduction of pituitary tumor size. Reduction of tumor size is inconstant and not always correlated with clinical or biological data (1). Activation of different somatostatin receptors probably plays a role with tumor shrinkage. Cell cycle arrest, induction of apoptosis, growth factor production and inhibition of angiogenesis have been proposed to explain the antiproliferative action of SA. But this possible effect of SA does not appear to be associated with striking morphological changes: necrotic changes are not noted but acidophilia and interstitial fibrosis occur. Moreover, some studies suggest that a biochemical response predicts tumor shrinkage and other not. Variety of methodologies to define tumor shrinkage, lack of standardization of measurements from MR, observer subjectivity, make difficult to compare results among studies. Thus, shrinkage is not regularly reliable; changes of T2 signal on MRI can be an additional marker of the efficiency of SA in acromegaly. This is particularly true with pituitary tumors invading the sphenoid sinus inferiorly, where MR is unable to differentiate tumor contours (before treatment) from persistent tumor imprint within the sphenoid sinus (after treatment).

Clinical cases: We present 3 cases of acromegalic patients with pituitary adenoma invading the sphenoid sinus. All 3 patients were treated with octreotide LAR 30 mg for 6 months before transsphenoidal surgery. At the 3 month-follow-up, the GH and IGF-1 levels were significantly decreased or normalized. In all 3 cases, MR did not demonstrate any tumoral shrinkage but striking changes of pituitary adenoma signal on MR were observed : hyposignal of the lesion on T2-weighted images turned to hypersignal at 3-month -follow-up.



Conclusion: Apart from tumor volume measurements standardization, we suggest that signal changes on T2- weighted MR images have to be considered in any protocol involving medical treatment of pituitary adenomas and particularly SA in the treatment of acromegaly.

(1) Freda PU, Katznelson L, Jan van Der Lely A, Reyes CM, Zhao S, Rabinowitz D. Long-acting somatostatin analog therapy of acromegaly : a meta-analysis. *J Clin Endocrinol Metab.*2005 ; 90 : 4465-73.

Nothing to Disclose: JFB, FC, AD, CB-G, FS, AB, GV

P3-281

Metastatic Growth Hormone Secreting Pituitary Carcinoma Treated with Peptide Receptor Radionuclide Therapy.

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Introduction: Pituitary carcinoma (PC) is a rare condition defined by the presence of adenohypophyseal neoplastic tissue outside the pituitary. Growth hormone (GH) secreting PC is even rarer and represents a particular challenge to clinical practice. Therapeutic modalities utilized to treat PC include surgery, radiation, hormonal therapy, and cytotoxic drugs. Peptide Receptor Radionuclide Therapy (PRRT) is an emerging therapeutic modality that involves the targeted delivery of an ablative dose of radiolabelled somatostatin analog. PRRT has been applied to various neuroendocrine tumors and results in improved morbidity and mortality. As yet, PRRT has not been applied to malignant pituitary tumors.

Aims: To present a case of GH secreting PC and suggest PRRT as an apparently effective therapeutic option.

Methods: A 56 year-old female with a 9 year history of GH secreting pituitary macroadenoma presented with worsening headache and marked acromegalic features. Surgical removal of the pituitary tumor was attempted twice, but GH and IGF-1 levels remained elevated and the pituitary mass re-expanded despite medical therapy with octreotide and cabergoline. The patient became severely debilitated with weight loss, musculoskeletal pain, and uncontrolled diabetes. Whole body CT exam demonstrated numerous osteoblastic bone lesions. A CT guided biopsy obtained from a bone lesion confirmed the diagnosis of metastatic GH secreting PC. Octreoscan demonstrated numerous somatostatin avid lesions in the pituitary and the skeleton. Pasireotide therapy was attempted but not tolerated; temozolamide was not available due to cost.

Results: Three courses of PRRT (¹¹⁷Lutetium-DOTATOC and ⁹⁰Yttrium-DOTATOC, 200 mCi each) were administered. Post treatment scan demonstrated a decrease in uptake intensity in several bone lesions with other metastases stable-appearing. However, the GH level continued to rise and palliative external radiation therapy to skeletal lesions was administered for local relief. The patient died 18 months after the initial PRRT and survived significantly longer than previously reported in PC patients.

Conclusions: The clinical course in this patient favors an aggressive therapeutic approach early in the management of malignant pituitary tumors. PRRT may be an effective therapeutic modality in PC. Additional studies are needed to examine the benefit of PRRT in selected cases of somatostatin receptor positive, unresectable, radiation resistant pituitary tumors.

Nothing to Disclose: SK, DJG, VD, RD-P

P3-282

An Unusual Case of Acromegaloidism Due to Beckwith-Wiedemann Syndrome Diagnosed as an Adult.

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Introduction: Beckwith-Wiedemann Syndrome (BWS) is an overgrowth disorder that usually involves increased rate of growth during the latter half of pregnancy and in the first few years of life. The presenting findings in children may include macrosomia (prenatal and/or postnatal gigantism), hemihyperplasia, macroglossia, and visceromegaly. The characteristic features tend to regress or normalize over time. It is considered to be a pediatric growth disorder and diagnosis is rarely made in adulthood.

Clinical Case: A 43 year-old African American male was referred for an endocrine evaluation for acromegaly that was suspected due to complaints of an increase in body size (increased total body weight, head size, shoe size and hand size) that was first noticed at the age of 37. He had severe obstructive sleep apnea (OSA) due to macroglossia and complaints of frequent tongue biting. Due to significant mandibular overgrowth and macroglossia, he recently had to undergo partial glossectomy (40%) and jaw reconstruction. Past medical history was significant for macrosomia (birth weight 10lbs 2 oz), macroglossia leading to speech difficulties and macrocephaly that always required him to wear a larger sized hat. Significant prognathism led to jaw reconstruction at the age of 16 yrs. Biochemical testing ruled out acromegaly. He was referred to the genetics clinic for further evaluation. Given his history of macrosomia, macroglossia and overgrowth in association with his physical findings, a diagnosis of BWS was considered. Abdomino-pelvic ultrasound was reported as normal. Genetic testing confirmed abnormal methylation of H19 in the 11p15.5 chromosomal region, consistent with a diagnosis of BWS. Chromosomal microarray analysis did not detect any rearrangements, particularly in the BWS region.

Conclusion: In this case report we describe a 43 year-old male with acromegaloid features with an apparent onset at birth, progressive but subtle over the years and with recent exacerbation of features 6 years ago now diagnosed with BWS. This case report suggests that BWS should be considered in the differential diagnosis of patients with acromegaloidism, even in adult patients where the diagnosis is much less common.

Nothing to Disclose: SMT, TNE, MFW, VRS, SUD, JMG

P3-283

Spontaneous Remission of Acromegaly after Infarctive Apoplexy with a Possible Relation to MRI and Diabetes Mellitus.

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Introduction: Spontaneous remission of acromegaly cases have been reported after pituitary apoplexy before, however pituitary apoplexy due to tumor infarction is very rare. **Clinical Case:** A 38 year-old man was admitted with complains of enlargement of his hand and foot size. He had been diabetic and hypertensive for about one year. Endocrine studies revealed a GH level of 80 µg/L and IGF-1 level of 747 ng/mL (100-494). His blood glucose level was 393 mg/dL. Basal hormone levels were within the normal range except gonadotropins and testosterone which were in low levels. Pituitary MRI showed a macroadenoma about 19x20 mm in size. He presented with a 2-day history of severe frontal headache, malaise accompanied by nausea and vomiting a week after insulin therapy was started and the day after a pituitary MRI was carried out. On admission, the patient's neurological examination and his visual field testing were normal. He was not hypotensive. Biochemical values revealed a glucose level of 330 mg/dL and a sodium level of 134 mEq/L. His urine ketone body was negative. Emergent MRI of the sella disclosed an enhancing intrasellar mass of 24x23mm, extending into the suprasellar cistern and compressing the optic chiasm, which was hyperintense on both T1 and T2 weighted images. Patient's sodium levels decreased from 134 mEq/L to 127 mEq/L and glucocorticoid treatment was immediately started in suspicion of acute ACTH deficiency. The patient underwent transsphenoidal decompression of the pituitary lesion urgently. Histological examination revealed that most of the tissue showed nonhemorrhagic coagulation necrosis and an adenomatous tissue positive of GH. Before surgery, his GH levels declined to 2.72 µg/L spontaneously and after surgery he was in remission (GH <0.05 µg/L and IGF-1:76 ng/mL). The patient developed panhypopituitarism after surgery. DM showed improvement and the insulin dose was reduced. Pituitary MRI taken three months after pituitary apoplexy showed no evidence of remaining tumor. **Conclusion:** DM and diabetic ketoacidosis are one of the precipitating factors of pituitary apoplexy. Also, our patient's symptoms developed the day after IV injection of contrast substance for MRI. The presence of contrast media induced endothelial swelling with the result of hypoperfusion and diabetes mellitus associated vasculopathy may be the precipitating factor for the infarction of the adenoma. Luckily, he developed spontaneous remission of acromegaly after apoplexy.

Nothing to Disclose: NUC, YM, SD, MZ, FS, TE

P3-284

Spontaneous Cerebrospinal Fluid Rhinorrhea as the Initial Presentation of Growth Hormone Secreting Pituitary Adenoma.

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Background

Patients with acromegaly usually present with characteristic clinical features or comorbidities associated with excess IGF-1/GH or at times may come to medical attention secondary to a mass effect such as visual field defect. We report a case with spontaneous cerebrospinal fluid (CSF) rhinorrhea as the presenting symptom of acromegaly.

Clinical Presentation

A 68-year old man presented to an outside facility with a two day history of headache associated with nausea, vomiting, dizziness, and clear nasal discharge. Examination on presentation to our hospital was significant for fluctuating level of consciousness. On subsequent exams, subtle coarse facial features could be appreciated. Upon detecting the sellar abnormalities, pituitary function tests were ordered which showed thyrotropin and gonadotropin deficiencies along with an elevated age and sex matched IGF-1 of 285(nl 59-225 ng/ml). Nadir GH during OGTT was 5.5 ng/mL and confirmed the diagnosis of acromegaly. Magnetic resonance (MR) imaging showed pneumocephalus, an enlarged sella with an elongated pituitary stalk and partial erosion of the anterior wall of the sphenoid sinus. A distinct adenoma was not identified radiographically. An endoscopic, transnasal, transsphenoidal exploration with a biopsy and reconstruction of the skull base to address the CSF leak which had resulted in a persistent low intracranial pressure state, was performed. At the same time, a random biopsy was obtained from the tissue that formed the lining of the cavity. Histological examination of the biopsy contents were consistent with a growth hormone producing adenoma. Post operatively the patient's fluctuating level of consciousness improved and returned to baseline once he started to retain CSF. During the follow up period, he had an IGF-1 of 713 ng/ml and recently started treatment with a somatostatin analogue.

Conclusion

To our knowledge, this is the first reported case of a growth hormone producing pituitary adenoma with an initial presentation of spontaneous CSF rhinorrhea. Pituitary adenomas should be considered in the differential diagnosis of patients presenting with spontaneous CSF rhinorrhea with abnormal sellar image and these patients should undergo a thorough hormonal evaluation.

Nothing to Disclose: VG, BS, AHH, BH

P3-285

Case Report: Gigantism - Odontological Changes.

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Background: Gigantism is an extremely rare condition, caused by a GH excess, there are approximately 100 cases reported, associated to secondary hypogonadism, which delays the epiphysial closure, thus allowing continued long bone growth.

Despite gigantism occurs before epiphysial closure, the growth is not necessarily symmetrical. Facial disproportions are present, such as macrocefalia, frontal bossing, prominent jaw and macrodontia. When dentist examine the patient, he can observe diastemas, disproportional jaw growth, increase of soft tissues causing a coarse facial features, another finding is macrodontia, which is a rare condition with unusually big teeth. Suspected diagnosis can be done by dentist, who most of times have the first contact with those patients.

Clinical cases: Two giants males, diagnosed with GH- secreting pituitary macroadenoma, were examined by dentist: ILS, 16 years old, diagnosed a year ago, the patient presented clinical signs when he was 8 years old, showing disproportional jaws growth, prominent jaw and diastemas in all mandibular arch, normal size teeth, however he did not initiate orthodontic treatment.

DML, 18 years old, diagnosed 2 years ago, without odontological changes at the moment, he has been receiving orthodontic treatment for 5 years, the treatment has not finished, probably because the active jaw growth complicating an optimal dental occlusion.

Conclusion: Some odontological changes can be noted before the accelerated growth. Macrodontia is a very rare clinic alteration which occurs when the tumor appears on childhood, disproportion jaw growth and increase of soft tissue is more frequent, the dentist training is very important, the first contact with those professionals is very common, and it could help the diagnosis as soon as possible, increase the cure rates once the diagnosis will be precocious.

Nothing to Disclose: FZL, FFI, DGF, DMN, GNA, MKPH, LCS, FFF

P3-286

A Case of Fugitive Acromegaly, Initially Presented as Invasive Prolactinoma.

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Fugitive acromegaly has known to be mostly caused by pituitary acidophil stem cell adenoma, characterized by relatively short clinical history, large and locally invasive tumor, and relatively low hormonal activity. Here we report an unusual case of fugitive acromegaly initially presented as invasive prolactinoma. Abstract termed fugitive AA 48-year-old man with a huge pituitary mass extending to the suprasellar area was referred in December 2007. He had undergone transsphenoidal surgery in November 1999 due to a large invasive prolactinoma. The tumor has grown progressively despite therapy with dopamine agonists. Subtle features of acromegaly were noted and measured serum IGF-1 level was also high (733.0 ng/ml). Oral glucose tolerance test revealed that basal and nadir levels of GH were 1.56 ng/mL and 1.0 ng/mL, respectively. As a therapeutic trial, long-acting octreotide LAR (20 mg IM, monthly) was added. The tumor size was markedly reduced in 6 months on MRI examination. Immunohistochemical staining of the tumor tissue obtained at the surgery in 1999 showed positive staining for GH and PRL. Double immunofluorescence staining showed mixed positivity for GH and PRL in the majority of tumor cells. However, two hormones were co-localized in the minority of tumor cells, indicating that tumors were composed of three different cells (GH, PRL and GH/PRL). This patient was overlooked initially for the diagnosis of fugitive acromegaly due to normal serum GH level and no acromegalic features although histological evidence for GH production was present. IGF-1 measurement would be helpful for the diagnosis of fugitive acromegaly.

Nothing to Disclose: MWL, JSL, CRK, MKL, TSK, SHK, EJJ

P3-287

An Unusual Clinical Presentation of a Woman with Acromegaly.

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A 20 years old Italian female with polycystic ovary syndrome was referred to our hospital for examination of clinical hyperandrogenism, acanthosis nigricans, menstrual irregularity, hypertension and headache. On admission she performed endocrine evaluation including serum levels of insulin-like growth factor 1 (IGF1), prolactin, luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, DHEA-S, androstenedione. She underwent hyperinsulinemic euglycemic clamp (HEC) and oral glucose tolerance test. As a result, IGF1 and prolactin levels were found to be elevated at 1273 ng/ml (normal range 240-540) and 57.1 ng/ml (normal range 3.5-26.5 ng/ml). Hormonal evaluation revealed an abnormal LH/FSH ratio of 0.83 (LH 0.5 MUI/ml FSH 0.6 MUI/ml), high values of DHEA-S (3007 ng/ml; normal range 800-3000), androstenedione (5.63 ng/ml; normal range 0.4-3) and testosterone (0.62 ng/ml; normal range 0.20-0.60). The GnRH stimulation test showed an exaggerated response of LH compared to FSH. The oral glucose tolerance test showed a normal glucose tolerance with hyperinsulinism. The HEC confirmed the presence of insulin resistance (M value: 3 mg/kg/min) The Ovary ultrasound revealed bilateral polycystic changes. Brain magnetic resonance imaging (MRI) with gadolinium enhancement revealed a pituitary macroadenoma in the sellar region. She underwent surgery for the partial remotion of macroadenoma. After surgery there was an amelioration of insulin resistance and hyperandrogenism. IGF 1 levels was reduced after surgery even if it did not was in the normal range. Thus, the patient is on somatostatin analogue therapy. The case reported here described is a unique combination of GH producing pituitary adenoma and PCOS: in the early preclinical phase the characteristics typical for acromegaly were not observed instead there were symptoms and signs suggesting PCOS as ovarian dysfunction, androgen overproduction and the presence of polycystic ovaries. The only sign suggesting acromegaly was the hypertension. This case supplies evidence that insulin resistance with IGF 1 overproduction related to GH excess may play a role in the onset of PCOS through stimulation of theca stromal cell proliferation. In conclusion GH excess could be involved in the pathogenesis of PCOS.

Nothing to Disclose: GM, CC, ES, AC, CF, BA, CP, GPS, AP, AP, AG, SDC

P3-288

Hyperthyroidism and Acromegaly Due to a TSH/GH-Secreting Microadenoma.

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Background: TSH secreting pituitary adenomas are rare (<1% of functioning adenomas) and generally present as aggressive macroadenomas with little chance for complete resection.

Clinical Case: We report a 36 year old Caucasian male presenting with hypogonadism and a goiter. He presented with fatigue, loss of libido, headache and 17 pound weight loss. He was found to have a low testosterone and mildly elevated prolactin. Pituitary MRI demonstrated an 8 mm on the left in a normal sized pituitary gland. Despite improvement of libido on testosterone replacement other symptoms continued, prompting an endocrinology referral. The patient had a goiter but no other physical signs of hormonal excess. His headache was debilitating interfering with work. Initial endocrine lab:

Preoperative Lab

| Component | 8 weeks Pre-Op | 5 weeks PreOp | PreOp | Reference Range |
|-----------------------|----------------|---------------|---------|-------------------|
| TSH | 2.180 | 1.230 | 1.180 | 0.400-5.500 uU/mL |
| Free T3 | 6.0 (H) | 5.1 (H) | 4.6 | 1.8-4.6 pg/mL |
| Free T4 | | 1.6 | 2.2 (H) | 0.7-1.8 ng/dL |
| Growth Hormone | | 0.2 | | 0.0-3.0 ng/mL |
| Ins. Like Grth Fact1 | 400 (H) | 409 (H) | | 117-329 ng/mL |
| IGFBP-3 | | 5.4 | | |
| Prolactin | | 11.4 | | 2.0-14.0 ng/mL |
| Alpha Sub Gonadotripi | | 0.2 | | |
| FSH | 0.2 (L) | 0.3 (L) | | 1-10 mU/mL |

Baseline GH was 0.5 ng/ml and suppressed to 0.2 ng/ml at 60 and 120 mins during OGGT. Because the patient was found to be hyperthyroid with an inappropriately normal TSH and elevated IGF-1, a TSH/GH secreting adenoma was suspected. The patient was sent for transnasal transphenoidal resection of his microadenoma after preparation with methimazole. At surgery an adenoma was identified on the left and resected. Immunostaining was positive for TSH and for GH. Six weeks post surgery the patient was endocrinologically normal without medications.

Post Operative Lab

| Component | 6 months Post-Op | Reference Range |
|---------------------|------------------|-------------------|
| TSH | 1.150 | 0.400-5.500 uU/mL |
| Free T3 | 2.7 | 1.8-4.6 pg/mL |
| Ins Like Grth Fact1 | 187 | 117-329 ng/mL |
| Prolactin | 4.7 | 2.0-14.0 ng/mL |
| Testosterone | 226 | 220-1000 ng/dL |

Headache had resolved at his 6 week follow-up visit but libido was subnormal. Testosterone was low at his 6 month follow-up so replacement was restarted.

Conclusion: With better TSH assays and high resolution MRI, make earlier identification of functional microadenomas possible and with this the greatest likelihood complete resection and possible afford cure with preservation of normal pituitary function.

(1)Socin HV, et al., European J Endo 2008;148:433.

Nothing to Disclose: IS, RJW, LO

P3-289

Huge Goiter Identified in a Patient with Acromegaly and Hashimoto's Thyroiditis.

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We present the case of a 68-year-old-woman who complained of huge goiter. The goiter was noted for the first time during a periodical health checkup at the age of 56 years. She consulted her family doctor who diagnosed her with Hashimoto's thyroiditis. She had mild primary hypothyroidism and received levothyroxine sodium as replacement therapy. Although she was asymptomatic, her goiter gradually increased. Therefore, she was referred to our hospital for further evaluation. Her appearance was acromegalic, and huge goiter was detected on palpation. The endocrinological tests revealed that her serum growth hormone (GH), prolactin (PRL), and insulin-like growth factor-I (IGF-I) levels were 9.16 mg/l, 15.9 mg/l, and 313 mg/l, respectively. She had low levels of antithyroid peroxidase antibody as well as antithyroglobulin antibody—0.6 IU/ml and 0.5 IU/ml, respectively. The serum GH level paradoxically increased after the administration of thyrotropin-releasing hormone. On the basis of the inadequate suppression of the serum GH level during the 75-g oral glucose tolerance test, we diagnosed her with acromegaly accompanied with Hashimoto thyroiditis. An X-ray film of her skull showed enlargement of the sella turcica, whereas the pituitary magnetic resonance image showed an empty sella without any mass lesion. Chest computed tomography revealed a huge diffuse goiter, with the right lobe of the thyroid gland extending into the mediastinum. The cabergoline administered for acromegaly slightly decreased her goiter as well as the serum levels of GH, PRL, and IGF-I to 1.83 mg/l, 0.60 mg/l, and 186 mg/l, respectively. On the basis of these results, we believe that chronic high levels of GH and IGF-I may be one of the factors causing enlargement of the thyroid gland.

Nothing to Disclose: KI, YH, TO, KC

P3-290

Pro-Opio-Melanocortin as a Candidate Antigen in a Patient with Biopsy-Proven Autoimmune Hypophysitis.

P Leporati MD^{1,2}, L Chiovato MD, PhD² and P Caturegli MD, PhD^{1,3}.

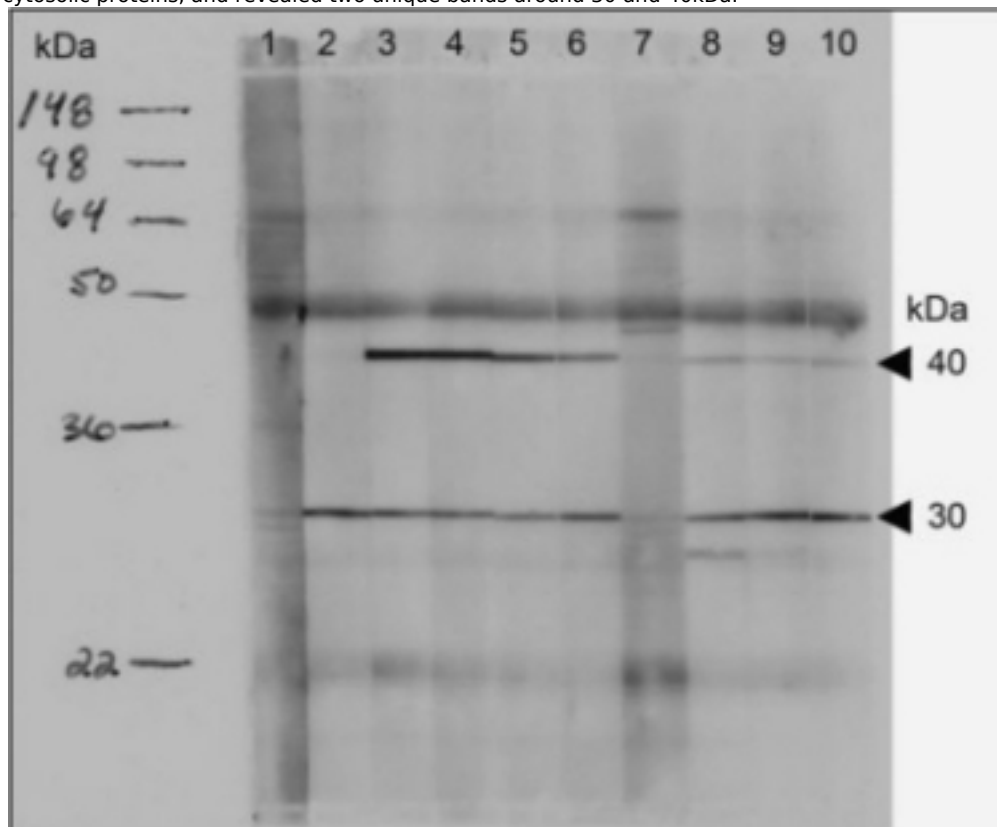
¹The Johns Hopkins Univ Sch of Med Baltimore, MD ; ²Fondazione Salvatore Maugeri IRCCS, Univ of Pavia Pavia, Italy and ³The Johns Hopkins Bloomberg Sch of Public Hlth Baltimore, MD.

Introduction. Autoimmune hypophysitis is a pituitary disorder typically presenting as a mass causing neurological and/or endocrinological symptoms. The pathogenic autoantigen(s) remain to be discovered and the diagnosis of certainty still relies on pituitary biopsy.

Clinical Case. We describe a 75years old man with a few months history of frontal headache, poorly controlled by non-steroidal anti-inflammatory drugs, fatigue, polyuria. Baseline assessment of pituitary hormones revealed central hypothyroidism (FT3 2.6pg/ml, FT4 4pg/ml, TSH 0.22mU/ml), central hypoadrenalism (morning cortisol 24ng/ml, ACTH 7pg/ml), hypogonadotropic hypogonadism (LH 0.3mU/ml, FSH 2mU/ml, testosterone 0.1ng/dl), decreased serum IGF-1 (43ng/ml), low serum prolactin (2.3ng/ml) and central diabetes insipidus (high urinary output, 4500ml/24h, with low osmolarity, 200mOsm/L). Patient began promptly hormone replacement therapy with cortisone, L-thyroxine and desmopressin. After 18months follow-up, the pituitary function was not restored, and substitutive therapy was continued.

Pituitary imaging and pathology. Cranial MRI at diagnosis showed an intra- and supra-sellar homogeneous mass that enhanced avidly after gadolinium injection; the stalk was thickened. Histopathology revealed a marked mononuclear infiltration of the adenohypophysis dominated by plasma cells and lymphocytes. Cranial MRI performed after 6 and 12 months, showed normal findings.

Pituitary Antibodies. Serum collected shortly after diagnosis tested negative for pituitary antibodies when assessed by immunofluorescence. IgM positive pituitary antibodies became detectable when immunoglobulins were purified from the serum by gel chromatography. Immunoglobulins were also used for western blotting against human pituitary cytosolic proteins, and revealed two unique bands around 30 and 40kDa.



Protein bands were fractionated by fast protein liquid chromatography, excised and sequenced by mass spectrometry. Sequencing revealed 4peptides from human GH in the 30kDa band, and 1peptide from pro-opio-melanocortin(POMC) in the 40kDa band.

Conclusions: we report the first case of autoimmune hypophysitis recognizing POMC as a candidate antigen.

Caturegli P et al., *Endocr Rev* 2005; 26:599

Lupi I et al., *Clin Endocrinol* 2008; 69:269

Caturegli P et al., *Autoimmun Rev* 2008; 7:631

Sources of Research Support: NIH grant DK080351.

Nothing to Disclose: PL, LC, PC

P3-291

Granulomatous Hypophysitis Mimicking Pituitary Adenoma: Report of 2 Cases.

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Introduction: Primary hypophysitis is rare sellar lesion classified as lymphocytic (the most frequent), granulomatous (GHYP), and xanthomatous necrotizant¹. GHYP is characterized by multinucleated giant cells, histiocytes, lymphocytes inflammatory infiltrate and calcium deposition. For diagnosis, secondary etiologies (tuberculosis, sarcoidosis, syphilis, mycotic granuloma, Wegener's granulomatosis and systemic immune diseases (Takayasu's or Crohn's disease) must be ruled out. It affects both genders and main symptoms result from mass effect, as headache and visual impairment. In MRI, pituitary gland may be diffusely increased (pyramidal or round shape) and pituitary stalk can be thickening.

However, typical image occurs infrequently, resulting in difficult differential diagnosis with pituitary adenoma¹. **Case reports:** case 1: 29 yrs-old female presented a severe headache. MRI depicted a sellar mass with suprasellar expansion and chiasmatic compression, with a right eye scotoma. Hormonal evaluation pointed to ACTH and TSH deficiencies, as well as mild hyperprolactinemia (PRL:39 ng/mL, n <26). Case 2: 54-yrs-old male presented weakness and loss of libido, with no visual complaints. Laboratory evaluation pointed to GH, LH, FSH, ACTH deficiencies and hyperprolactinemia (PRL 36 ng/mL). MRI depicted a sellar mass with suprasellar extension and pituitary stalk thickening (Fig. 1a). Surgery was performed in both cases and histopathologic analysis pointed to GHYP (Fig. 1b). Histochemistry for fungi and koch bacilli were negative.

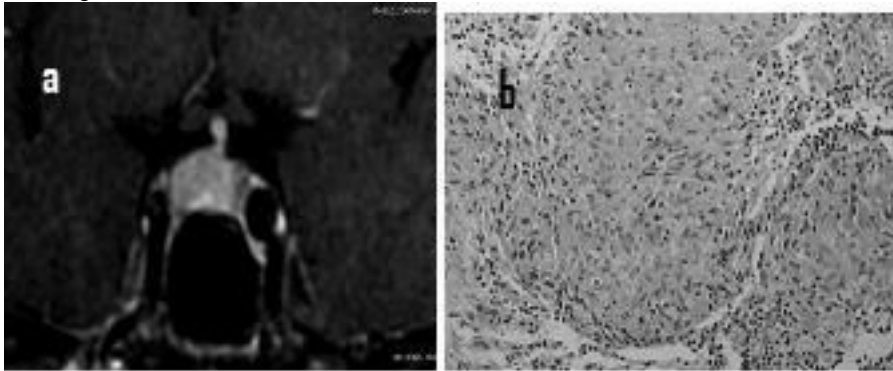


Fig. 1: (a)Sellar MRI coronal T1-weighted depicted a lesion with homogeneous enhancement and thickening of infundibulum. (b)Anatomopathological examination revealed a giant cell granuloma.

Conclusion: GHYP was first described before *mortem* in 1980 and until now, there are other 15 case reports. In all of them the diagnosis was difficult. Most frequent symptoms were headache and visual defects, but diabetes insipidus and cranial nerve palsy were also reported. Diagnosis was made after transsphenoidal biopsy or surgery, as our cases. Although rare, GYPH diagnosis should be considered when there is a pituitary mass with symptoms suggestive of meningitis (headache, fever) or diabetes insipidus. After clinical suspicion, pituitary mass biopsy is indicated and systemic corticosteroid therapy can be tried².

(1)Shi, J. Et al. Granulomatous hypophysitis: two case reports and literature review. J of Zhejiang Univ Sci B. 2009,10:552-8.

(2)Yamagami, K et al. Treatment of lymphocytic hypophysitis by high-dose methylprednisolone pulse therapy. Intern Med.2003, 42:168-73.

Nothing to Disclose: TPS, AG, DV, PC, SR, MRG, PNF

P3-292

Lymphocytic Hypophysitis Mimicking Aseptic Meningitis.

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BACKGROUND

Lymphocytic hypophysitis is an uncommon autoimmune disease in which the pituitary gland is infiltrated with lymphocytes, plasma cells and macrophages. It usually occurs during pregnancy or postpartum, presenting as headache, visual field impairment or hormonal deficiencies. We describe a patient with lymphocytic hypophysitis who posed a diagnostic challenge with the unusual presentation of aseptic meningitis accompanied by papilloedema and raised cerebrospinal fluid (CSF) pressure.

CLINICAL CASE

A 23-year old non-pregnant female from Myanmar was admitted with headache, vomiting, fever and neck stiffness. She had similar symptoms a year prior, with CSF analysis showing lymphocytic pleocytosis and sterile cultures. As her mother had pulmonary tuberculosis (TB), she was presumptively treated for TB meningitis. Symptoms resolved with a short course of Prednisolone and nine months of quadruple anti-TB medications.

The current illness was associated with papilloedema and CSF pressure of 31 cm H₂O. Multiple CSF analyses showed elevated white cell counts with lymphocytic predominance, but bacterial, viral and fungal cultures and serology; TB PCR; angiotensin converting enzyme (ACE) level, autoimmune screening and computed tomography (CT) scans from head to abdomen were all negative. Magnetic resonance imaging (MRI) of the pituitary showed a 14 mm intrasellar lesion, with an upward convexity in contact with the optic chiasm. There was central hypothyroidism (FT₄ 9.1 pmol/L, TSH 0.4 mIU/L) and central hypocortisolism (8 am cortisol 23 nmol/L, ACTH 3.6 pmol/L) with attenuated response to Synacthen testing. She had no clinical evidence of pituitary hormone deficiencies and had intact visual fields. Meningitic doses of Ceftriaxone were ceased with the negative culture results. Pituitary biopsy was deferred by the neurosurgeons because of "difficult anatomy". Rapid relief of symptoms and normalization of papilloedema, CSF pressure and white cell count were noted after starting Dexamethasone; repeat MRI showed normalization of pituitary size and only prominence of the pituitary stalk.

CLINICAL LESSON

Lymphocytic hypophysitis can present dramatically as aseptic meningitis, with papilloedema and raised intracranial pressure. While histological proof was not obtained in this patient, the negative tests for other differential diagnoses such as TB and lymphoma and the rapid resolution of symptoms and MRI pituitary changes with steroid treatment support its diagnosis.

(1) Caturegli P, Newschaffer C, Olivi A, et al. Autoimmune hypophysitis. *Endocrine Review*. 2005; 26(5): 599-614.

(2) Bellastella A, Bizzarro A, Coronella C, et al. Lymphocytic hypophysitis: a rare or underestimated disease? *European Journal of Endocrinology*. 2003; 149: 363-376.

Nothing to Disclose: YLMZ, SBC

P3-293

Lymphocytic Hypophysitis Presenting with Hyponatremia and Lateral Rectal Palsy in a Male Patient.

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A 53 year-old male with past medical history of ankylosing spondylitis and psoriasis presented to the ER with a chief complaint of double vision that started suddenly one day before his admission and two weeks of mild headache and fatigue.

His physical examination was consistent with right rectus muscle paresis. Vision fields were intact, and no other deficits were detected. Laboratory data showed severe hyponatremia (Na: 121), central hypothyroidism (TSH: 0.36, FT4: 0.4), and central adrenal insufficiency (ACTH: 7, R. Cortisole: 0.7).

Head MRI showed enlarged pituitary gland, so a dedicated pituitary gland MRI was done which showed again enlarged gland with mild thickening of the stalk and non visualization of the posterior pituitary bright spot. There was no enhancing mass in the sella. Those finding were consistent with a diagnosis of lymphocytic hypophysitis. Patient's hyponatremia responded to fluid restriction as well as thyroid and adrenal replacement therapy . Patient was discharged on 60 mg of prednisone daily His lateral ocular muscle palsy and double visioin improved significantly in 3 weeks.

Occurrence of lymphocytic hypophysitis is rare especially in males(1). It is initially characterized by lymphocytic infiltration and enlargement of the pituitary; this stage is followed by destruction of the pituitary cells. It occurs most often in late pregnancy or the postpartum period. The natural history typically involves progressive pituitary atrophy with replacement of pituitary tissue by fibrosis. However, at least partial spontaneous recovery of both anterior and posterior pituitary function can occur. High-dose pulse glucocorticoid therapy has been reported to reduce the mass effect in a small number of patients(2). Response to High dose steroids can avoid the more invasive diagnostic procedures, including surgical intervention and pituitary biopsies.

(1). Cosman, F, Post, KD, Holub, D, et al. Lymphocytic hypophysitis: report of 3 new cases and review of the literature. *Medicine* 1989; 68:240.

(2). Kristof, RA, Van Roost, D, Klingmuller, D, et al. Lymphocytic hypophysitis: non-invasive diagnosis and treatment by high dose methylprednisolone pulse therapy?. *J Neurol Neurosurg Psychiatry* 1999; 67:398.

Nothing to Disclose: HH, WA, SN, MHH

P3-294

Use of Recombinant Human Growth Hormone for Adult Onset Severe Growth Hormone Deficiency as a Consequence of Lymphocytic Hypophysitis Does Not Appear To Affect Progression of the Underlying Pituitary Lesion. Case Series.

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¹Royal Liverpool Univ Hosp Liverpool, UK.

BACKGROUND: Lymphocytic hypophysitis (LH) is a rare cause of pituitary hypofunction originally thought to be limited to women in the peripartum period but increasingly reported in men, children and women with no temporal association with pregnancy. It is an inflammatory process of presumed autoimmune aetiology resulting in a pituitary mass lesion and variable disruption of pituitary function depending on the site of the lesion. The lesion may be difficult to distinguish from an adenoma on MRI although there are characteristic findings of lymphocytic and plasma cell infiltration on biopsy. Severe adult onset growth hormone (GH) deficiency is estimated to occur in up to 54% of reported cases.(1,2) It is not known whether the use of recombinant human growth hormone (rhGH) affects progression of the underlying pituitary lesion in LH.

CLINICAL CASES: Case 1. A 49-year old man was found to have panhypopituitarism including severe GH deficiency (peak GH 3.0mU/L on glucagon testing, normal peak response >40mU/L, equivocal response 20-40mU/L) after presenting to his general practitioner with erectile dysfunction. He had a medical history of neurological signs consistent with intracranial mass effects which had spontaneously resolved. MRI of his pituitary revealed a 3.5mm pituitary stalk lesion which on excision was confirmed as LH. He was treated with rhGH for 34 months with no change in the pituitary lesion on MRI.

Case 2. A 44-year old woman with diabetes insipidus was found to have a 7x5x5mm inflammatory pituitary stalk lesion on MRI. She developed severe GH deficiency (peak GH 5.4mU/L on insulin tolerance testing) 12 months after initial presentation and was commenced on rhGH. Follow up pituitary MRI confirmed reduction in size of the pituitary lesion to a maximal diameter of 4mm following 19 months of treatment with rhGH.

Case 3. A 37-year old man initially presented with diabetes insipidus, hypogonadotropic hypogonadism and severe GH deficiency (peak GH 2.0mU/L on insulin tolerance testing) and was noted to have a 4mm pituitary stalk lesion on MRI. Progressive loss of pituitary function was noted 3 months after commencing rhGH and the pituitary lesion increased in size to 6.6x5.5x8mm. However, there was no further increase in size over the subsequent 22 months of follow up and therapy.

CONCLUSION: Use of rhGH for adult onset severe GH deficiency as a consequence of LH does not appear to have consistent effects on tumour mass or progression in this case series.

(1)Rivera JA., Pituitary 2006; 9:35-45

(2)Molitch ME, Gillam MP., Horm Res 2007; 68(5):145-150

Nothing to Disclose: LPO, RA, JPV

P3-295

Unusual Case of Panhypopituitarism Following Cerebral Aneurysm Embolization.

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Background: Suprasellar aneurysms and subarachnoid hemorrhages are known to cause hypothalamic-pituitary dysfunction. Here we report a case of profound panhypopituitarism following embolization of an anterior communicating artery aneurysm.

Clinical case: A 40-year-old man presented with bilateral hemianopsia and severe headache, and was diagnosed with a ruptured aneurysm. He was treated with angiographic embolization of the anterior communicating artery. One month later, he was referred to the Endocrinology clinic for an assessment of pituitary function. In addition to his visual problem, he complained of erectile dysfunction and mild fatigue. Results showed an undetectable morning serum cortisol, and a cosyntropin stimulation test demonstrated a peak cortisol level of 60 nmol/L (2.2 mcg/dL). Testing also showed central hypothyroidism (free thyroxine 7.6 pmol/L, normal 10-23), central hypogonadism (total testosterone <1.0 nmol/L, normal 10-30; LH <0.1 UI/L, normal 1.5-9.3) and mild hyperprolactinemia. Following adrenal replacement, he developed overt diabetes insipidus. MRI was not possible in this patient but reviewing the angiographic studies we identified the embolization coil entirely filling the sella turcica.

Conclusions: Hypopituitarism is a well recognized complication of cerebral aneurysm rupture. The pathogenesis is thought to be ischemic damage to the hypothalamus and/or pituitary. This case illustrates the rare occurrence of direct mechanical pituitary injury by an embolization coil. This emphasizes the need to assess pituitary function when a subarachnoid hemorrhage is diagnosed or an aneurysm is successfully treated.

Nothing to Disclose: CL, JL, JR, CB

P3-296

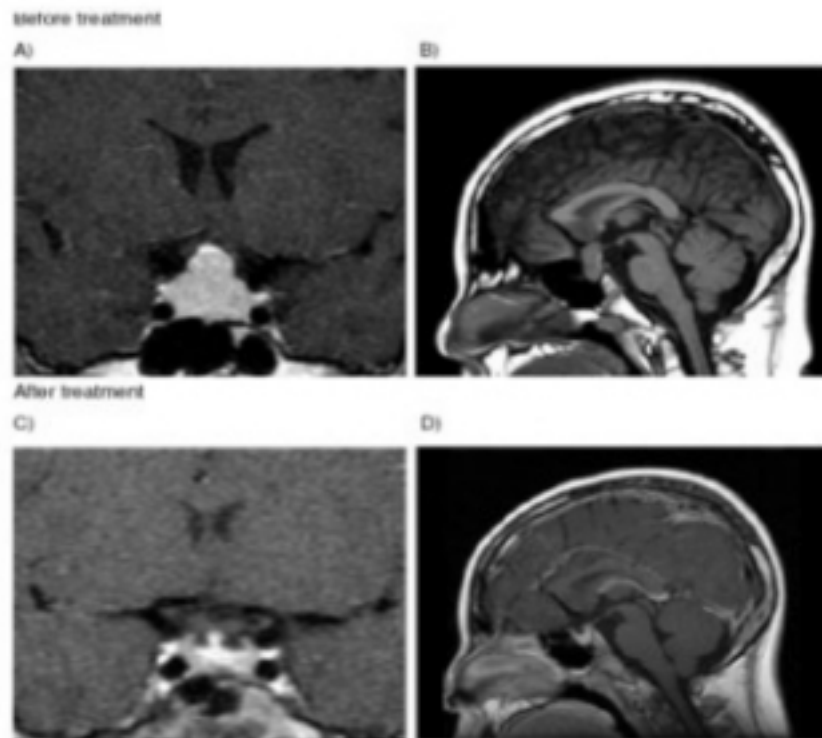
Reversible Pituitary Hyperplasia Due to Hashimoto's Thyroiditis Related Severe Hypothyroidism.

JK Park M.D.¹, EJ Lee M.D.¹ and KJ Kim M.D.¹.

¹Yonsei Univ Coll of Med Seoul, Korea.

A 15-year-old boy presented with headache and short stature. Sellar magnetic resonance imaging (MRI) revealed a pituitary mass with homogeneous pituitary tissue (fig. 1). Neurological examination showed intact reflexes and no visual field defect. He was prepubertal. His height was on the less than 3rd centile. Results of serial endocrinologic parameters show that severe primary autoimmune hypothyroidism with secondary pituitary hyperplasia and growth hormone deficiency. He was found to have a TSH of 50mIU/mL, fT4 of 0.05ng/dL GH < 0.01, and Microsome Ab > 3000U/mL. The patient started on oral thyroxine and growth hormone replacement. The patient became clinically and biochemically euthyroid and repeated MRI after 3 months of treatment showed dramatic reduction in the size of the pituitary gland.

Fig 1. Brain MRI before treatment and after treatment



Interval change of brain MRI at presentation (A,B) and after 3 months of thyroxine treatment (C,D)
(Enlarged pituitary glands with homogeneous pituitary tissue, mimicking a pituitary macroadenoma)

His height initially increased to the level of 10th centile, then continued to increase along the 50th centile. In addition, he had signs of early pubertal development.

Pituitary hyperplasia associated with untreated primary hypothyroidism is a rare condition. (1) There are only few reports on this condition in adolescent, especially accompanied with Hashimoto's thyroiditis. MRI have proven to have a low specificity in distinguishing pituitary hyperplasia from pituitary adenomas. (2) Therefore, the diagnosis of pituitary hyperplasia secondary to hypothyroidism relies on a thorough history, clinical course, and endocrinologic evaluation. (3) Generally, therapy with levothyroxine results in rapid improvement of the clinical signs and resolution of the pituitary hyperplasia on MRI. Thus, reexamination of thyroid functions in patients with sellar and suprasellar masses may prevent unnecessary operations in adolescent.

Nothing to Disclose: JKP, EJJ, KJK

P3-297

Pituitary Dysfunction in a Patient with Rosai-Dorfman Disease.

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Background: Rosai-Dorfman disease (Sinus histiocytosis with massive lymphadenopathy, SHML) is an uncommon histioproliferative disorder with unknown etiology, characterized by growing histiocytes which occurs most commonly in the lymph nodes but also occurs in extranodal sites like skin and soft tissue, nasal cavity, orbit, bone and central nervous system (1).

Clinical case: 25-year-old Caucasian Marine Corp male developed symptoms of partial left eye vision loss and dry ejaculate 3 months before his evaluation as he was serving his last month of 7 months in Iraq. He was then found to have multiple foci of abnormal signal intensity in the brain including the areas of the hypothalamus and the pituitary stalk which on biopsy confirmed the diagnosis of SHML. Additional lesions were identified and biopsied with confirmed histopathology of the same disease, including left cornea and bilateral flank skin lesions.

He was treated initially with pulse methylprednisolone therapy then maintained on prednisone dose of 20mg twice daily with minor improvement of his brain lesions on follow up MRI done later. His endocrine work up started later and showed hypogonadotropic hypogonadism (total testosterone 99ng/dl, free testosterone 10.4 pg/ml, FSH 4 mIU/ml, LH 3.7 mIU/ml), elevated prolactin (41.55 ng/ml, n<18.77) and growth hormone deficiency (IGF-1 68 ng/ml, n=126-382 ng/ml and GH 0.9 ng/ml, n≤10ng/ml). He had an appropriate morning cortisol, ACTH and Free T4 levels. The patient was started on testosterone replacement therapy with 5mg patch every 24 hours with significant improvement in his ejaculate volume and maintenance of erection.

Conclusion: Multiple pituitary dysfunctions can result from Rosai-Dorfman disease involvement in the central nervous system but since it's a rare disease, no investigational or therapeutic consensus was ever described or published.

(1) Gaitonde S. Multifocal, extranodal sinus histiocytosis with massive lymphadenopathy: an overview. Arch Pathol Lab Med 2007; 131: 1117-1121

Nothing to Disclose: TMA

P3-298

Granular Cell Tumor as a Differential Diagnosis of Suprasellar Mass: A Case Report.

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Introduction: Granular Cell Tumor (GCT) is rare, most affecting women in the 4th and 5th decades, and usually located in the tongue and proximal digestive tract¹⁻². GCT can sporadically be found in the central nervous system and more rarely affects sellar region, mainly neurohypophysis and/or pituitary stalk. Tumor grows slowly leading to mass effect symptoms. **Case Report:** a 30 yrs-old female with galactorrhea and secondary amenorrhea, no visual disturbances. Mild serum PRL increase (PRL 29 ng/mL, n 2-15) and low IGF-I were the sole lab abnormal result. MRI (Fig. 1- a,b) depicted a suprasellar mass with chiasmatic compression. Carotid angiograph revealed a lesion "blush". Craniotomy was indicated and pathological analysis depicted GCT (Fig 2). No mass reduction in post operative MRI (5mo) (Fig. 1-c,d) was observed.

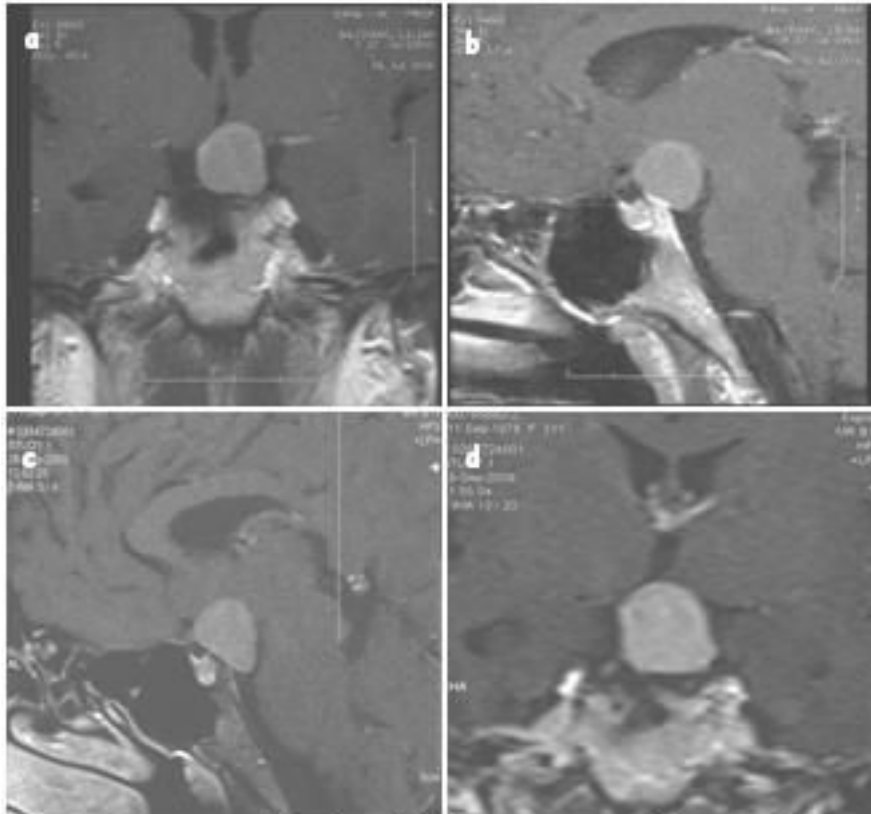


Fig 1: MRI T1 weighted gadolinium contrasted images in coronal (a) and sagittal (b) views showing a 1.7 x 1.6 x 1.5cm suprasellar mass. Lesion features were maintained after surgery (c, d)

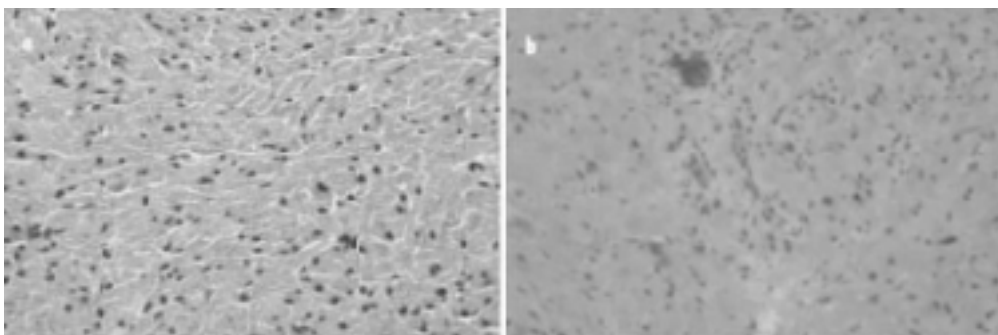


Fig 2: Histological aspects of GCT: HE (a) and IHC for CD68 (b), optic zoom 400x

Conclusion: GCT pathogenesis is still unknown and received other names as pituicytoma, infundibuloma and choristoma². MRI usually shows a suprasellar mass with gadolinium enhancement. Tumor size varies from 1.5-6cm. As there is no specific clinical or imaging characteristics, GCT diagnosis is usually post surgical. In 11 reported cases², the most frequent symptom was visual defect and hyperprolactinemia was present in two. MRI showed an isointense mass in T1 with homogeneous gadolinium contrast in 6 cases. Regarding pathology, GCT consists of densely packed polygonal cells with abundant granular eosinophilic cytoplasm. Neoplastic cells frequently express S100 and CD 68¹ in immunohistochemistry. Due to its firm consistence and rich vascularization, surgery is usually non curative². We reported a rare cause of suprasellar lesion, with clinical and image features comparable to the literature². Due to the tumor compressive effect, surgical approach was recommended and histopathological analysis was conclusive.

(1)- Brian H. Le et al. Granular cell Tumor. Arch Pathol Lab Med 2004;128:771-5

(2)-Aaron A. Cohen-Gadol et al. Granular Cell Tumor of the Sellar and Suprasellar Region: Clinicopathologic Study of 11 Cases and Literature Review. Mayo Clin Proc. 2003;78:567-73.

Nothing to Disclose: TPS, AG, DV, WC, SR, MDB

P3-299

Central Diabetes Insipidus as the First Manifestation of Myelodysplastic Syndrome.

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Background: Central diabetes insipidus (DI) has several etiologies, which include neurosurgery, trauma and infiltrative or neoplastic diseases. Amongst the latter, lymphomas and leukemias have been reported, but few cases of myelodysplastic syndrome have been identified.

Clinical case: An 81 year-old female with no known illnesses presented to the emergency room with a two-month history of increased thirst, polyuria and sudden abdominal pain. Investigations revealed a sodium of 156 mmol/L (normal 135-145 mmol/L) with an inappropriate urine osmolarity of 165 mmol/kg. The diagnosis of central DI was confirmed with a dehydration test and a good clinical and biochemical response to DDAVP. The classic «bright spot» of the posterior pituitary was absent on MRI, and the stalk was normal. Further evaluation showed central hypothyroidism, and all other anterior pituitary hormones were normal, including morning plasma cortisol at 556 nmol/L (20.2 ug/dL) and prolactin 8.3 mcg/L (normal 3-29). The patient was treated with DDAVP 0.025 mg PO q HS. Abdominal CT scan revealed a left renal vein thrombosis. She also presented with severe macrocytic anemia (hemoglobin 72 g/L) as well as leukopenia (white blood cells $2.7 \times 10^6/L$). Bone marrow biopsy confirmed the diagnosis of myelodysplastic syndrome with 10% blasts.

In the literature, most cases of central DI attributed to hematologic malignancies were caused by lymphomas and non-lymphocytic leukemias. There have been a few reports of myelodysplastic syndrome causing central DI, some of them after transformation into acute myeloid leukemia. The proposed mechanism is infiltration by neoplastic cells of the posterior pituitary and/or the pituitary stalk.

Conclusion: Myelodysplastic syndrome should be included in the differential diagnosis of central DI in patients presenting with hematologic abnormalities such as anemia.

Nothing to Disclose: JA, SV, HB, RC, CB

P3-300

Central Diabetes Insipidus: A Rare Presentation of Acute Myelogenous Leukemia Relapse.

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Introduction: The association of central diabetes insipidus (DI) with acute myelogenous leukemia (AML) is not well recognized. DI has been reported to occur at variable times in the course of AML, some cases associated with chromosomal anomalies. Our case highlights this important association in a patient with AML relapse who presented with only symptoms of DI.

Case: A 55-year-old female with Graves' disease treated with methimazole was diagnosed with AML (M4 morphology) 27 months ago with no chromosomal anomalies; Cerebrospinal fluid (CSF) analysis done at that time showed no leukemic CNS involvement. After systemic chemotherapy, the patient underwent successful autologous stem cell transplant (ASCT). She presented 22 months later with polyuria, polydipsia, and headaches for 10 days. Laboratory results showed: serum sodium 141 mmol/L (135-148 mmol/L); glucose 100 mg/dL; serum osmolality 296 mOsm/kg (280-295mOsm/kg); urine osmolality 131 mOsm/kg (500-800mOsm/kg); urine specific gravity 1.004. Hematologic findings: leukocyte count of 5.3 k/mcL (4.8-10.8 k/mcL) with 11% blasts. A 4-hour water deprivation test confirmed DI: serum sodium 151 mmol/L; serum osmolality 307 mOsm/kg; urine osmolality 108 mOsm/kg with 1.8 liters of urine output. Hormonal assays did not demonstrate any other pituitary dysfunction. MRI of the brain revealed normal pituitary gland size and homogenous enhancement; however a posterior pituitary bright spot on T1 images was not seen. CSF analysis performed did not show leukemic involvement. Administration of Desmopressin (DDAVP) resolved the patient's symptoms and normalized laboratory abnormalities: serum sodium 143 mmol/L; serum osmolality 293 mOsm/kg; urine osmolality 613 mOsm/kg with a decrease in urine output. Bone marrow biopsy showed AML relapse with no chromosomal anomalies. Salvage and consolidation chemotherapy was administered via systemic and intrathecal routes. Despite achieving AML remission, the patient continues to have persistent DI.

Conclusion: DI presenting as a symptom of AML relapse is rare. Our case highlights this rare association in a patient after successful ASCT with no chromosomal anomalies. Increased clinical awareness of this association in patients presenting with symptoms of DI is important to allow for the timely diagnosis and initiation of treatment.

Nothing to Disclose: SDH, AG, MCG

P3-301

Diabetes Insipidus in the Context of Langerhans Cells Histiocytosis - A Case Report.

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Introduction: Central diabetes insipidus is associated with a deficient secretion of anti-diuretic hormone. It is frequently idiopathic, although it can also result from a traumatic brain injury, surgery, ischemia, tumour, or an infiltrative disease like Langerhans cells histiocytosis. This entity has a prevalence of 1-2/1000000 and is characterized by an aberrant proliferation of these cells, being capable of affecting different tissues, mainly bone, skin, lung, liver, spleen, bone marrow, lymphatic nodes and the hypothalamic-hypophyseal region.

Case Report: A female patient 45 years old, with Langerhans cells histiocytosis, to whom had been prescribed corticosteroids was sent to the Endocrinology appointment of São João Hospital because of polyuria and polydipsia. The patient referred polyuria, polydipsia and nocturia since 8 months. She said that sometimes she ingested 10 water litres in one single day. She denied other relevant complaints, namely a history of brain injury, headaches, vomits or diminished visual acuity. The analytic study revealed plasma osmolarity=285mOsmol/Kg (N:282-300) and urinary osmolarity=78mOsmol/Kg (N:50-1200); urinary volume=12L; plasma electrolytes, renal function and thyroid function were normal. She didn't have other hormonal deficits. Hypophysis MRI: adenohypophysis of normal dimensions, infundibular thickening (about 5mm); absence of the normal hyperintense T1 signal over the neurohypophysis. Water restriction test confirmed the suspicion of diabetes insipidus: urinary osmolarity 0/1h/2h=111/106/108mOsmol/Kg and plasma osmolarity 0/1h/2h=285/293/298mOsmol/Kg. After the administration of 1µg of desmopressin there was a 260% rise in the urinary osmolarity at 60'. It was initiated intranasal DDAVP solution 5+0+5µg.

Conclusion: In patients that already have the Langerhans cells histiocytosis diagnosis it is important to look for the appearance of new symptoms, namely those related to hormonal disturbances.

Nothing to Disclose: JSM, ASV, AMM, DCB, FGG, DMC, JLM

P3-302

Diabetes Insipidus and Hypopituitarism Secondary to Hypothalamic Metastatic Lesions from Large Cell Lung Carcinoma.

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Background: Metastatic lesions to the hypothalamus and pituitary gland account for 1 to 2 % of sellar masses, and are commonly associated with lung cancer in men. While there are reported cases of pituitary metastasis from lung cancer, there are only few cases in the literature describing hypothalamic metastasis, and none from large cell lung carcinoma (1,2). We present a case of diabetes insipidus (DI) and hypopituitarism due to hypothalamic metastasis from large cell lung carcinoma.

Clinical Case: A 54 year old man with no significant past medical history presented with a history of polyuria and polydypsia of 2 weeks duration. He was found confused, nauseous and vomiting following a presumed seizure. Physical exam was normal except for mild confusion. An MRI of the brain revealed several brain lesions including one in the hypothalamic area along the pituitary recess of the third ventricle measuring 15 x 12 x 14 mm, pituitary was reported to be normal. The patient was started on dexamethasone IV. On evaluation, his urine output was 6 L/24 hr(1-2L/24hrs), serum sodium was 141mEq/L(135-145mEq), and urine sodium was 16mEq/L(10-40mEq/L). A plasma osmolality of 300 mOsm/kg (276-295mosm/kg) and urine osmolality of 177mosm/L(80-1300mosm/L) suggested the diagnosis of DI. Diagnosis was confirmed with an increase in urine osmolality to 423 and a decrease in urine output to 950 ml/24 hours after 1 mcg of desmopressin subQ. Further work up revealed low serum levels of the following; TSH 0.11 mcUnit/ml(0.5-5mcunit/ml), FreeT4 0.5 ng/dl(0.5-2.1ng/dl), testosterone 12.6 ng/dl(300-1300ng/dl), FSH 0.5 mcUnit/ml(0-20mcUnit/ml), and LH < 0.1 mcunit/ml(0-25mcUnit/ml). Prolactin was normal at 8.2 ng/ml(0-15ng/ml). Adrenal axis could not be assessed as the patient was on dexamethasone. Further work up with a CT chest revealed a 2cm left upper lobe lung mass. CT guided FNA showed large cell carcinoma. The patient was started on levothyroxine replacement and underwent one session of palliative whole brain radiation. However, he went downhill soon thereafter due to septic shock and had a cardiac arrest. **Conclusion:** Although metastatic lesions involving the hypothalamus are rare, patients with non small cell lung primary cancers may present with DI and panhypopituitarism from hypothalamic metastasis as shown in this case. These patients should be evaluated for hypothalamic and pituitary hormonal dysfunction, as well as other derangements in temperature and thirst regulation.

1. Guerra A.M, Garrido L.P, Pachón V, Martínez MJ, González M, A, Vaz S MA. Endocrinol Nutr. 2009 Apr;56(4):201-4. Epub 2009 Jun 11. Secondary hypopituitarism due to hypothalamic metastasis from small cell lung cancer.
2. Taniguchi H, Inomata M, Abo H, Miyazawa H, Noto H, Izumi S, Ann Endocrinol . 2006 Jun;67(3):259-64. Hypopituitarism caused by hypothalamic metastasis.

Nothing to Disclose: MJAK, JGB, TY, FH

P3-303

Natural History of Meningitis-Induced Syndrome of Inappropriate Anti-Diuretic Hormone.

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Introduction: The time course of the evolution and resolution of meningitis-induced SIADH is poorly understood. We present a 42-year-old man with SIADH induced by meningitis following excision of a subarachnoid cyst, whose clinical course allowed precise documentation of onset and resolution of SIADH.

Case presentation: A 42-year-old man presents with fever and confusion which starting 25 days after cranial surgery for an arachnoid cyst. On examination, the temperature was 101.7 degrees F, and he was tachycardic with a blood pressure of 133/83 mmHg. He was disoriented with nuchal rigidity. A diagnosis of meningitis was confirmed by lumbar puncture (LP). The CSF showed: glucose 16 mg/dL, protein 150 mg/dL, WBC 3410.

Treatment with ceftriaxone and vancomycin were initiated. On (hospital day {HD} 1) the serum sodium (SS) was 136 mmol/L. This decreased to 127 mmol/L on HD-8 and the endocrine service was consulted. Further evaluation showed urine osmolality 585 mOsm/kg, serum osmolality 270 mOsm/kg, and urine sodium 186 mmol/L. He was clinically euthyroid, euadrenal and free from peripheral edema.

Diagnosed with SIADH, his fluids were restricted to 1000cc/day. Antibiotics were continued. On HD-10 the SS remained 127 mmol/L, prompting further fluid restriction to 600cc/day. On HD-13 with SS decreased to 124 mmol/L, demeclocycline (300mg orally every 6 hours) was added to fluid restriction. The SS remained between 124 - 127 mmol/L. Clinically, the meningitis continued and serial LPs showed persistent pleocytosis.

The patient's condition improved on HD-22 and LP showed WBC of 16. Coinciding with resolution of meningitis on HD-22 was return of SS to 136 mmol/L. Demeclocycline was weaned off over 4 days while maintaining fluid. Fluid was liberalized gradually over the following 4 days. With normal SS, SIADH was adjudged to have resolved.

Conclusion: Based on our experience with this patient, the onset of meningitis induced SIADH was approximately eight days from diagnosis of meningitis. It appeared to be refractory to fluid restriction and relatively refractory to demeclocycline during persistence of the active infection. Clinical and cytologic resolution of meningitis was accompanied by prompt resolution of SIADH and normalization of SS, which occurred approximately fourteen days from diagnosis of SIADH. This case emphasizes the need to achieve complete resolution of the underlying meningitis before meningitis associated SIADH can be resolved.

Nothing to Disclose: DEW, SED-J

P3-304

Two Cases of Pituitary Abscess.

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Background: Pituitary masses have a wide differential diagnosis. When the patient presents with diabetes insipidus (DI), one should consider the diagnosis of pituitary abscess. Here we present two patients with this pathology.

Clinical Case (1): A 52 year-old woman complained of excessive thirst (intake of up to 9 liters per day) and polyuria. Her endocrinological investigation revealed a decreased FSH at 0.3 UI/L (normal in menopause >23), hyperprolactinemia (93 mcg/L - normal: 3-29), and normal free T4 and cortisol. A 3-hour dehydration test resulted in hypernatremia at 148 mmol/L (normal: 135-145 mmol/L) with a urinary osmolality of 100 mmol/kg. The MRI showed a 2 cm pituitary lesion, with a thickened stalk. She underwent partial transsphenoidal resection of her lesion and pathology confirmed a pituitary adenoma expressing prolactin and GH. Postoperatively she developed adrenal insufficiency and central hypothyroidism. Three weeks later she developed bitemporal hemianopsia and imaging revealed a suprasellar lesion compatible with hypophysitis. She was treated with up to 40 mg of Prednisone daily, with intermittent improvements in her visual fields. The lesion progressed and therefore she underwent a second surgery. Pathological and microbiological analyses showed infection with *staphylococcus aureus*. The patient was treated with meropenem and cloxacillin, and improved thereafter.

Clinical case (2): A 71-year-old woman was admitted to the ICU for severe community acquired pneumonia. She was diagnosed with central adrenal insufficiency and was treated with intravenous hydrocortisone. During her hospital stay she developed polyuria (5 liters per day), with a serum sodium of 161 mmol/L and an inappropriate urinary osmolality of 161 mmol/kg. She responded well both clinically and biochemically to intranasal DDAVP. A 1.5 cm pituitary lesion with suprasellar extension was found on MRI. At transsphenoidal surgery, a yellow opaque material was drained, quite similar to pus. Unfortunately, insufficient material was sent to pathology, which only revealed the presence of a moderate quantity of polymorphonuclear cells, and cultures remained sterile. Post-operatively, the adrenal insufficiency resolved, but her DI persisted.

Conclusion: The diagnosis of pituitary abscess must be considered when a pituitary mass is found in a patient presenting with DI.

Nothing to Disclose: JA, JL, RM, CB

P3-305

Cranial Nerve Palsy as a Clue to Pituitary Metastases Diagnoses.

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Pituitary metastases (PM) are an unusual complication of systemic cancer occurring in 1 to 3.6% of patients with malignance(1). In autopsy series PM occurred in 0.14 to 28.1% of all brain metastases. They affect, mainly, elderly patients with no clear sex predominance(2). Based on previous autopsies studies, the majority of PM is clinically silent: symptoms are reported in only 2.5-18.2% of cases (2). In 85% of cases the PM are located in the posterior lobe (in 34% combined with the anterior lobe). This distribution justifies the usual symptoms of diabetes insipidus (DI) identified in 70 to 100% of patients (2,3). Symptomatic anterior pituitary failure and cranial nerve deficits, like ophthalmoplegia, have been less frequently described(1-3). Five unusual cases of PM [four women (27, 63, 66 and 80 years old) and one man (63 years-old)] are here reported. They were admitted to a General Hospital, Functional Neurosurgery Division, from 2004 to 2009 due to recent ophthalmoplegia. Malignant disease was not previously diagnosed in all, except one with skin cancer not related to the pituitary metastases. No patients complained about DI. Anterior hypopituitarism, in some degree, was confirmed in all cases. The metastases were secondary to pancreatic carcinoma, lymphoma, lung adenocarcinoma, small cell adenocarcinoma and cystic adenoid carcinoma. Discussion: Although MP occurred usually in elderly patients with generalized metastatic disease, they may be the first manifestation of an occult primary tumor and may occur in early adulthood, even without diabetes insipidus. One must be aware of this possibility when cranial nerve palsy is present.

(1)Fasset DR et al. Neurosurg Focus [serial online] 2004; 16: 1

(2)Komninos J et al. J Clin Endocrinol Metab 2004; 89:574

(3)Goglia U et al. Pituitary. 2008; 11:93

Nothing to Disclose: VASC, LAB, DRD, GOS, MBCC-N, NRdCM

P3-306

Late Recurrence of Craniopharyngioma - Case Report.

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Background: Craniopharyngiomas are uncommon tumors, accounting for approximately 2% to 3% of primary intracranial neoplasms. Craniopharyngiomas arise from remnants of the embryonic craniopharyngeal duct and, although benign in nature, can contribute to significant morbidity. When located near structures such as the optic chiasm, pituitary and hypothalamus, these tumors can potentially cause visual, neurological and endocrine deficits. Craniopharyngiomas tend to recur, often at the primary site and contiguous areas.

Clinical case: We report a 59-year-old female patient admitted to the hospital in 1979 with a history of primary amenorrhea and decreased visual acuity predominantly in her left eye. Neurological examination revealed bitemporal hemianopsia, anisocoria (left > right) and bilateral optic atrophy. Neuroimaging by CT scan showed a space-occupying suprasellar lesion, with pituitary stalk involvement, that was producing optic chiasm compression. She was submitted to partial surgical resection of the lesion and histologic examination established the diagnosis of a craniopharyngioma. Postoperatively she developed left abducent nerve palsy and panhypopituitarism and is being followed by neurosurgery and endocrinology. In January 2008 MR imaging follow-up revealed growth of the lesion. Neurosurgical reintervention was performed and histologic examination confirmed recurrence of the craniopharyngioma. She is on treatment with levothyroxine and hydrocortisone and maintains regular clinical, laboratory and neuroimaging surveillance.

Conclusion: Craniopharyngiomas can present with a variety of symptoms, such as headaches, decreased visual acuity, visual field defects and/or hormonal abnormalities. Endocrine manifestations are usually the initial symptoms both in children and adults. To facilitate early diagnosis and treatment, all endocrinologists should raise the possibility of a craniopharyngioma when evaluating a patient with one or more endocrine deficits. Treated patients are at risk for recurrence and need to be followed up on a long-term basis.

Nothing to Disclose: PR, JLC, JLM

P3-307

Pituitary Apoplexy during Pregnancy.

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Introduction

Pituitary apoplexy is caused by hemorrhage and/or infarction in the pituitary gland. It usually presents with headaches, nausea/vomiting, visual disturbance and altered mentation. It occurs in up to 10% of all pituitary adenomas. Apoplexy during pregnancy is rare and there are only few reports in the literature. It is attributed to temporary enlargement of pituitary adenoma occurring during pregnancy which compromises blood supply.

Clinical Case

A 19 year old primigravida in her 27th week of gestation was admitted for 1 week history of headache, nausea/vomiting and blurry vision in right eye for 3 days. She denied any similar complaints in the past. Menarche was at age 13. She denied irregular menstrual periods, hirsutism and galactorrhea before pregnancy.

On physical exam vital signs were stable. Neurological exam showed left superior quadrantanopia in right eye. No other cranial nerve deficit was noticed. Formal visual field testing showed left superior quadrantanopia and right inferior quadrantanopia.

MRI brain showed cystic tumor of pituitary gland with hemorrhage. Laboratory results were as follows: TSH 1.58 (nl 0.34-0.56), FT4 10.7mcg/dl (nl 6-12.2), prolactin 56.9 9ng/ml (nl 3.3-26.7), somatomedin C 112ng/ml (nl 128-488), cortisol 19mcg/dl (nl 6.7-22.6).

Neurosurgery was consulted and recommended no surgical intervention since visual deficit was stable and patient did not have any altered mentation. Patient was kept under observation and started on dexamethasone. Repeat formal visual testing in 4 days showed no changes in visual deficits. Patient was subsequently discharged with instruction to return immediately if there is worsening of headache, visual deficits or any mental status changes.

Patient was seen in outpatient clinic, she reported improvement in her symptoms including visual deficit, headache and vomiting.

Conclusion

Pituitary apoplexy is rare endocrine emergency with significant morbidity and mortality. It is known to be associated with pituitary adenoma, radiation, pregnancy, oral contraceptives, head trauma and GnRH analogue therapy. Treatment consists of replacement of deficient hormones and transsphenoidal surgery. However, some advocate conservative management in cases with no altered consciousness and stable visual field deficit. Our patient, since she was pregnant and her symptoms were stable we took conservative approach. In these patients close monitoring, in respect to visual fields and hormonal status, is required.

Nothing to Disclose: RSS, FMR

P3-308

Pituitary Apoplexy: From Symptoms to Pathologic Confirmation.

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Introduction: Typically, the diagnosis of pituitary apoplexy is made on the basis of clinical suspicion on a background of pituitary tumor/empty sella. We here present a 72-year-old man in whom a clinical diagnosis of pituitary apoplexy was confirmed in real time by pathological evidence of hemorrhagic necrosis in the resected tumor.

Clinical Case: 72-year-old male with history of hypertension presents with severe headache described as "the worst headache of his life". He denied photophobia, neck stiffness, blurred vision, weakness, or change in mentation. However, he admitted to cold intolerance and weight gain over the preceding three months. Decreased libido resulted in no sexual activity for two years.

On physical examination, the patient had stable vitals and appeared uncomfortable. He exhibited mild bitemporal field deficits with a normal fundoscopic examination. He had cushingoid features including buffalo hump, moon faces, and truncal obesity, with small extremities.

Laboratory evaluation revealed a Triiodothyronine level of 0.7 ng/mL, free T4 0.51 ng/dL, thyroid-stimulating hormone 0.72 ng/dL, luteinizing hormone 1.2 mIU/mL, follicle-stimulating hormone 2.1 mIU/mL, prolactin 2.0 ng/mL, serum cortisol 64, and ACTH 1210 pg/mL.

A computerized tomography of head showed a large sellar/suprasellar mass. Magnetic Resonance Imaging showed 3.3cm x 2.7cm pituitary mass with suggested hemorrhage. A clinical diagnosis of pituitary apoplexy was made and treatment with high-dose hydrocortisone was started.

Four days following stabilization, the patient underwent an uneventful resection of the pituitary tumor. Histological examination revealed an ACTH staining pituitary adenoma with evidence of infarction. Follow-up labs indicated a decrease of ACTH from 1210 pg/mL to 34 pg/mL.

Conclusion: There are many postulated theories as to the exact cause of pituitary apoplexy (1,2). First, imbalance of regional pressures within the wall of a rapidly increasing tumor volume induces bleeding in the tumor. Second, the rate of increase in tumor size outstrips the ability of the blood supply to the pituitary, leading to ischemia. Third, the anatomy of the hypophyseal portal system and stalk is such that a tumor can compress the stalk and provoke ischemia. Unlike many clinical cases of suspected pituitary apoplexy, our case illustrates one in which clinical suspicion was proven in real time by pathology of hemorrhagic necrosis in the resected tumor.

(1) Nawa R et al., J Intens Care Med 2008; 23:75-90

(2) Murad-Kejbou S et al., Curr Opin Ophthalmol. 2009 Nov;20(6):456-61

Nothing to Disclose: DEW, BJW-C, AEK, SED-J

P3-309**Successful Treatment of Intracranial Germinoma Coexisted with Sjögren Syndrome by Radiotherapy: Case Report and Review of the Literature.**

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Background: Sjögren's syndrome (SS) is a chronic inflammatory systemic autoimmune disease. The association with malignancies in central nervous system in SS is seldom documented. We reported the successful treatment of intracranial germinoma coexisted with SS by radiotherapy. **Clinical case:** A 16-year-old male was referred to the rheumatic outpatient with dryness of eyes and mouth. Together with the positive results of the laboratory investigations the patient was diagnosed as SS, and treated with methylprednisolone for three months without improvement. He further complained of a severe polyuria, headache, visual disturbance. The endocrinological evaluation revealed that diabetes insipidus centralis, hyperprolactinemia, and complete anterior pituitary insufficiency (TSH:0.008 mIU/L, FT₃:2.93 pmol/l, FT₄:12.16 pmol/l; FSH<0.10 mIU/ml, LH<0.10 mIU/ml, T<0.69 nmol/L; ACTH<1.11 pmol/l, COR<27.6 nmol/L. TSH:0.27<n<4.2 mIU/L; FT₃:3.1<n<6.8 pmol/L; FT₄:12<n<22 pmol/l; FSH:1.27<n<12.96 mIU/ml; LH:1.24<n<8.62 mIU/ml; T: 9.08<n<55.23 nmol/L; ACTH<10.13 pmol/L; COR:138<n<690 nmol/L). A MRI demonstrated diffuse thickening of the pituitary stalk. Two months later, the MRI showed mass lesions in the suprasellar and pineal regions, and the obstructive hydrocephalus. No biopsy was able to be performed when the willings of patient's parents, not to accept a potential higher risk of brain hernia. Neither tumor cells, nor α -AFP, nor HCG, nor CEA were detected in the cerebrospinal fluid and in the serum. The patient received a diagnostic RT. The dose to the gross tumor volume was 20Gy. CT scan showed a significantly tumor shrink in brain. Then we modified the clinical target volume as craniospinal and 5-field conformal technique was used. The dose to craniospinal was 25.6Gy RT. The MRI scan one month after radiotherapy showed the tumor and the obstructive hydrocephalus disappeared. Complete improvement of the clinical symptoms of SS and diabetes insipidus centralis were observed. No recurrence occurred during follow-up for one year. **Conclusion:** The typical clinical manifestation, multiple midline tumours and the level of tumour markers could be considered compatible with the clinical diagnosis of intracranial germinoma without biopsy. The appearance of the intracranial germinoma in the setting of SS is unusual. Recognition of this uncommon complication of SS is very important because early RT can result in individualized treatment of both intracranial germinoma and SS.

- (1) Louis DN, et al (eds): WHO Classification of Tumours of the Central Nervous System, ed 4. Lyon: IARC Press, 2007
- (2) Echevarria ME, et al., The Oncologist 2008;13:690:699
- (3) Mavragani CP, et al., Nature clinical practice rheumatology. 2006;2:252:261
- (4) Kassan SS, et al., Arch Intern Med. 2004;164:1275-1284.
- (5) Ogawa K, et al., Int.J.Radiation Oncology Biol.Phys.2004; 58:705-713

Nothing to Disclose: SS, YB, DZ, HY, JW, GT, WC, LL, HW, HZ, JJ

P3-310

Unmasking of Undiagnosed Pre-Existing Central Diabetes Insipidus after Renal Transplantation.

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Introduction: Acquired central diabetes insipidus (DI) often occurs abruptly after a cranial event causing hypothalamic and pituitary insult. We present a case of a pre-existing and clinically unapparent central DI which was unmasked after renal transplantation.

Clinical Case: A 60 year old woman with end-stage renal failure due to autosomal dominant polycystic kidney disease (ADPKD) underwent renal transplantation. Recovery was unremarkable.

After 1 week she noted polydipsia and polyuria. During a ward admission she was markedly polyuric and polydipsic with fluid intake of 10 L/d. Fluid restriction of 6 L/d resulted in marked patient distress and intense thirst. Serum sodium (Na⁺) on admission was normal. Serum osmolality (Osm) the morning after a relative fluid restriction (6 L/d) was 295 mmol/kg (280-295), with urinary Osm of 136 mmol/kg. Serum glucose and calcium were normal. The renal team diagnosed probable psychogenic polydipsia.

She was referred for endocrine review. She was noted to have had an intracerebral aneurysm rupture with subarachnoid haemorrhage 31 years ago requiring craniotomy with partial R frontoparietal lobectomy and aneurysmal clipping. No polyuria, polydipsia or pituitary deficiency was noted after the surgery. On examination she was mildly dehydrated. She had divergent strabismus and L homonymous hemianopia, present since neurosurgery.

A fluid deprivation test was started after 1.5 hours of fast. Her weight was 72.3 Kg, serum Osm 297 mmol/Kg, Na⁺ 143 mmol/L, and urinary Osm 93 mmol/Kg. 2 hours later (3.5h fast), serum Osm was 305 mmol/kg, urine Osm 100 mmol/kg, serum Na⁺ 145 mmol/L, and weight 71.2kg. UO during first and second hour of test were 350 and 400 mls respectively. Desmopressin was given, and at 2 hours post, urine Osm rose to 374 mmol/kg and UO reduced to 60 ml/h. She was diagnosed with central DI and was treated with intranasal desmopressin. UO improved to 1.5 L/d with normal serum Na⁺ and Osm. Her thirst resolved. An MRI of the brain showed a midline defect in the hypothalamus presumably resulting from her previous neurosurgery.

Conclusion: This case demonstrates a rare instance of pre-existing but clinically unapparent central DI unmasked after renal transplantation. It is likely that renal damage from her ADPKD disguised her vasopressin deficiency prior to transplantation. There is a single previous report of unmasking central DI after renal transplantation (1).

(1) Henne T et al., *Pediatr Nephrol* 2001;16:315-7

Nothing to Disclose: DDK, IMH

P3-311

A Possible Temporal Relationship between Tacrolimus and Cranial Diabetes Insipidus: A Case Series.

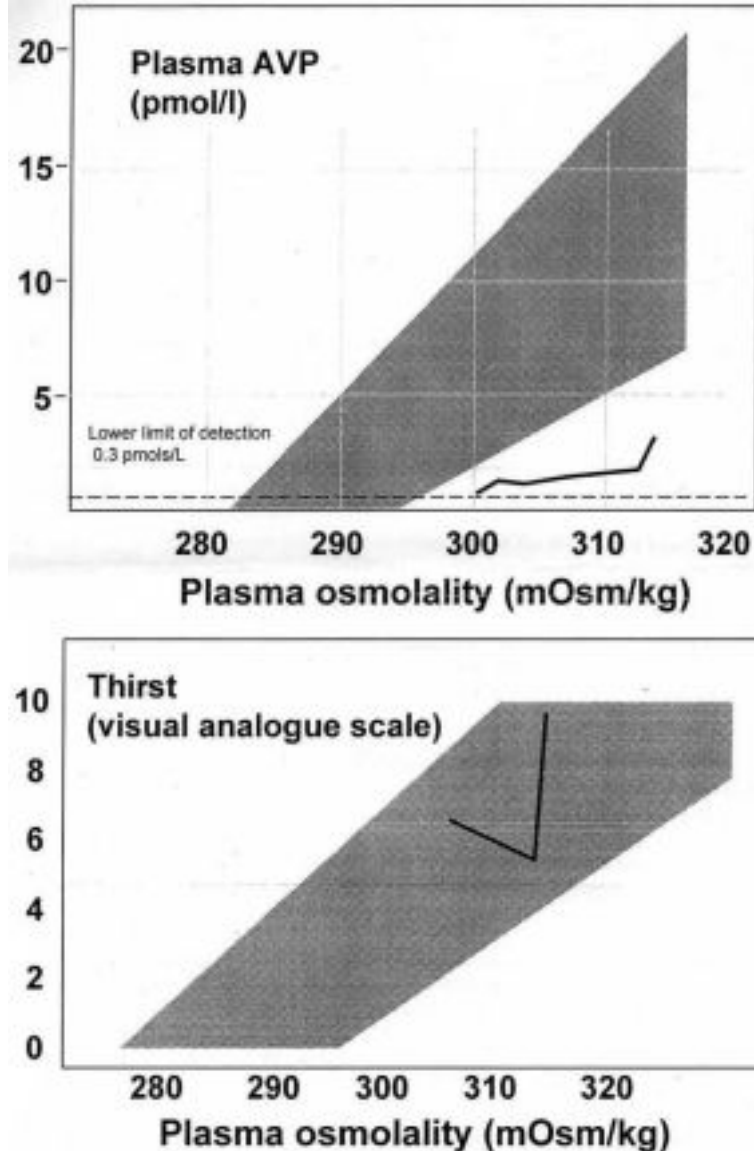
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Background

Cranial Diabetes insipidus (CDI) is a rare disorder secondary to deficient arginine vasopressin (AVP) secretion. Tacrolimus is a macrolide immunosuppressant and inhibits interleukin 2 (IL-2) transcription.

Case 1. A 41-year old woman with a familial medullary cystic kidney related end stage renal failure (ESRF) was started on tacrolimus after cadaveric renal transplant. She developed polyuria and polydipsia the next day. Her plasma osmolality was 265 mosmol/L and urine osmolality was 194 mosmol/L. 5% saline loading test showed suppressed AVP response but intact thirst response indicating CDI.



Her baseline pituitary function tests and magnetic resonance imaging (MRI) pituitary were normal.

Case 2. A 42 year old male who was commenced on tacrolimus after live related renal transplant for focal segmental glomerulosclerosis related ESRF developed polyuria and polydipsia. His plasma osmolality was 248 mosmol/L, urine osmolality was 168 mosmol/L & urine specific gravity was 1.005. The water deprivation test confirmed CDI. His baseline pituitary function tests and MRI pituitary were normal. Investigations to exclude secondary causes of CDI, which included chest X ray (CXR), serum angiotensin converting enzyme (ACE) activity, autoantibodies and chorioembryonic antigen (CEA) were normal.

| Time (hours) | Plasma osmolality (mosmol/L) | Urine osmolality (mosmol/L) |
|--------------|------------------------------|-----------------------------|
| 0 | 297 | 442 |
| 2 | 296 | 464 |
| 4 | 294 | 446 |

| | | |
|------------|-----|-----|
| 6 | 289 | 457 |
| Post DDAVP | 292 | 714 |

Case 3. A 29-year old male who was started on tacrolimus after live related renal transplant developed polyuria and polydipsia. Urine output was in excess of 7 litres per day. Plasma osmolality was 297, urine osmolality was 381 mosmol/L, urine specific gravity was 1.003 indicating CDI. He was started on desmopressin, which gave dramatic relief of polyuria and nocturia. MRI pituitary was normal as were CXR, ACE activity and CEA concentration.

Conclusion

We present 3 patients who developed CDI soon after commencing tacrolimus. Although establishing a temporal correlation between the two events does not establish a causal relationship, we tentatively conclude, mainly due to lack of secondary causes, that the development of CDI may be related to tacrolimus.

Nothing to Disclose: AM, YMK, TSP

P3-312

Valvular Abnormalities Associated with Cabergoline Use and Cumulative Exposure in Persons with Hyperprolactinemia.

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Background. Cardiac valvular abnormalities are associated with high-dose dopamine agonist therapy (pergolide, cabergoline) for Parkinson's disease. Whether lower dose cabergoline therapy used for hyperprolactinemia increases risk of valvular dysfunction has not been well characterized.

Methods. The CATCH Study examined the prevalence of valvular abnormalities in a community-based sample of adults with hyperprolactinemia and no prior cardiac disease treated with cabergoline (CAB) or bromocriptine (BCR). Patients receiving dopamine agonists ≥ 12 mos for hyperprolactinemia were selected from Kaiser Permanente Northern California, a large integrated health system. We used health plan databases and a survey to obtain data on dopamine agonist exposure and patient characteristics. Transthoracic echocardiography was used to examine valve morphology and function using standard criteria.

Results. In 174 participants, 62 received CAB only, 63 received BCR only, and 49 received both. Median CAB use was 2.8-3.2 yrs; median BCR use was 5.5 yrs in BCR only users and 1.1 yrs for those exposed to CAB+BCR. In CAB users, median cumulative exposure was 115 mg. Mean age was 49 \pm 13 yrs; 63% were women; 43% white, 19% black, 15% Hispanic, 18% Asian; 70% were overweight or obese; and 36% current or former smokers. We found prevalence of valvular thickening and regurgitation varied by exposure status (Table). Compared with BCR only exposure (1.6%), regurgitation of 2 valves was more common for CAB (11.3%, P<0.05) and CAB+BCR (12.6%, P<0.05) use. After age-sex adjustment, this association persisted for CAB only (OR 3.3, 95% CI: 1.4-7.7) but was not statistically significant for CAB+BCR (OR 1.7, 0.8-3.8). Cumulative CAB exposure >115 mg was associated with much higher age-sex adjusted odds of valvular regurgitation (OR 9.6, 1.1-81.2).

Conclusions. In a diverse, contemporary community-based sample of adults treated for hyperprolactinemia, CAB use and greater cumulative exposure of CAB were associated with a higher prevalence of valvular regurgitation but not valvular thickening.

Dopamine Agonist Therapy and Valvular Abnormalities

| | Bromocriptine (BCR)* n=63 | Cabergoline (CAB) n=62 | CAB+BCR n=49 |
|----------------------------|------------------------------|---------------------------|-----------------|
| Thickening, % | | | |
| Aortic | 11.1 | 16.1 | 4.1 |
| Mitral | 7.9 | 4.8 | 18.4 |
| Pulmonic | 0 | 0 | 2.0 |
| Tricuspid | 0 | 0 | 0 |
| Regurgitation ≥ 2 , % | | | |
| Aortic | 0 | 6.5, P=0.058 | 4.1 |
| Mitral | 1.6 | 3.2 | 8.2 |
| Pulmonic | 4.8 | 24.2, P<0.01 | 16.3, P=0.056 |
| Tricuspid | 12.7 | 17.7 | 14.3 |

*Referent group

Sources of Research Support: Kaiser Permanente Northern California Community Benefit Fund.

Nothing to Disclose: TCT, AB, GH, JY, JGZ, MC, ASG, JCL

P3-313

Valvular Heart Disease with Long-Term Cabergoline Therapy for Prolactinomas: A Follow-Up Study.

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Background

We ¹and others have shown in case-control studies that cabergoline used in patients with hyperprolactinemia does not appear to be associated with a risk of developing clinically significant cardiac valve dysfunction. However, there are no long term echocardiographic studies of such patients.

Objective

To assess the change (if any) of cardiac valve function in patients with prolactinomas treated with long term cabergoline and reevaluated longitudinally two years after a first echocardiogram.

Methods

We recruited 27 patients with prolactinomas treated with cabergoline from subjects attending the outpatient endocrine clinic of our institution. All patients had a prior echocardiographic evaluation in 2007 and were reassessed by a second transthoracic echocardiogram performed by the same cardiologist as in the first study. The results were interpreted blindly by a second echocardiographer. Valvular regurgitation was graded according to American Society of Echocardiography recommendations as mild, moderate or severe. Differences between proportions of patients with echocardiographic findings were analyzed using the chi-square test.

Results

Patients (66% females, mean age 44 yr, SD 13 yr) were treated with cabergoline for a mean duration of 7.2 yr (SD 1.3 yr). Mean cumulative dose at follow-up was 301 mg (SD 230 mg). Mean interval between the two echocardiograms was 25 months (SD 4.7 months). No patient had severe valve regurgitation at any time. Moderate regurgitation of any valve was present in 4 patients at initial evaluation and in only two at follow-up assessment. Prevalence of mild regurgitation of mitral, aortic and tricuspid valves were not significantly different between initial echocardiogram (25%, 18%, 39%) and follow-up (15%, 11%, 26%).

Conclusion

Our study shows that there was no increase in prevalence nor progression of any valve regurgitation in patients with prolactinomas treated with cabergoline over a two year follow-up with echocardiographic assessment.

(1) Vallette, S et al. Long-term cabergoline therapy is not associated with valvular heart disease in patients with prolactinoma. *Pituitary*. 2009;12;153-157

Nothing to Disclose: PD, PS, KS, NA-J, HB, CB, SV, OS

P3-314

Efficacy of Primary Treatment with Dopamine Agonists on Decreased Visual Acuity in Invasive Macroprolactinomas with Suprasellar Invasion: Results, Delay and Predictive Factors of Response.

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Surgery is the first-line treatment of macroadenomas with decrease of visual acuity (VA) except for prolactinomas since tumor shrinkage is rapidly observed in patients treated with dopamine agonists (DA). To evaluate the efficacy of primary DA treatment on the decrease of VA in macroprolactinomas, we studied 25 patients (22 men, PRL: 6224 ± 7666 ng/ml), with decrease of VA, treated with quinagolide (n = 21), bromocriptine (n = 3) or cabergoline. VA was $\leq 5/10$ in 72 % of them and decrease of VA was bilateral in 52 %. Neuro-ophthalmological examinations were performed 3-6 days, 10-15 days, 1, 3, 6 and 12 months after the initiation of DA and MRI after 1 month of treatment. VA recovered in 84% of the patients. Improvement of visual field defects was rapidly observed in all of them. Normalization of the VA was obtained in 68% of the patients and improvement in 16 %. Normalization of VA was observed after only two weeks of treatment in 35 % of the patients, after 1 month in 35 % and after more than 3 months in 30 % but improvement of VA was observed in every patient during the two first weeks of treatment. VA normalized in all patients with unilateral (n = 5) or bilateral (n = 2) VA > 5/10 but also in 10 patients with unilateral (n = 7) or bilateral (n = 3) VA $\leq 5/10$ and in 5 patients with decreased VA for at least 6 months. Significant tumor shrinkage was observed in 82 % of the patients after 1 month of treatment but tumor remained close to the chiasm and/or optic nerves in 50 %. In spite of the good ophthalmologic response 4 patients were secondarily operated on (absence of tumor shrinkage (n = 3), apoplexy (n = 1) or DA intolerance (n = 1)). Field defects and VA didn't improved or worsened in 16 % of the patients who were operated on 6 to 30 days after the beginning of DA. Post-operative improvement of VA was observed only in one patient. PRL levels and tumor invasiveness were not significantly different between patients who normalized or not VA. Conclusion: primary treatment with DA is frequently and rapidly efficient on decreased VA in macroprolactinomas even when visual disturbances are severe and present for a long time. Absence of improvement of VA during the two first weeks of treatment is a factor of bad prognosis. In our experience surgical decompression had poor efficacy in patients not improved with primary DA therapy. Prolonged neuro-ophthalmological examinations are mandatory particularly in patients with persistent compressive adenomas.

Nothing to Disclose: SGG, SDD, EMM, LSS, EYY, GSASA, RAA, PFF, CCRC-R

P3-315

Individualized High-Dose Cabergoline Therapy for Hyperprolactinemic Infertility in Women with Micro- and Macroprolactinomas.

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Context: Cabergoline is effective for hyperprolactinemic hypogonadism. However, the rate of cabergoline-induced pregnancy in women with prolactinoma remains unknown. Also unknown is whether cabergoline can control tumor growth and thereby achieve successful pregnancy in patients with macroprolactinomas.

Methods: Eighty-five women with macroprolactinomas (n=29) or microprolactinomas (n=56) received prospective, high-dose cabergoline therapy for infertility according to individual prolactin suppression and/or tumor shrinkage. The patients included 31 bromocriptine-resistant, 32 bromocriptine-intolerant, and 22 previously untreated women. Conception was withheld until three regular cycles returned in women with microadenoma and until tumors shrank below 1.0 cm in height in women with macroadenoma. Cabergoline was withdrawn at the 4th gestational week.

Results: Cabergoline normalized hyperprolactinemia and recovered the ovulatory cycle in all patients. All adenomas contracted, and 11 macroadenomas and 29 microadenomas disappeared. Eighty patients (94%) conceived 95 pregnancies, two of which were cabergoline-free, second pregnancies. The dose of cabergoline at the first pregnancy was 0.25-9 mg/week overall and 2-9 mg/week in the resistant patients. Of the 93 pregnancies achieved on cabergoline, 88 resulted in 85 single live births, one stillbirth, and 2 abortions; the remaining 7 were ongoing. All babies were born healthy, without any malformations. No mothers experienced impaired vision or headache suggestive of abnormal tumor re-expansion throughout pregnancy.

Conclusion: Cabergoline achieved a high pregnancy rate with uneventful outcomes in infertile women with prolactinoma, independent of tumor size and bromocriptine resistance or intolerance. Cabergoline monotherapy could be a substitute for the conventional combination therapy of pregestational surgery or irradiation plus bromocriptine in macroprolactinomas.

Nothing to Disclose: MO, NM, TS, RM

P3-316

Low Dose Dopamine Agonist in Treatment of Invasive Prolactinoma Eroding into the Sphenoid Sinus.

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Background

Use of dopamine agonists in treatment of prolactinoma eroding the skull base and infiltrating sphenoid sinus carries a risk of cerebrospinal fluid (CSF) fistula due to tumor shrinkage. Radiotherapy is associated with similar dangers and risk of damaging the surrounding structures. We describe four patients who presented with prolactinoma infiltrating sphenoid sinus and responded well to treatment with small doses of dopamine agonist cabergoline.

Patients and methods

Four male patients presented to our department with invasive prolactinoma eroding the skull base and invading the sphenoid sinus (age at presentation 32-42). Prolactin range at diagnosis was 86913- 927538 IU/l (range 53- 360). One of the patients presented with CSF fistula and has undergone surgical repair of the fistula prior to starting cabergoline. All were treated with low dose D2 agonist cabergoline (dose 1- 2 mg weekly in divided doses) and all have experienced significant tumor shrinkage. All patients remain well on this dose of cabergoline, and all now have normal or low prolactin level. Mean duration of treatment was 29.5 months (range 11-43 months).

One of the patients had a tumor co-secreting growth hormone which also responded to treatment with cabergoline.

None of the four has experienced CSF rhinorrhea after starting treatment. Despite tumor shrinkage all patients remain testosterone deficient and one is cortisol deficient.

Conclusion

In treatment of prolactinomas, dopamine agonists can cause tumor shrinkage of up to 80%. In patients with prolactinoma eroding the skull base and infiltrating the sphenoid sinus, this can result in life threatening CSF fistula. We have used low dose cabergoline in four patients presenting with prolactinoma infiltrating sphenoid sinus. All have responded well to treatment, and none had any serious complications of the treatment.

In patients with prolactinoma eroding the skull base and infiltrating sphenoid sinus, treatment priorities are preserving integrity of the skull base and normalizing prolactin levels. Low dose dopamine agonist is a treatment of choice in such patients.

Nothing to Disclose: NDS, EC, KHN, SC, JB, JP

P3-317

Gender Differences in Prolactinoma: Presentation and Response to Treatment.

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Context Prolactinomas are the most common functioning pituitary adenomas & it is recognised that gender has an influence on presentation and management of this condition.

Objective To examine the effects of gender on presentation and response to treatment in a large cohort of adults with confirmed prolactinoma (MRI performed and macroprolactin excluded).

Design & patients This retrospective cohort study design used an electronic database (Diabeta3) to identify current adult patients with prolactinoma attending the endocrinology department of a University Teaching Hospital from October 2007-February 2008. Data including tumour size, prolactin levels, age at diagnosis, current management and gender were recorded.

Results 369 patients (326 females, 43 males; 7.3:1 females to males) with prolactinoma were included in the study. In the total cohort the ratio of microprolactinoma-to-macroprolactinoma was 8.2:1. Males presented significantly later in life than females (43.7±16.5 vs 31.4±8.5 years (mean±SD), p=<0.0001), with higher prolactin levels (53,339±104240 vs 3799±11020 mU/L, p=<0.0001) and a larger tumour size at diagnosis (16.4±15.3 vs 7.7±4.23 mm, p=<0.0001). The majority of patients were receiving cabergoline therapy (51.5%). A higher proportion of males than females achieved normoprolactinemia (62.8% vs 44.2%, p=0.03) with men receiving a higher average dose of cabergoline (1000µg (250-6000) vs 500µg (250-3000); p=0.0003).

Ratio of females-to-males according to decade of life in which diagnosis of prolactinoma was made

| Decade | Ratio of patients (F:M) |
|----------|-------------------------|
| 2nd | 18.0 : 1 |
| 3rd | 22.0 : 1 |
| 4th | 8.4 : 1 |
| 5th | 7.7 : 1 |
| Post 5th | 1 : 2.4 |

Conclusions Prolactinomas occur more frequently in females but males present at a later age with higher PRL levels and larger tumour size at diagnosis. In this study the overall rates of biochemical normalisation were relatively low, although males had higher rates of normoprolactinemia during treatment. This may reflect more aggressive treatment of the relatively larger prolactin secreting adenomas seen in males and a 'tolerance' of modest hyperprolactinaemia in females without clinical symptoms from prolactin excess.

Nothing to Disclose: SLL, JKP, SMT, BMM, PVC

P3-318

Effect of Dopaminergic Drug Treatment of Prolactinomas on Surgical Findings.

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OBJECTIVE: It has been reported that prolactinomas treated with Bromocriptine (BROM) show a certain degree of fibrosis, as described by neurosurgeons and histopathologists, that may interfere with complete surgical resection. The same finding has not been reported to for Cabergoline (CAB). In this retrospective study we analyzed the relationship between tumor fibrosis and treatments with CAB only vs. BROM (only, or in combination with CAB). **PATIENTS AND METHODS:** We retrospectively analyzed 24 consecutive patients (13 F, mean age 40, range 16-60) with histopathologically confirmed prolactinomas undergoing surgical resection at Johns Hopkins Hospital between 1992 and 2009. We compared them to 34 patients (22 F, mean age 42.9, range 15-75) with GH-secreting adenoma undergoing surgery in the same period of time. The operative notes from 7 different neurosurgeons were reviewed to catalog the tumors according to the surgeon's description. We classified as "fibrous" any tumor in which the surgeon had mention the adenoma being fibrous, hard, firm, or of increased consistency. When the adenoma was described as soft, or was not otherwise described, we classified as "non fibrous". Correlation with the exposure to Dopamine Agonists (DA) was investigated. **RESULTS:** Of the 24 prolactinoma patients, 21 (87.5%) were previously treated with DA, while 3 needed urgent surgery due to local compressive symptoms. Indication for surgery in DA-treated patients was: DA resistance (n.5), DA intolerance (n.6), persistent mass effect (n.7) and CSF leak (n.3). Of the 34 patients with GH-secreting adenomas, 28 (85.3%) had not received any medical therapy before surgery, and 5 (14.7%) were exposed to DA and/or somatostatin analogs. We found that 54% of prolactinomas and only 6% of GH-secreting adenomas were described as fibrous (not exposed to DA). 10/12 (77%) of prolactinomas exposed to BROM for at least 1 month, 2/9 (22%) of subjects exposed to CAB only, and 1/3 (33%) not previously treated were fibrous ($p < 0.05$ by χ^2 test). The mean BROM cumulative dose was 406 mg (range 75-1375) (minimum 1 month) and the CAB was 133 mg, range 2.5-1,080). Only 18% of non-fibrous prolactinomas had been exposed to BROM. Only 4 of the patients had persistent biochemical remission (3 treated with CAB and 1 not treated). **CONCLUSIONS:** Patients exposed to BROM for at least one month are more likely to have tumor fibrosis than patients that are treated only with CAB, or are not treated with any DA agent.

Nothing to Disclose: MBM, AQ-H, RS

P3-319

Prospective Assessment of Pituitary Function in Patients with Macroprolactinoma Treated with Cabergoline.

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Background: Patients with macroprolactinoma often present with impairment of a number of anterior pituitary hormones attributed to the hyperprolactinaemia or to the mass effect. Achievement of normal prolactin and tumour shrinkage by dopamine agonist treatment is expected to reverse, at least partially, the pituitary dysfunction. Studies assessing prospectively the pituitary function in subjects with macroprolactinoma responsive to cabergoline are lacking. We described the first prospective study to assess changes in pituitary function during treatment of macroprolactinoma with cabergoline.

Aim: To check the time course of recovery of the anterior pituitary reserve in patients with macroprolactinoma not resistant to cabergoline.

Patients/Methods: All patients presenting to our Department with macroprolactinoma between 10/2005 and 6/2007 were studied prospectively. The subjects underwent assessment of their pituitary function at diagnosis and at yearly intervals [insulin tolerance or glucagon test (GH and ACTH reserve), FSH/LH, testosterone or oestradiol, TSH, fT4, fT3]. The serum prolactin was checked at regular intervals during the titration of the dose of cabergoline and 6 months after the achievement of normoprolactinaemia. Pituitary imaging was performed at 3 months after commencing on treatment and at yearly intervals thereafter.

Results: Twelve patients were identified, one of which was lost to follow-up [final group 10 males and 1 female, median age at diagnosis 38 years (range 17-56), mean serum prolactin at diagnosis 27.247 mU/L (range 17.168-305.847)]. Nine patients were followed-up for 3 years and three for 2 years. All except one, achieved normal prolactin and all had significant tumour shrinkage. Hormone deficits at diagnosis and at last evaluation were GH (severe): 9/11 (82%) and 9/11 (82%), FSH/LH: 10/10 (100%) and 6/10 (60%) (female on oral contraceptive pill excluded), ACTH: 2/11 (18%) and 2/11 (18%), TSH: 4/10 (40%) (patient with primary hypothyroidism excluded) and 4/10 (40%).

Conclusions: In this first prospective study of pituitary function in treated macroprolactinoma subjects, we clearly show no improvement, apart from LH and FSH changes, which because growth hormone reserve has not changed, must be related to the treatment of the hyperprolactinaemia. This study also clearly shows that pituitary function does not recover in treated macroprolactinoma and therefore, replacement therapy should be commenced at the outset of treatment.

Nothing to Disclose: NK, JAHW

P3-320

Probably Benign Breast Lesions Associated with Prolactinoma and/or Macroprolactinemia.

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Background: Any kind of breast lesion is assumed to be an etiologic factor for hyperprolactinemia. Whether the prolactinoma with and without macroprolactinemia is also associated with benign breast lesions, not known. Here we aimed to document the prevalence of probably-benign breast lesions in persistent hyperprolactinemia caused by an etiologic factor other than breast-lesions like prolactinoma and/or macroprolactinemia.

Methods: Forty-six premenopausal women (age: 31.7 ± 9.1 years, prolactin: 77.2 ± 48.9 ng/ml) with persistent hyperprolactinemia having prolactinoma and/or macroprolactinemia, matched with 29 premenopausal healthy controls (age: 33.6 ± 5.6 years, prolactin: 15.7 ± 10.1 ng/ml). Study parameters including prolactin levels and ratio of recovery after polyethylen glycol precipitation, breast imaging with USG, and pituitary MRI findings were obtained and compared in a case-control study.

Results: Probably benign breast lesions were found in 57% of cases (28/49) while 34% of controls (10/29), (p: 0.044, odd's ratio: 2.53, CI: 0.98-6.56). Presence or absence of macroprolactinemia among the cases, did not show any statistical significance in regard to presence of probably benign lesions (58% vs 56%, p>0.05).

Conclusion: Our study showed that hyperprolactinemia due to a non-breast lesion like prolactinoma and/or macroprolactinemia, increased the prevalence of probably-benign breast lesions.

Nothing to Disclose: SAC, SD, ITS, MGA, TE, AU

P3-321

Long-Term Follow-Up of Macroprolactinaemia.

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Macroprolactinaemia is a common cause of hyperprolactinaemia in which more than 60% of serum prolactin (PRL) is precipitated with 12.5% polyethylene glycol (PEG) as an IgG-bound form. Several reports have suggested that macroprolactinaemia is a benign condition, which should first be considered in the differential diagnosis of hyperprolactinaemia. So far, there are no reports showing that macroprolactin appeared or disappeared during a long term period.

Objectives

To examine if macroprolactinaemia is a transient condition or a long lasting one.

Subjects and Methods

Macroprolactinaemia was screened using PEG precipitation method in 625 hyperprolactinaemic serum samples collected between March 2004 and September 2009 at a city hospital. PEG-precipitated PRL ratio greater than 60% (recovery as a free PRL after PEG precipitation less than 40%) was defined as having macroprolactin. Since blood was repeatedly taken in some patients, there were 308 individuals with hyperprolactinaemia. Protein G column was used to examine if macroprolactin was composed of PRL-IgG complex.

Results

Among the 308 patients with hyperprolactinaemia, macroprolactinaemia was identified in 40 patients (13.0%) [37 females and 3 males, 44 ± 12 y.o. (mean \pm SD)]. There were 11 patients with macroprolactinaemia whose sera were examined more than twice with the interval more than one year (3.0 ± 1.7 years). Among the other 29 macroprolactinaemic patients, two patients were found to have been evaluated for macroprolactinaemia 17 and 15 years ago. PEG-precipitated PRL and IgG-bound PRL ratios were compared between the paired serum samples of the 13 macroprolactinaemic patients. There were significantly positive correlations between the paired samples as to PEG-precipitated PRL ratio ($r=0.79$, $p<0.01$) and IgG-bound PRL ratio ($r=0.74$, $p<0.01$), suggesting that those who have high proportion of macroprolactin remain to possess it at a high level during the long term, and so do those who have low macroprolactin ratio. The PEG-precipitated PRL ratios remained high (from 95% to 78% and from 90% to 81%) in two patients followed for 17 and 15 years, respectively. Macroprolactin neither appeared nor disappeared during the observation periods in all patients except two whose PEG-precipitated PRL ratios were close to borderline (from 33% to 61% and from 63% to 57%).

Conclusions

These results suggest that macroprolactinaemia is a long lasting phenomenon.

Nothing to Disclose: NH, TI, YS, AS

P3-322

Prolactinoma: A Condition Associated with Hypoadiponectinemia.

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Introduction: Adiponectin is an adipocytokine secreted from adipose tissue and its serum levels decrease with obesity and insulin resistance (IR). Hyperprolactinemia has been otherwise described as possibly associated with obesity and IR (1). The aim of this study was to evaluate adiponectin levels and IR in prolactinoma patients with uncontrolled (UPRL) and controlled (CPRL) disease as compared to healthy subjects. **Methods:** Forty patients with prolactinoma (20 UPRL and 20 CPRL) were included in this transversal study. Forty healthy subjects matched for age, gender and body mass index (BMI) were included as controls (CG). Diagnosis of prolactinoma was confirmed by elevated prolactin (PRL) levels and adenoma image. Exclusion criteria were other causes of hyperprolactinemia, diabetes, hypertension, and smokers. Controlled prolactinomas were defined as patients without hyperprolactinemia symptoms and PRL < 30 ng/mL and UPRL as patients with hyperprolactinemia symptoms and PRL > 30 ng/mL. Clinical history and anthropometric measures (BMI and waist-hip ratio - WHR) were recorded. The following variables were analyzed: PRL, adiponectin, lipid profile, glucose, insulin and HOMA-IR. **Results:** There were no significant differences in lipid profile among groups. The mean prolactin levels were 224,92 ± 211; 17,00 ± 9,69 and 13,43 ± 6,1 ng/ml for UPRL, CPRL and CG, respectively (p<0,05 for UPRL vs CPRL and CG). The female WHR was 0,92 ± 0,09; 0,85 ± 0,07 and 0,85 ± 0,06 for UPRL, CPRL and CG respectively (p<0,05, UPRL vs CPRL and CG), whereas no significant difference could be detected for the male groups. The HOMA-IR results were 2,48 ± 1,72; 1,30 ± 1,02 and 1,03 ± 0,81 for UPRL, CPRL and CG, respectively (p<0,01, UPRL vs CPRL and CG). The mean adiponectin levels were 12,03 ± 7,83; 19,73 ± 8,39 e 33,81 ± 18,37 ng/mL for UPRL, CPRL and CG, respectively (p<0,01 among groups). The correlation between prolactin and adiponectin was statistically significant (p<0,01). **Conclusion:** Hyperprolactinemia in uncontrolled prolactinomas is associated with insulin resistance as demonstrated by HOMA-IR elevations, and WHR changes in females. Controlled prolactinomas have HOMA-IR and WHR similar to healthy subjects, but still show lower adiponectin levels. These data altogether suggest that prolactinomas are per se a condition associated with decreased adiponectin levels, which may be aggravated by insulin resistance when higher prolactin levels are achieved in non-controlled patients.

(1) Ben-Jonathan N et al, Endocr Rev 2008; 29:1

Disclosures: AR-O: Clinical Researcher, Novartis Pharmaceuticals.

Nothing to Disclose: LFAAR, SMSC, PACM, MFB, AVG

P3-323

Prolactin Levels in Patients with Weight Gain.

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Although actions of prolactin (PRL) on metabolic parameters and body fat have been object of interest of several studies, controversy still surrounds the issue of PRL and weight gain.

Objectives: to evaluate PRL levels and correlate them with biochemical and clinical data of patients with weight gain.

Patients & methods: This cross-sectional study analyzed anthropometric measures (body mass index - BMI; abdominal circumference - AC), PRL, glucose (G), uric acid (UA), total cholesterol (TC), LDL, HDL, triglycerides (TG), and insulin (IN) levels of 187 women (age= 40.2 ± 13.2 years, BMI= 29.3 ± 5.5 kg/m²) and 70 men (age= 36.4 ± 11.9 years, BMI= 31.7 ± 7.4 kg/m²) who sought Endocrine evaluation due to weight gain from January/2005 to October/2009. Most women were overweight (41.2%) or obese (38.5%), while 20% still had normal BMI despite the weight gain. Among men, 48.6% were obese, 41.4%, overweight, and 10% had normal BMI. Biochemical and clinical data were analyzed simultaneously in order to identify correlations.

Results: PRL levels were above the normal range in 4.3% of the women (subgroup prevalence: obese= 2.8%; overweight= 5.2%; normal BMI= 5.3%) and 1.4% of the men (1 obese patient). Obese women had lower PRL levels than non-obese ones (10.9 ± 6.7 vs 12.9 ± 6.4 ng/mL, p= 0.01). No difference in PRL levels was obtained when obese (10.3 ± 4.2 ng/mL) and non-obese men (9.9 ± 3.3 ng/mL) were compared. Obese patients presented higher levels of G, UA, TC, LDL, TG, and IN than non-obese ones. In addition, these parameters were positively correlated with BMI in both women (r: G= 0.46, UA= 0.34, TC= 0.18, LDL= 0.19, TG= 0.32, IN= 0.49) and men (r: G= 0.34, UA= 0.39, TC= 0.43, LDL= 0.40, TG= 0.43, IN= 0.76). In women, negative correlations were detected between PRL levels and BMI (r= -0.21), AC (r= -0.19), G (r= -0.22), TC (r= -0.16), and LDL levels (r= -0.19); the correlations between PRL levels and those of G and LDL persisted after the adjustment for BMI. In men, negative correlations were detected between PRL and G (r= -0.30), TC (r= -0.36), LDL (r= -0.27), and TG levels (r= -0.37); the correlations between PRL and TC, LDL, and TG levels also persisted after the adjustment for BMI.

Conclusion: In women with weight gain, PRL levels and body weight were inversely correlated. The relationship between PRL and some metabolic parameters seemed to be independent of body weight. PRL evaluation may be useful for the investigation of patients with weight gain.

Nothing to Disclose: LBF, HRC, VTO, LCLCF, TRF, LRMO, CFM, ECON

P3-324

Efficacy and Safety of Intranasal Desmopressin Acetate Administered Orally for the Management of Infants with Neurogenic Diabetes Insipidus (DI).

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Background: The regulation of sodium and fluid balance in infants with neurogenic DI is challenging. Intermittent antidiuresis in an infant with obligatory fluid intake can lead to rapid fluctuation between fluid overload and dehydration, and compromises nutrition. Treatment with subcutaneous, nasal, or oral tablet desmopressin acetate (DDAVP) is difficult to titrate and administer. Other therapeutic approaches, such as thiazide diuretics in combination with low renal solute load formulas or compensatory administration of free water, may have decreased efficacy and safety. Effective alternatives to current therapy could prevent morbidity and improve growth and development.

Objective: Assess the efficacy and safety of intranasal DDAVP given orally for the management of infants with neurogenic DI.

Design and methods: Retrospective chart review of clinical and laboratory data of patients with neurogenic DI (n=10) of various etiologies. All patients were less than one year old. Treatment was with diluted intranasal DDAVP formulation, but given orally.

Results: After an initial inpatient titration period lasting 60-80 hours, DDAVP doses at time of discharge ranged from 1 mcg twice daily to 5 mcg once or twice daily, given orally. Mean sodium concentration at diagnosis of DI was 164.1 ± 6.2 mmol/L (158-178), improving to 143.5 ± 4.2 mmol/L (138-150) after inpatient DDAVP titration. Serum sodium was maintained during outpatient management at a mean of 145.2 ± 5.8 mmol/L. During the initial titration period, sodium concentrations reached normative values gradually and did not fluctuate significantly while approaching steady state. Subjects remained clinically stable, without signs and symptoms of electrolyte disturbances, and demonstrated improved linear growth and weight gain compared to pre-treatment.

Conclusions: We propose an approach to therapy for neurogenic DI in infancy using oral administration of the intranasal formulation of DDAVP. Our clinical experience demonstrated ease of dosing and titration of therapy, and avoidance of fluctuation between hypo- and hypernatremia. Such a treatment approach should facilitate appropriate weight gain and linear growth.

Nothing to Disclose: IG-L, DRR, PFB

P3-325

Familial Glucocorticoid Deficiency (FGD) as a Cause of Hyperthyrotro-Pinemia (Hyper-TSH).

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During paediatric age, hyper-TSH can be the consequence of several different mechanisms, including autoimmune thyroid disease (AITD) or genetic defects in thyroid hormonogenesis. The identification of the underlying cause has several implications including the decision for treatment. We recently had the opportunity to study two families whose probands presented non-AITD hyper-TSH. The first child was born from consanguineous Pakistan parents and came to our attention at the age of 3 years for a mild speech retardation accompanied by convulsive equivalents. Hormonal screening revealed an elevated serum TSH (12.4 mU/L) and normal freeT4 in the absence of AITD. L-thyroxine was started. Few months later he was admitted to the emergency department for an episode of acute hypoglycemia. Corticotropin levels were high (ACTH, 3654 ng/L) and cortisol= 20 ng/mL. The child was put on glucocorticoids. The sequence of MC2R gene revealed a homozygous inactivating mutation (R146H). Due to the diagnosis of non-AITD hyper-TSH, TSH receptor (TSHR) gene was analyzed revealing a novel heterozygous substitution (T607I). In vitro, this variant was found to generate cAMP levels similar to those of wild-type TSHR. Soon after, another child born from consanguineous Pakistan parents came to our attention for a severe adrenal insufficiency. Also in this case, non-AITD hyper-TSH was present (7.0 mU/L) and both glucocorticoids and L-thyroxine treatments were started. Due to the coexistence of hyperpigmentation and alacrimia, Allgrove's syndrome was suspected and the analysis of ALADIN gene revealed a homozygous nonsense mutation (R194X). Both parents were heterozygous, but his elder brother who never experienced hypoglycemic events was also found to be homozygous for ALADIN mutation and had both adrenal (ACTH= 1250 ng/L; Cortisol= 99 ng/mL) and thyroid defects (TSH= 4.6 mU/L). TSHR gene was normal in this case. On these bases, L-thyroxine administration was stopped in the two probands and the 3 children showed a persistent TSH normalization (1.5-3.75 mU/L) during glucocorticoid substitution. In conclusion, reversible hyper-TSH should be included among the clinical findings of FGD and always be suspected in severe adrenal insufficiency. This is relevant because L-thyroxine treatment may be unwarranted in these patients. These data support the relevant role played by glucocorticoids in the regulation of hypothalamic-pituitary-thyroid axis.

Nothing to Disclose: MB, DVL, DFK, GR, LP

P3-326

Reduced Central Nervous System Serotonergic Responsivity Is Associated with High Free Fatty Acids.

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Background Reduced central serotonergic responsivity is associated with mood disorders (1), eating habits (2), atherosclerosis (3) and the metabolic syndrome (4). In addition, the presence of obesity in depressed men is associated with a diminished therapeutic response to selective serotonin re-uptake inhibitors (SSRIs) (5). However, the association remains unexplained.

Purpose Since increased free fatty acids (FFAs) is a hallmark in the metabolic syndrome, we hypothesized that FFAs may interfere with central serotonergic response. Therefore we studied whether an increase in FFAs is associated with lower serotonergic responsivity.

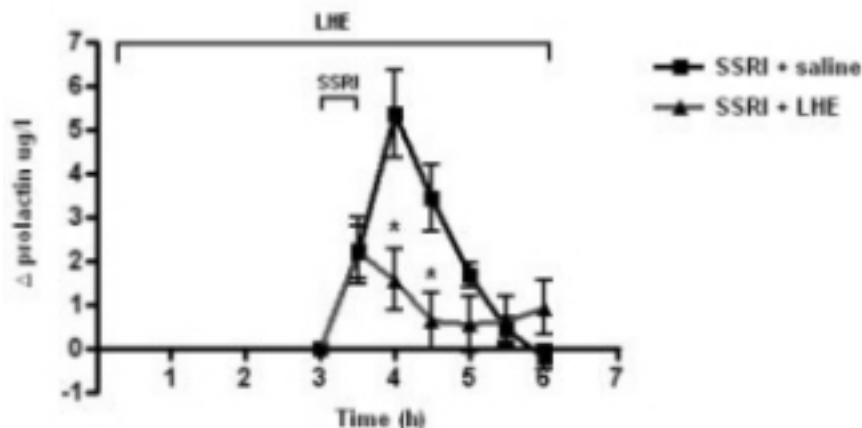
Methods Subjects were 10 lean healthy men, 23.6 ± 4.7 years of age and free of any clinical disease. The serotonergic response was measured with the well validated serotonergic challenge test (6). Stimulation of hypothalamic serotonin receptors with administration of SSRIs promotes the pituitary to subsequently release prolactin and provides an index of the serotonergic responsivity in the hypothalamic-pituitary axis (7). In random assignment lipid (Intralipid 20%) /heparin emulsion (LHE) or saline was administered while serum prolactin was measured for 3 hours after administration of the SSRIs.

Participant Characteristics

| | |
|--------------------------------|--------------------|
| N | 10 |
| Age, y | $23,6 \pm 4,72$ |
| Waist circumference, cm | $81,45 \pm 10,5$ |
| BMI, kg/m ² | $22,55 \pm 1,83$ |
| Total cholesterol, mmol/L | $4,45 \pm 0,91$ |
| LDL cholesterol, mmol/L | $2,45 \pm 0,70$ |
| HDL cholesterol, mmol/L | $1,55 \pm 0,37$ |
| Triglycerides, mmol/L | $1,0 \pm 0,64$ |
| HbA1c, % | $5,27 \pm 0,22$ |
| Systolic blood pressure, mmHg | $127,40 \pm 12,01$ |
| Diastolic blood pressure, mmHg | $76,40 \pm 7,89$ |
| Kreatinine umol/l | $77,7 \pm 8,97$ |
| Hemoglobin mmol/l | $9,4 \pm 0,42$ |
| Baseline prolactin ug/l | $8,9 \pm 4,26$ |

Results Change in plasma prolactin response to SSRIs was significantly reduced with high plasma FFA's compared to saline ($P < 0.001$).

Fig 1. Changes in prolactin levels during 2.5 hours after SSRI challenge test with (▲) and without (●) lipid (Intralipid 20%) heparin emulsion (LHE) infusion in healthy subjects. Values are mean \pm SEM. * $p < 0.01$ LHE vs saline.



Conclusion Serotonergic hypothalamic pituitary response is reduced when plasma levels of FFA are increased. This

may explain the association between lower serotonergic reponsivity in the metabolic syndrome and the low therapeutic response in depressed and obese patients.

- (1) Ogilvie AD et al., Lancet 1996; 347: 731-733
- (2) Tecott LH et al., Nature 1995; 374: 542-546
- (3) Muldoon MF et al., Stroke 2007; 38: 2228-2233
- (4) Muldoon MF et al., J Clin Endocrinol Metabol 2006; 91:718-721
- (5) Kloiber S et al., Biological Psychiatry 2007;62:321-326
- (6) Lotrich EF et al., Psychopharmacology 2005; 178: 268-275
- (7) Johnston CA et al., Endocrinology 1986; 118: 805-810

Nothing to Disclose: BMS, CFKT, ESGS, MJMS, TBT

P3-327

Multinational Randomized Trial on Patients with Childhood Craniopharyngioma (KRANIOPHARYNGEOM 2007) - Update after 27 Months of Recruitment.

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Despite high overall survival rates (92%) in patients with childhood craniopharyngioma (CP), health-related quality of life (QoL) is frequently impaired due to sequelae resulting from hypothalamic involvement of CP such as severe obesity. Based on the results of the multicenter prospective study **KRANIOPHARYNGEOM 2000** radical surgery is no appropriate treatment strategy in patients with hypothalamic involvement of CP. Furthermore, tumour progression and relapses are frequent and early events in CP patients. The analysis of event-free survival rates (EFS) in 117 prospectively evaluated patients with CP showed a high rate of early events in terms of tumour progression after incomplete resection (EFS: 0.31 ± 0.07) and relapses after complete resection (EFS: 0.63 ± 0.09) during the first three years of follow-up. Therefore, innovative treatment strategies are warranted for patients with hypothalamic involvement of CP after incomplete resection.

Accordingly, in **KRANIOPHARYNGEOM 2007** QoL, and survival rates in CP pts are analyzed after randomization of the time point of irradiation (XRT) after incomplete resection (immediate XRT versus XRT at progression of residual tumour). All patients with completely resected CP and patients of an age < 5yrs, regardless of the degree of CP resection, will be recruited in a surveillance study. Up to now (12/09) 52 pts with CP have been recruited in KRANIOPHARYNGEOM 2007 (28 pts in the randomization arm; 19 pts in the surveillance arm; 5 pts in the process of review of imaging). 10 of 28 pts were randomized. 7 pts could not be randomized due to parental decision, delays in schedule (7 pts) and due to decision of the physician (4 pts).

In conclusion, KRANIOPHARYNGEOM 2007 represents the first randomized multicenter trial in patients with CP and the first study in children and adolescents with a CNS tumour analyzing health-related QoL as an endpoint. Aim of the study is to analyze the appropriate time point of XRT after incomplete resection in order to improve QoL in patients with hypothalamic involvement. The recruitment rate is high. However, the compliance to randomization has to be increased. Problems in the organization and timing of the randomization process have been improved during the first year of recruitment in **KRANIOPHARYNGEOM 2007** (www.kraniopharyngeom.net).

Sources of Research Support: Deutsche Kinderkrebsstiftung, Bonn, Germany (www.kinderkrebsstiftung.de).

Nothing to Disclose: HLM, UG, SS, FP, RDK, IZ, AF, MW-M, TP, GC, RK, CW, NS

P3-328

Clinical, Biochemical Characteristics and Treatments of Patients with TSH Secreting Pituitary Adenomas.

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Background:

Thyroid stimulating hormone (TSH)-secreting pituitary adenoma (TSHoma) is very rare and represents 1~2% of all pituitary adenomas. TSHoma should be distinguished from the syndrome of resistance to thyroid hormone. Patients with TSHoma may also be misdiagnosed as primary hyperthyroidism and often receive inappropriate treatment directed towards the thyroid gland.

Method:

We analyzed clinical characteristics of the patients with TSHoma who were presented to Severance hospital, Yonsei University College of Medicine, Seoul Korea from 2005 to 2009.

Results:

Among 484 pituitary tumor patients who underwent pituitary tumor resection, 8 (1.65%) were revealed to be TSHoma. Five were women and 3 were men. Mean age was 40.6±8.9 years at diagnosis (range 28-55 years). The median duration from the onset of symptom to diagnosis was 9.8 months (range 4-36months). Four patients had overt symptoms of hyperthyroidism and 2 had visual field defect. Six patients had elevated free thyroxine levels with elevated, or inappropriately normal TSH levels and 2 patients were associated with Hashimoto's thyroiditis. One had normal FT4 normal TSH and the other had normal FT4 with high TSH. The serum levels of free α subunit measured in 2 patients were elevated. MRI showed that 6 were macroadenomas (>10mm) and 2 were microadenomas. Complete tumor removal was achieved in all patients. Immunohistochemical staining performed in 6 specimen revealed TSH positivity. Five patients had preoperative anterior pituitary dysfunction. Anterior pituitary function was recovered in 3 after operation. Three patients were lost to follow-up and 5 patients had no evidence of recurrence or hyperthyroidism for 30.8 months (mean, range 3-57months) of follow-up.

Conclusion:

Early diagnosis and complete removal of tumor mass may improve the neurological and endocrine defects.

Nothing to Disclose: WKL, SH, YHL, JWH, YDS, SHK, EJL

P3-329

Partial Response to Clomiphene Citrate in Males with Type 2 Diabetes.

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Type 2 diabetes in males is characterized also by low libido and by erectile dysfunction(ed). Aging males with and without diabetes exhibit a gradual decline in Total Testosterone(TT) levels, without elevation of gonadotropins. Previous studies suggest either a hypothalamic defect, or an increase in sex hormone binding globulin(SHBG), causing increase binding of TT and thus low free Testosterone(fT). A private practice setting was used to recruit males with both types of diabetes for the purpose of performing the Clomid Stimulation Test(CST). All type 2 diabetic patients were found to have a basal sub-normal fT level along with normal LH and a low-normal TT levels. All type 1 diabetic patients had normal basal fT levels along with normal LH and TT levels. Both groups underwent the CST which consisted of levels of fT, LH, and TT both pre and 10 days post 100mg BID of Clomiphene Citrate. Patients with type 1 diabetes showed a normal (doubling) response of LH and fT, whereas patients with type 2 diabetes showed a marked decline in response of both LH and fT to CST. The results of this small study suggest a double defect in type 2 diabetic males. They seem to have a sluggish hypothalamic-pituitary response to the drug which blocks the negative feedback loop, as well as a defect in their ability to elevate fT. Interestingly, type 1 diabetic patients do not exhibit these defects. Further testing is necessary in order to elucidate the mechanisms causing these subtle defects.

Nothing to Disclose: YL

P3-330

Preliminary Experience with LHRH Receptor Blockade for Postmenopausal Flushing.

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Introduction:

Flushing is the most common complaint for which postmenopausal women seek medical help. Severe flushing, defined as more than 10 flushes per day, occurs in 30% of women in natural menopause, and in 50% of women who are postmenopausal after treatment of breast cancer. Oestrogen antagonists and aromatase inhibitors markedly increase the frequency and severity of flushing.

Oestrogen replacement therapy is the most effective treatment of flushing, but its use is contraindicated in many cases. Non-hormonal treatment that is currently used has limited effectiveness and is often discontinued because of side effects.

Aim of the Study:

Our hypothesis is that LHRH is involved in the pathogenesis of postmenopausal flushing. Therefore, we explored short- and long-term efficacy of cetrorelix, which blocks the effects of LHRH.

Materials and Methods:

Open-label treatment with cetrorelix subcutaneously was given once or twice a day in eight postmenopausal women with severe flushing, which was defined as more than 10 flushes/day. Flushing characteristics were recorded by diary and by miniature hygrometer. Serum LH, FSH and oestradiol responses were monitored.

Initial treatment occurred in an in-hospital setting for a period of 5-12 days, followed by outpatient long-term treatment. Serum bone marker levels and bone density were measured during the long-term treatment.

Results:

In all eight subjects serum LH and FSH levels decreased to premenopausal levels. The daily number of diary reported hot flushes decreased markedly in seven out of eight women. Serum oestrogen levels did not change. Discontinuation of treatment was associated with a rapid return of serum LH and FSH to pre-existing postmenopausal levels. Recurrence of flushing was delayed by several weeks.

Conclusion:

This preliminary experience suggests that LHRH receptor blockade may become a useful tool to reduce postmenopausal flushing, particularly in women with severe flushing and a contraindication for oestrogen replacement therapy. In contrast to other non-hormonal treatments, no side effects were observed.

Nothing to Disclose: PMvG, MvdZ, DT, AvS, HdB

P3-331

Infected Rathke's Cleft Cysts: Distinguishing Factors and Factors Predicting Recurrence.

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OBJECTIVE: Rathke's cleft cysts (RCC) are benign sellar lesions that are generally asymptomatic but sometimes warrant transsphenoidal drainage. Small case reports have described infected RCCs, but this phenomenon remains uncharacterized. We reviewed RCCs over 23 years at our institution to determine factors predicting infection and recurrence.

METHODS: We retrospectively reviewed MRIs, labs, microbiology, and pathology of 176 RCC patients (1985-2008) who underwent initial operation at our institution (n=170) or at another institution followed by recurrence managed at our institution (n=6).

RESULTS: There were 3 RCC categories: cysts cultured intraoperatively during initial surgery (n=21); cysts not cultured during initial surgery but cultured during subsequent surgery (n=9); and cysts that were never cultured (n=146). Cultured cysts were larger (1.6 cm vs. 1.2 cm, $P=0.002$) and had more frequent pituitary dysfunction (76% vs. 30%; $P<0.00001$) than non-cultured cysts. Restricted diffusion was also more common in cultured cysts (50% vs. 0%, $P=0.02$). Of cysts cultured at initial or subsequent surgery, 48% and 44%, respectively, had positive cultures (n=14) and were treated with antibiotics. The most common organisms were *Staphylococcus epidermidis* (64%) and *Propionibacterium acnes* (57%). Kaplan-Meier recurrence rates were 13% (culture-positive/antibiotic-treated), 31% (culture-negative/non-antibiotic-treated), and 9% (non-cultured) ($P=0.002$ cultured versus non-cultured; $P=0.002$ culture-negative/non-antibiotic-treated versus non-cultured; $P=0.5$ culture-positive/antibiotic-treated versus non-cultured).

CONCLUSION: In our series, suspected RCC infection, independent of culture results, is a strong predictor of recurrence and may warrant antibiotic treatment. With antibiotic treatment, the recurrence rate of infected RCC approaches that of non-infected cysts. The higher recurrence rates reported in other series may reflect under-recognition of occult infection.

Nothing to Disclose: MKA, SK, MCT, LSB

P3-332

Rathke Cleft Cyst "Apoplexy": A Distinct Clinical Entity That Mimics Pituitary Tumor Apoplexy.

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Most Rathke's Cleft Cysts (RCC) remain asymptomatic, although some present with compression of surrounding structures and pituitary hormone dysfunction. However, a few cases of hemorrhage into an RCC have been reported and these included very limited data. The presentation in the latter setting includes sudden bleed into an RCC, clinically manifested by sudden onset of headaches, visual disturbances, impairment in pituitary function, and rarely alterations in mental status; a compilation of symptoms that mimics pituitary tumor apoplexy. Hence we recommend to use the term "Rathke's Cleft Cyst Apoplexy" to describe the syndrome. We reviewed the literature on hemorrhagic RCC and included our own experience with 11 such cases over a 10 year period. In each case, the diagnosis was confirmed intra-op and on histologic exam. Patients with RCC apoplexy present at different ages (8-72y, mean = 40y) with a F:M ratio of 3:1. Table 1 summarizes the classical clinical manifestations of RCC apoplexy.

Table1. Clinical Manifestations

| | Altered Mental Status/ Meningismus | Headaches | Visual Deficits | Cranial Nerve Palsies |
|-----------------------|---------------------------------------|-----------|-----------------|-----------------------|
| Current series (N=11) | 3/11 | 10/11 | 3/11 | 1/11 |
| Published Data (N=10) | 1/3 | 8/9 | 6/9 | 3/9 |

In comparison to patients with pituitary tumor apoplexy (1), those with RCC apoplexy tended to be more females, have smaller size sellar lesions (1.4 cm vs 2.4 cm), but have similar age at presentation (40.2y vs 50.9y). On MRI, RCC apoplexy appears as a hyperintense lesion on T1 weighted non-contrast images in most of the patients. As shown in table 2, pituitary dysfunction at presentation is less prevalent than in patients with pituitary tumor apoplexy and most of deficient patients recover function postop.

Table 2. Pituitary Function (Normal Function/Total)

| | Our series (N=11) | | Published Data (N= 10) | |
|-----------|-------------------|--------|------------------------|--------|
| | At Presentation | Postop | At Presentation | Postop |
| Adrenal | 7/11 | 9/11 | 8/8 | N/A |
| Thyroidal | 10/10 | 11/11 | 6/8 | N/A |
| Gonadal | 7/11 | 7/11 | 8/8 | N/A |
| Prolactin | 8/11* | 10/11 | 7/8* | N/A |

* The remaining patients had mild hyperprolactinemia

In conclusion, RCC apoplexy can mimic the classical presentation of pituitary tumor apoplexy and differentiating the two entities can be difficult. However, RCC apoplexy presents with less severe symptoms, lower prevalence of pituitary dysfunction and smaller sellar mass. The final confirmatory diagnosis can be made intraoperatively with subsequent histopathological confirmation. Management is similar to that of pituitary tumor apoplexy.

(1)Nawar RN et al., J Intensive Care Med 2008;23:75

Nothing to Disclose: JTC, DKA, MC, WRS, BMA

P3-333

Prevalence of Pituitary Tumors in the Health Care System of a Private Hospital in Buenos Aires (Argentina).

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There seems to be an increase in the prevalence of clinically relevant pituitary tumors in latest decades and an excess of mortality due to coronary and cerebrovascular factors among the affected patients (1-2).

This is a retrospective cross-sectional study of the prevalence of hypophysial tumors in a University Hospital (*Hospital Italiano de Buenos Aires*) featuring a prepaid health care system with over 100,000 members and electronic database of clinical records since 2001. Data were gathered from the clinical records of patients aged ≥ 18 diagnosed as having acromegaly, Cushing's disease, prolactinoma, non-functioning pituitary adenoma. A considerable number of the patients had unspecified diagnoses (hyperprolactinemia, hypophysial tumor, sellar tumor, pituitary adenoma, etc.) which were reviewed later. This information was included in a data base to estimate the frequency of every diagnosis, age and gender. The term prevalence, as defined in cancer epidemiology, means "the number and/or proportion of people with a past or present diagnosis of a pituitary adenoma within a well-defined population at a fixed point in time".

Results: 249 patients aged 50.0 ± 28.6 , F/M 69.4/30.5 %, out of 123,811 members were identified.

| | Prolactinoma | Non- functioning | Acromegaly | Cushing | Not studied | Other lesions |
|--------------|-------------------|------------------|-----------------|-------------|-------------|---------------|
| Percentage % | 56 (n 140) | 20 (n 56) | 12 (n 30) | 2.8 (n 7) | 3.2 (n 7) | 4.4 (n 11) |
| Age | 42.5 \pm 13.9 * | 65.9 \pm 17.5 | 59.3 \pm 16.2 | 59 \pm 20 | 57 \pm 15 | 58 \pm 12 |
| Gender F/M % | 75.5 / 24.5** | 62.3/37.7** | 63.5 /36.5** | 76.6/23.4** | 70 / 30 | 68 / 32 |

* Lower age within the group of patients with prolactinoma ($p < 0.001$) **Higher prevalence of females in all etiologies ($p < 0.001$)

Based on the figure of 123,811 members, the finding of 230 patients with a diagnosis of pituitary tumor yields a prevalence of 1 case/538 members, i.e. 185 cases/100,000 members. The higher prevalence of females in all tumors and the lower age of incidence of prolactinomas are data found in other populations. The prevalence of tumors is about 2-3 times higher than that estimated in a population of a region precisely delineated in Belgium (94 cases/100,000 inhabitants) and the United Kingdom (Bandbury) (77 cases/100,000 inhabitants).

The prevalence of clinically relevant hypophysial tumors is be much higher than that known a few decades ago. It is uncertain whether this is due to an actual increase of the pathology in addition to the access to MRI and the assessment of serum hormones.

(1) Bo Nilsson et al, JCEM 2000; 85:1420

(2) Adrian F Daly et al, JCEM 2006; 91:4769

Nothing to Disclose: PFD, MG, DK, SL, VCD

P3-334

First Pituitary Unit in Uruguay: Evaluation of Pituitary Tumors in a 3.2 Million Inhabitant Country.

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Pituitary tumors (PT) form about 15% of intracranial tumors. Surgery is the initial treatment of most PT, excluding prolactinomas (PRL). A pituitary unit (PU) was formed in 2006, were patients are now followed by a team of Endocrinologists and Neurosurgeons. We retrospectively evaluated clinical presentation, diagnosis, endocrine status, treatment and outcomes in 72 patients followed in it until 11/2009. Mean age at diagnosis was 39.84 (12-79) years, with F/M ratio of 2.1/1. Mean time of follow up was 5.9 ± 0.86 years. PRL were the most common PT (table 1), mostly in females (93.1%, p=0.003). Since 2006 ACR was diagnosed more often (12 vs. 6, p=0.025). PRL presented often with galactorrhea (p=0.010) and amenorrhea/impotence (p=0.003); ACR with acral enlargement (p=0.000); CD with weight gain (85.7%) and NFA with hypocortisolism symptoms (p=0.047). Most PT were macroadenomas (71%, p = 0.035). At least one hormone deficiency was present at diagnosis in 34% of PT, and 15 new developed after treatment (Table 1). No association between hypopituitarism (includes hypogonadism in PRL) and tumor type or size was seen. TS was the most common treatment, except in PRL (Table 1). Postoperative (post-op) complications appeared in 11/41 PT, most often CSF rhinorrhea (n=4), meningitis (n=3) and DI (n=1). RT was used most often in ACR (table 1); octreotide in 5 ACR. Initially all PRL received DA; now 58% are on cabergoline and 24% on bromocriptine. Most PT did not achieve remission (table 2); yet, there was a trend to increase number of remissions along time. No association between tumor size, post-op complications, type of DA and remission rates was found. In conclusion, despite the small number of PT involved there was a trend to improve outcomes along time after the creation of a PU. We stress the importance of a skilled multimodality team to improve outcomes.

| | PRL | Acromegaly (ACR) | Non-Functioning Adenomas (NFA) | Cushing's Disease (CD) | Mixed GH/Prolactin (ACR+PRL) | Rathke Cyst |
|---------------------------------------|------------|------------------|--------------------------------|------------------------|------------------------------|-------------|
| No. of patients | 29 (40.2%) | 18(25%) | 14 (19.4%) | 7 (9.7%) | 3 (4.1%) | 1 (1.3%) |
| Microadenoma | 8 | 5 | - | 3 | 1 | - |
| Macroadenoma | 14 | 11 | 13 | 3 | 2 | 1 |
| Hypopituitarism (HP)Pre-Treatment(Rx) | 14(25) | 7(17) | 9(13) | 3(3) | 1(3) | 0 |
| HP Post-Rx | 3(23) | 5(15) | 4(8) | 1(7) | 1(3) | 1 |
| Transphenoidal (TS) surgery (Sx) | 3 | 15 | 11 | 7 | 2 | 1 |
| Transcranial Sx | 1 | - | 1 | - | - | - |
| Radiotherapy (RT) | 1 | 7 | 6 | - | 1 | - |
| Dopamine Agonists(DA) | 29 | 5 | 1 | 0 | 3 | 0 |

Outcomes

| | PRL | ACR | NFA | CD | ACR+PRL | Total |
|----------------|-----|-----|-----|----|---------|-------|
| Remission | 9 | 3 | 1 | 4 | 0 | 17 |
| Active Disease | 19 | 9 | 6 | 2 | 3 | 39 |
| | 28 | 12 | 7 | 6 | 3 | 56 |

Nothing to Disclose: MMP, MLC, NS, MRF, EH, RL, SW, AS, SP, MCB

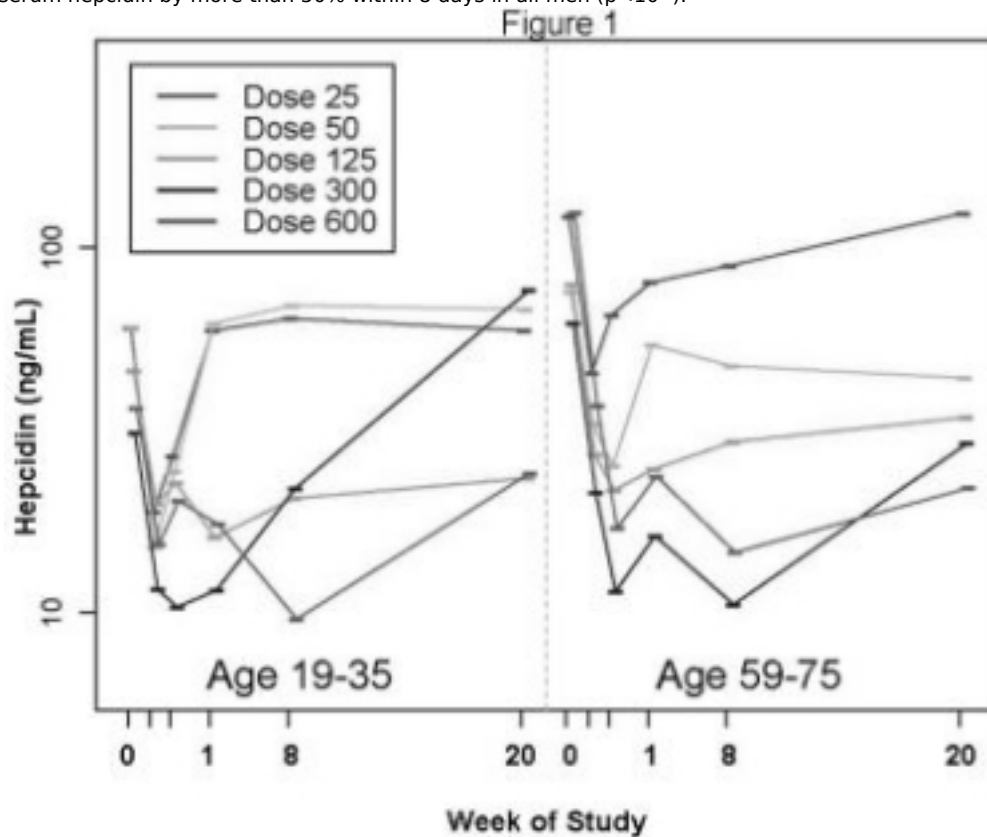
P3-335

Testosterone Suppresses Hepcidin in Men: A Potential Mechanism of Testosterone-Induced Erythrocytosis.

ES Bachman MD PhD¹.

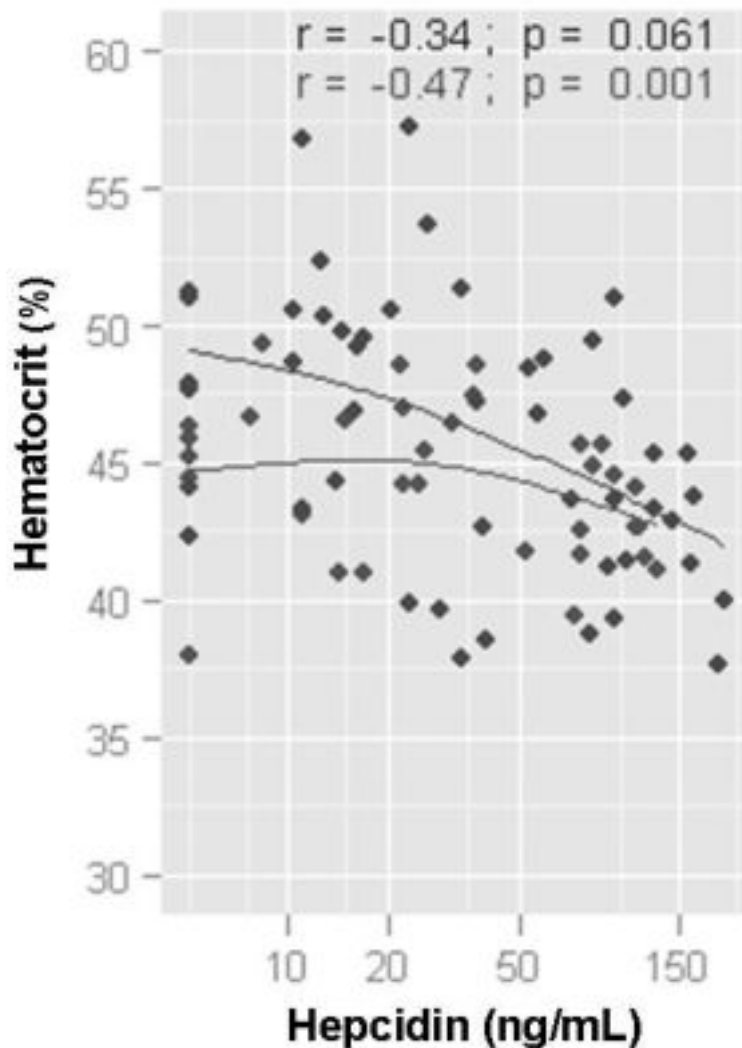
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An increase in hematocrit (polycythemia) is the most frequent adverse effect of testosterone (T) therapy. The hypotheses that T stimulates erythropoietin (Epo) and/or proliferation of erythroid progenitors lacks experimental support. For example, Epo levels remain unchanged during T-induced erythrocytosis in humans (1) and T has no direct effect on CD34+ erythroid progenitor cells (2). Hepcidin is a liver-derived peptide that plays a major regulatory role in iron bioavailability and red blood cell production (3). We tested the hypothesis that T regulates hepcidin. In order to test this hypothesis, we measured serum hepcidin in cohorts of healthy younger and older men who received varying doses of T (25, 50, 125, 300, 600 mg/week) for 20 weeks while endogenous T was suppressed by GnRH agonist administration (4,5). Baseline serum hepcidin levels were higher in older men ($p < 0.0001$). Testosterone administration suppressed serum hepcidin by more than 50% within 8 days in all men ($p < 10^{-8}$).



Suppression of hepcidin correlated with T dose ($p < 0.0001$), was significantly greater per dose and overall in older men ($p < 0.001$), and corresponded to the greater rise in hematocrit in older men. Serum hepcidin levels at mid-study were predictive of change in hematocrit at end of study.

Figure 2A



We conclude that altered iron bioavailability, via suppression of hepcidin, occurs in response to T administration and older men have a greater response. These data support the hypothesis that T-induced suppression of hepcidin, and increased iron bioavailability, underlies the basis for androgen-induced erythrocytosis. The biological consequences of T-regulated alterations in hepcidin/iron bioavailability, which may include non-hemoglobin related processes such as myoglobin synthesis, mitochondrial metabolism and cell division, remain to be elucidated.

1. Coviello et al. Effects of graded doses of testosterone on erythropoiesis in healthy young and older men. *JCEM*. 2008; 93(3):914-9195.
2. Kim et al. Direct and indirect effects of androgens on hematopoietic cells in vitro. *J. Korean Med Sci*. 2005; 20(3): 409-416.
3. Fleming, M. The regulation of hepcidin and its effects on systemic and cellular iron metabolism. *Hematology*. 2008; 151-158.
4. Bhasin, S. et al. Testosterone dose-response relationships in healthy young men. *Am J Physiol Endocrinol Metab*. 2001; 281:E1172-E1181.
5. Bhasin, S. et al. Older men are as responsive as young men to the anabolic effects of graded doses of testosterone on the skeletal muscle. *JCEM*. 2005; 90(2):678-688.

Sources of Research Support: Evans Medical Research Foundation and NIA grant U01AG14369.

Nothing to Disclose: ESB

P3-336

Do the Effects of Testosterone on Muscle Strength, Physical Function, Body Composition, and Quality of Life Persist Six Months Post-Treatment in Intermediate-Frail and Frail Elderly Men?.

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Introduction: Frailty is associated with increased risks of adverse outcomes and further functional deterioration in the elderly. The age-related decline in circulating testosterone (T) levels has been linked to the development of frailty. Short-term T treatment in frail elderly men can improve lean body mass and muscle strength. It is unclear if these effects of T can be maintained after treatment.

Methods: 262 intermediate frail and frail elderly men aged 65-90 mean(SD) 73.8(6.0) with total T \leq 346 ng/dL or free T \leq 250 pmol/L participated in a randomised double-blind placebo controlled trial to investigate the effects of transdermal T (2.5 - 7.5 mg daily) for 6 months. Participants were assessed at the end of treatment and 6 months following the cessation of treatment (i.e. 12 months after start of treatment). Outcome measures included body composition, muscle strength, physical function, and quality of life.

Results: Mean T increased from 316.8 (92.2) ng/dL at baseline to 529.9 (267.8) ng/dL at 6 months, then declined to 302.4 (121.0) ng/dL at 12 months, in the T-treated group. Isometric knee extension peak torque increased in the T-treated group compared to placebo to give an adjusted mean difference (95% CI) between groups of 8.6 (1.3 to 16.0) Nm at 6 months. This decreased to 4.0 (-3.9 to 11.9) Nm at 12 months, and was no longer significant ($p=0.32$). Lean mass increased significantly in the T group giving a difference between groups of 1.1 (0.6 to 1.5) kg at 6 months, this decreased post-treatment to a non significant difference at 12 months of 0.3 (-0.095 to 0.8) kg. Physical function did not improve significantly during or after treatment. Somatic and sexual symptom scores decreased during treatment, but there was no difference between groups at 12 months. Prostate Specific Antigen (PSA) levels and haematocrit increased within the physiological range during T treatment but returned to baseline by 12 months.

Conclusion: The effects of 6 months T treatment on muscle strength, lean mass and quality of life in frail men are not maintained at 6 months post treatment. Effects of T on muscle function are not sustained beyond the period of treatment.

Nothing to Disclose: MDLO, SAR, US-S, AT, MJC, JEA, JAO, FCWW

P3-337

Demonstration of Testosterone Effect on Ubiquitin and Ubiquitin-Protein Expression in Human Seminal Plasma by Top-Down Proteomic Strategy.

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Ubiquitin is a 76-residues protein, involved in protein degradation by the ubiquitin-proteasome system. Ubiquitination is important in processes such as apoptosis and antigen presentation. Ubiquitin is also involved in spermatogenesis and sperm-oocyte interactions and was recently reported as an indicator of mammalian sperm quality or fertility. Ubiquitin was also described in seminal plasma in greater concentration than spermatozoa. A possible role in control of sperm quality and of proteins secreted by the epididymal cells was proposed for ubiquitin conjugating enzyme of epididymal origin. We performed proteomic studies on seminal plasma by a top-down approach to investigate the role of testosterone on sperm ubiquitin and ubiquitin-protein expression. Semen samples were collected by 3 fertile normospermic men (sperm concentration (mean±SD) $56.67 \pm 15.27 \times 10^6$ /ml; progressive motility $54.0 \pm 5.29\%$; normal morphology $39.0 \pm 6.56\%$) and 3 patients affected by secondary hypogonadism (sperm concentration $50 \pm 34 \times 10^6$ /ml; progressive motility $32.0 \pm 13.4\%$; normal morphology $15.3 \pm 6.21\%$). Hormonal blood assay were: testosterone 1.59 ± 0.18 ng/ml (n.r. 3.5-8.0), estradiol 10.0 ± 2.0 pg/ml (n.r. 20-40), LH 1.57 ± 0.06 UI/l (n.r. 2.5-10.0), FSH 1.7 ± 0.1 UI/l (n.r. 2.5-8) in hypogonadal men and testosterone 4.9 ± 1.3 ng/ml, estradiol 25 ± 4.93 pg/ml, LH 5.1 ± 2.12 UI/l, FSH 4.2 ± 1.2 UI/l in controls. An aliquot of seminal plasma was mixed with aqueous trifluoroacetic acid, and centrifuged. The upper acidic supernatant was analyzed by an Ultimate 3000 Nano/Micro-HPLC apparatus equipped with an FLM-3000-Flow manager module coupled to an LTQ Orbitrap XL apparatus. The LTQ-Orbitrap mass spectrometer was operated in data dependent mode in which each full MS scan was followed by three MS/MS scans. The most abundant molecular ions were dynamically selected and fragmented by collision-induced dissociation. Tandem mass spectra were searched against the Swiss-Human.fasta database. Filtering criteria were XCorr versus charge 1.8, 2.5, for 2+, 3+ ions; mass accuracy 3 ppm; high value peptide confidence. Among the proteic spectrum we compared ubiquitin and ubiquitin expression among the hypogonadal group and controls. Ubiquitin-conjugating enzyme was identified in 3 control samples and was absent in all hypogonadal patients. This report describe, with a new proteomic approach, the role of testosterone in modulating ubiquitin proteic pattern, involved in sperm and proteic quality control.

Nothing to Disclose: DM, GG, FV, AB, AG, GP, MC, AP, RM, LDM

P3-338

Ganciclovir Induced Apoptosis of Germ Cells in HSV1-*tk* Transgenic Rats.

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We have reported that the transgenic (TG) rat, which was established using the chimera gene of porcine FSH beta subunit promoter fused to the Herpes simplex virus 1 thymidine kinase (HSV1-*tk*) gene, showed an ectopic expression of HSV1-*tk* gene in round spermatids in testes in addition to the expected tissue specific expression in the pituitary gland [1]. HSV1-TK phosphorylates nucleoside analogs such as ganciclovir (GCV) into monophosphorylated molecules. They are then converted into triphosphorylated substrates and are incorporated into elongating DNA, resulting interruption of DNA synthesis and apoptosis of dividing cells [2]. The aim of this study was to investigate cell toxicity of the metabolite of GCV to the germ cells in testes.

The adult TG and WT rats were intraperitoneally injected 30mg/kg GCV dissolved in 0.1M NaOH-saline (pH11.0) twice in daily for 2 weeks. Control animals were injected with 0.1M NaOH-saline (pH11.0). The weights of testes and epididymides in the GCV treated TG rats were significantly decreased in comparison with those of control animals. As apoptosis was not only observed in round spermatids but also in spermatogonia and spermatocytes, finally, only Sertoli cells and a few apoptotic spermatids were present. Together with our previous observation that the transgene is only detected in round spermatids but not in dividing spermatogonia, these results suggested that GCV is first phosphorylated in round spermatids, then is incorporated into Sertoli cells through phagocytic mechanism or cell-cell junctions, and thereafter leaks to neighboring spermatogonia. Incorporated metabolite might interrupt DNA synthesis and lead cell death of dividing germ cell line but not Sertoli cell which does not proliferate in adult rats. Thus the current study confirmed that communications between Sertoli cells and germ cells were disrupted by GCV administration. To clarify the mechanisms of Sertoli cell-mediated ablation of germ cells may contribute to further understanding in the interactions between germ cells and Sertoli cells in the rat.

[1]. Cai LY, *et al.* J Reprod Dev. 2007;53:201-209.

[2]. Moolten FL. Cancer Res. 1986;46:5276-5281.

Nothing to Disclose: KI, LYC, AO, TK, YK

P3-339

A New Missense Mutation in the Leptin Gene Causes Mild Obesity and Hypogonadism without Affecting T Cell Responsiveness.

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Introduction: Leptin, a protein-product of adipocytes, plays a critical role in the regulation of body weight, immune function, pubertal development and fertility. So far, only three homozygous mutations in the leptin gene in a total of 13 individuals have been found leading to a phenotype of extreme obesity with marked hyperphagia and impaired immune function.

Methods: Serum leptin was measured by ELISA. The leptin gene (OB) was sequenced in patient DNA. The effect of the identified novel mutation was assessed using HEK293 cells.

Results: We describe a 14 year old child of non-obese Austrian parents without known consanguinity. She had a body mass index of 31.5 kg/m² (+2.46 SDS) and undetectable leptin serum levels. Sequencing of the leptin gene revealed a hitherto unknown homozygous transition (TTA to TCA) in exon 3 of the LEP gene resulting in a L72S replacement in the leptin protein. RT-PCR, Western blot and immunohistochemical analysis indicated that the mutant leptin was expressed in the patient's adipose tissue, but retained within the cell. Using a heterologous cell system we confirm this finding and demonstrated that the side chain of L72 is crucial for intracellular leptin trafficking. Our patient had signs of a hypogonadotropic hypogonadism. However, in contrast to the literature, she showed only mild obesity and a normal T cell responsiveness.

Conclusions: These findings shed a new light on the clinical consequences of leptin deficiency.

Congenital leptin deficiency should be considered possible in pediatric patients with mild obesity even if parents are lean and unrelated.

Sources of Research Support: Ministry of Science, Research and Arts Baden-Wuerttemberg and European Social Fund to PFP; German Federal Ministry of Education and Research (National Genome Research Network, BMBF 01GS0824 and BMBF 01GI0851) to MW.

Nothing to Disclose: PF-P, JvS, BM, GL, GS, TB, JK, HM, PM, K-MD, PG, MW

P3-340

Expression of Androgen and FSH Receptors and AMH in Testis of Lambs Prenatally Exposed to an Excess of Testosterone.

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We (1) previously showed that prenatal exposure to an excess of testosterone (PET) induces a decrease in sperm count and motility together with a reduced number of germ cells and a higher number of Sertoli cells in seminiferous tubules in the adult ram. It is not clear whether these alterations are triggered before adulthood. The expression of antimüllerian hormone (AMH), androgen receptor (AR) and FSH receptor (FSHR) are crucial during normal testicular development and may be markers of the normal development of Sertoli cells. Our aim was to evaluate the effect of the PET on the expression of AMH, AR and FSHR in prepubertal lambs. Therefore, we evaluated mRNA levels of AMH, AR and FSHR by real time PCR in testis of male lambs at 4 weeks of age. We studied 8 lambs (T-males) born to mothers treated with 30 mg testosterone propionate (TP) between 30 and 90 days of pregnancy, followed by 40 mg TP between 90 and 120 days of pregnancy (birth at term: 147 days). As control we studied 6 lambs (C-males) born to mothers treated with vehicle. No differences were found in the body or testis weight between C- and T-males. AMH expression was similar between groups (mean \pm SEM; 880.9 \pm 97.5 fg/mg total RNA in C-males vs. 763.3 \pm 67.1 fg/mg total RNA in T-males). FSHR expression was significantly lower in T-males (447.0 \pm 17.4 fg/mg total RNA in C-males vs. 367.0 \pm 23.7 fg/mg total RNA in T-males; $P \leq 0.05$). Although the expression of AR was similar between groups (mean \pm SEM; 10.1 \pm 0.9 fg/mg total RNA in C-males vs. 8.7 \pm 0.9 fg/mg total RNA in T-males; $P=0.156$), 5 of 8 T-males (mean \pm SEM; 6.9 \pm 0.2 fg/mg total RNA) showed expression levels below the range of expression shown by the C-males. A positive correlation was found between the level of AR expression and testis weight in the C-males ($r=0.951$; $P \leq 0.05$), but not in the T-males. These results suggest that the prenatal exposure to excess testosterone impairs the normal postnatal development of the testis in lambs, by affecting the expression of key reproductive receptors expressed by Sertoli cells during early prepuberty

(1) Recabarren SE et al. Endocrinology 2008;149:6444

Sources of Research Support: Fondecyt Grant 1090031.

Nothing to Disclose: PPR-G, SP, MM, MPR, HT, RR, TS-P, SER

P3-341

Evaluation of the Gonadal Axis Function in Males Exposed to Excess Exogenous Testosterone during Fetal Development.

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¹Univ of Concepcion Chillan, Chile and ²Univ of Chile Santiago, Chile.

The prenatal exposure to an excess of Testosterone (T) has a profound impact on reproductive and metabolic functions in female sheep. These alterations resemble those exhibited by women with the Polycystic Ovary Syndrome, giving experimental support to the hypothesis of an involvement of an excess of T in the fetal programming of this syndrome. Nevertheless, few studies have addressed the effect of the prenatal exposure to an excess of Testosterone (PEET) on reproductive and metabolic function in males. The aim of the present study was to assess the impact of PEET on the pituitary-testis axis based on the LH and T response to a GnRH analogue test in male sheep. Control Suffolk Down male sheep (C-males, n=6) and males born to mothers exposed to twice weekly injections of 30 mg T propionate from day 30 to 90 and of 40 mg T propionate from day 90 to 120 of gestation (T-males, n=6) were studied at 20 and 30 weeks of age, ages which represent peripubertal and adult stages of sexual development. The GnRH analogue test consisted in administrating a bolus of a GnRH analogue by means of one indwelling jugular catheter (leuprolide acetate, 10 ug/kg BW) and collecting blood samples using a second catheter at 0, 0.5, 1, 1.5, 2.0, 2.5 and 3 hours of the GnRH challenge to determine the acute response and followed by further sampling at 6, 9, 12, 18, 24, 30, 36, 42 and 48 hours of the start of the test to recognize the long lasting effect of GnRH analogue on LH and T secretion. In each sample, LH and T concentrations were determined by radioimmunoassay (RIA). LH and T concentrations during the first three hours and during the whole experiment (48 hours) were converted in area under the curve (AUC). Basal plasma T concentrations were higher in T-males. There was a higher secretion of LH (P <0,05) in T-males of 20 weeks of age compared to C-males and to T-males of 30 weeks of age. However, T secretion was similar between C- and T-males of either age. The T/LH ratio was higher in C-males of 30 weeks of age than in T-males of the same age suggesting different Leydig cell sensitivity to LH. Results suggest that the PEET produces an alteration in the gonadal axis of peripubertal males which is translated in an increase in the responsiveness of the pituitary to the GnRH stimulus in terms of LH secretion which is not accompanied by a same responsiveness to LH in the T secretion

Sources of Research Support: Fondecyt Grant 1050915;Fondecyt Grant 1090031.

Nothing to Disclose: MPR, SER, PPR-G, FA, TS-P

P3-342

Initial Characterization of the Human Seminal Plasma Proteome in 3 Fertile Normogonadic Men by Top-Down Strategy.

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Human seminal plasma contains a large array of proteins required for the normal physiology of spermatozoa and fertilization. To provide informations about the physiological mechanisms of male fertility we performed proteomic studies on human seminal plasma by a top-down approach. Semen samples were collected by 3 fertile normospermic men (sperm concentration (mean±SD) $56.67 \pm 15.27 \times 10^6$ /ml; progressive motility $54.0 \pm 5.29\%$; normal morphology $39.0 \pm 6.56\%$). Hormonal blood assay were performed: testosterone (mean±SD) 4.9 ± 1.3 ng/ml (n.r. 3.5-8.0), estradiol 25 ± 4.93 pg/ml (n.r. 20-35), LH 5.1 ± 2.12 UI/l (n.r. 2.5-10.0), FSH 4.2 ± 1.2 UI/l (n.r. 2.5-8). An aliquot of seminal plasma was mixed (1:40) with aqueous trifluoroacetic acid (TFA/H₂O 0.2% v/v), and centrifuged. The upper acidic supernatant was analyzed by an Ultimate 3000 Nano/Micro-HPLC apparatus equipped with an FLM-3000-Flow manager module coupled to an LTQ Orbitrap XL apparatus. The column was a Dionex C18 with 3 µm particle diameter. The chromatography eluents were A TFA/H₂O 0.056% (v/v) and B CH₃CN+ 0.050% TFA. The applied gradient was linear from 0 to 50% of solvent B in 60 min, at flow rate of 4.5 µl/min. The LTQ-Orbitrap mass spectrometer was operated in data dependent mode in which each full MS scan (60000 resolving power) was followed by three MS/MS scans. The most abundant molecular ions were dynamically selected and fragmented by collision-induced dissociation (CID) using a normalized collision energy of 35%. Tandem mass spectra were searched against the Swiss-Human.fasta database using SEQUEST. The results were filtered using the following criteria: XCorr versus charge 1.8, 2.5, for 2+, 3+ ions; mass accuracy 3 ppm; high value peptide confidence. A total of 114 proteins were present in all samples. Based on the molecular function and biological process of the proteins, as reported in literature, 43 were classified as sperm structural, 36 proteins as intracellular (including cell enzymes, cell cycle proteins and transcriptional factors), 16 secreted, and 6 as sperm movement proteins. 12 proteins didn't present known molecular function. This report is the first identification, based on new approach and stringent criteria, of seminal plasma proteome in fertile men. Proteomics may give new insight about the molecular mechanisms of reproduction and may permit the identification of molecular markers in infertile patients.

Nothing to Disclose: DM, GG, FV, AG, AB, ALA, MC, AP, LDM, RM

P3-343

Retinoblastoma Protein pRb Is Required in Sertoli Cell To Support Male Germ Cell Development.

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The functional importance of retinoblastoma protein (pRb) and E2F family partners in testicular development has been enigmatic. We reasoned that the Rb-deficient mouse would be a useful model to elucidate the effects of sustained Rb deficiency on Rb pathways in the developing testis. The phenotypic analysis of Rb+/- mice showed a reduced spermatogenesis at both qualitative and quantitative levels. Furthermore, we found that pRb has an anti-apoptotic function and plays a role in the chromatin remodelling process during spermatogenesis. In the mouse seminiferous epithelium, Sertoli cells (SC) produce pRb in a cyclic fashion, suggesting that pRb regulates spermatogenesis via controlling SC proliferation and function. We generated a SC-Rb specific knockout (SC-RbKO) mouse line using the Cre-Lox system. The most marked phenotype in SC-RbKO mice is the progressive impairment of spermatogenesis resulting in a significant decrease in testis size in adult mice, loss of germ cell - SC contact, an increase in germ cell apoptosis, and infertility. To better understand the role of *rb* in testicular development and maintenance of male fertility, we have performed a microarray analysis to explore its downstream genes in SC-RbKO, heterozygous, and control animals. Expression profile indicates alterations of several genes involved in DNA replication, G1/S transition of mitotic cycle, single strand break repair, mismatch repair, and homologous recombination. Our results suggest that there is a tight control of specific E2F transcription factor expression by Rb during spermatogenesis. A thorough molecular analysis of our mouse model, having dysregulated Rb and E2F proteins will provide insights into the role of pRb in SC maturation and function. Moreover, SC-RbKO model will help us to clarify the mechanisms underlying the link of Rb and E2F proteins with testicular tumorigenesis.

Sources of Research Support: Academy of Finland; Finnish Cultural Foundation; Sigrid Jusélius Foundation; Pediatric Research Foundation and Turku University Hospital.

Nothing to Disclose: SB, ER, AR-M, MN, MP, NK, JT

P3-344

Cytochromes P450 2C19, 3A7, POR and PXR Transcription Factor Polymorphisms on the Pharmacogenomics of Testosterone in Hypogonadal Males.

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Context: Testosterone replacement for hypogonadism restores quality of life and sexual function. Although, the testosterone therapy is well established, an interindividual variability in dose requirement is observed, which could be genetically determined. Single nucleotide polymorphisms in cytochromes P450 and PXR transcription factor have been shown to have a significant effect on requirement of many drugs in clinical use, such as warfarin and omeprazole. To date, there is no study in the pharmacogenetics of testosterone in hypogonadal patients, in order to obtain an individualization of testosterone therapy. **Objectives:** to evaluate if PXR, POR*28 (A503V), CYP3A7*1C, CYP2C19*17 variants could account for differences in testosterone levels during therapy for hypogonadism. **Patients and Methods:** We selected 31 hypogonadal patients who presented basal testosterone levels < 84 ng/dL. Testosterone cypionate (200 mg IM every 2 or 3 weeks) was the standard therapy for all patients. Chronic illness was ruled out in all patients. DNA samples were extracted from peripheral leukocytes and the entire PXR, exons 12-13 of POR, CYP3A7 and CYP2C19 promoter regions were PCR amplified and products were submitted to sequencing. Testosterone levels were assessed by a fluoroimmunoassay at 11-15 days after IM testosterone administration. Comparisons were performed by Student's t or Mann-Whitney tests. Statistical significance was set at p<0.05. **Results:** polymorphism frequencies were 23% for POR*28, 11.3% for CYP2C19*17, 1.8% for CYP3A7*1C and P27S PXR and 0.6% for G36R PXR. There were no differences in testosterone levels after 11-15 days between patients harboring the POR*28 allele (306 ± 223, ranging from 70 to 785 ng/dL) and wild-type (348 ± 128, from 127 to 519 ng/dL; NS), and between patients carrying the CYP2C19*17 allele (459 ± 263, from 70 to 649 ng/dL) and wild-type (295 ± 160, from 175 to 785 ng/dL; NS). One patient carried the POR*28 in homozygosis and two patients the CYP2C19*17 allele in homozygosis; their testosterone levels were 489, 338 and 538 ng/dL, respectively. **Conclusion:** We did not identify any influence of CYP2C19, CYP3A7, PXR allelic variants in the testosterone levels during the androgen replacement for hypogonadism in this cohort. The absence of significant differences suggests that other factors influence the pharmacogenetics of testosterone in our cohort.

Sources of Research Support: FAPESP# 05/04726-0.

Nothing to Disclose: BBA, LCSL, RPPM, LCK, EMFC, VNB, LFGS, TASSB, BBM

P3-345

Stress Induces Glucocorticoid-Mediated Apoptosis of Leydig Cells in the Rat.

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Background: The impairment of long term excessive stress or big pressure of spirit on male reproductive health is a fact without any argument. One of reasons is that stress can disrupt and suppress endocrine signaling in the male reproductive axis through high level of glucocorticoid, the hallmark of stress. Leydig cells, located within the interstitium of the testis, are the primary source of testosterone in male. Our previous work revealed that stress level of exogenous glucocorticoid can induce apoptosis of Leydig cell in adult male rats. The aim of this study is to investigate whether stress can directly induce rat Leydig cell apoptosis in vivo and whether the process is the result of direct effect of glucocorticoid.

Methods: The rat model of chronic stress was established through treating adult male Sprague-Dawley (SD) rat with 21-day continuous unpredictable stress. Serum concentrations of corticosterone (glucocorticoid in rat, CORT) and testosterone in stressed rats were assayed with enzyme immunoassay. Apoptosis was identified by electron microscopy and FACS analysis of annexin V-FITC labeling. The localization of glucocorticoid receptor (GR) in Leydig cells of stressed rats was observed with immunofluorescence by laser confocal microscopy. Knockdown of GR in Leydig cells was performed through RNA interference, to identify whether the glucocorticoid-induced apoptosis of Leydig cells is mediated by GR.

Results: Our results showed that serum CORT and catecholamine concentrations in stressed rats were significantly increased. However, testosterone showed an adverse change. The apoptotic frequency of Leydig cell in stressed animals was increased significantly. Ultrastructural analysis confirmed that Leydig cells subjected to stress treatment showed characteristic signs of apoptosis. The catecholamine of stress level could not increase the apoptotic frequency of Leydig cells in vivo and in vitro. The activation of GR was involved in stress-induced Leydig cell apoptosis. Western blot analysis revealed the expression of GR was decreased obviously in Leydig cells infected with recombinant adenovirus expressing microRNA. Downregulation of the expression of GR alleviated the CORT-induced increase in apoptosis of Leydig cells.

Conclusions: These results suggest that stress can induce rat Leydig cell apoptosis and the process is the result of direct effect of glucocorticoid.

Nothing to Disclose: HBG, YC, PZ, QW

P3-346

Effect of Ethane 1,2-Dimethane Sulfonate(EDS) on the Histology of Epididymis and Seminal Vesicle in Adult Rats.

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Ethane 1,2-dimethane sulfonate(EDS) is a well-known alkylating agent used as selective rat Leydig cell(LC) toxicant to create a testicular dysfunction model. Previous studies clearly demonstrated the dramatic weight loss of the androgen dependent accessory sex organs such as epididymis, seminal vesicle and prostate gland in this 'LC knock-out' rats. The present study was performed to evaluate the effect of EDS administration on histological changes of the epididymis, seminal vesicle and prostate in adult rats.

Adult male Sprague-Dawley rats(350~400g B.W.) were injected with a single dose of EDS(75mg/kg, i.p.) and sacrificed on weeks 0, 1, 2, 3, 4, 5, 6 and 7. Tissue weights(testis, epididymis, seminal vesicle and prostate gland) were measured, numbers of epididymal sperms were counted, and serum testosterone levels were determined by specific radioimmunoassay. The histological changes of the tissues were observed by a light microscopy using Hematoxylin & Eosin staining.

Weights of the reproductive and accessory organs progressively declined after the EDS treatments(weeks 1, 2 and 3). After this, the decrements were stopped, then gradually returned to the normal levels in all tested organs except epididymis. There was a partial(about 60%) recovery of the epididymis weight during weeks 6-7. Serum testosterone levels decreased significantly on week 1 after EDS treatment, then returned to the initial levels during weeks 5-7. The cross section of epididymis revealed that remarkable increase in thickness of the epithelium during weeks 1-3. In contrast, considerable reduction of epithelial thickness in seminal vesicle was observed during the same period. Similarly, a noticeable reduction in thickness of prostate epithelial layer was found during weeks 1-3, then back to normal thickness after week 4.

Taken together, the present study demonstrated that the temporally induced androgen-deficiency by EDS administration could result the prominent alterations in histology of the accessory sex organs. Further studies on the physiological and molecular regulation of these androgen-sensitive organs using EDS model will be helpful to understand the normal and pathological development and differentiation mechanism of these organs.

Nothing to Disclose: SHL, WYL

P3-347

Curcumin Derivatives Inhibit Testicular 17 β -Hydroxysteroid Dehydrogenase 3.

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17 β -hydroxysteroid dehydrogenase 3 (17 β -HSD3) is a steroidogenic enzyme to catalyze the conversion of androstenedione into testosterone. 17 β -HSD3 is expressed in Leydig cells as well as in prostate cancer. 17 β -HSD3 is an attractive target for inhibitors for the treatment of prostate cancer or the development of male contraceptive. We screened many small molecules purified from herbs and found that curcumin a selective inhibitor of 17 β -HSD3 with the half maximal inhibitory concentration (IC₅₀) of 9.0 ± 1.0 (mean \pm SEM, n =4), 2.3 ± 1.2 and 67.3 ± 11.9 μ M in rat intact Leydig cells, rat and human testis microsomes, respectively. We further synthesized forty curcumin analogues and tested their potencies of inhibiting 17 β -HSD3. We found that compound C3 is a novel potent and selective inhibitor of 17 β -HSD3 with an IC₅₀ 100 nM for human testis microsomes.

Nothing to Disclose: GXH, GL, YHC, XKL, QQL, HL, YDH, DOH, RSG

P3-348

Corticotropin-Releasing Hormone Acts on CRHR1 To Differentially Modulate $[Ca^{2+}]_i$ in Human Myometrium Cell before and during Labour.

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Corticotrophin-releasing hormone (CRH) has been implicated in modulation of uterine contractility during pregnancy. Intracellular Ca^{2+} ($[Ca^{2+}]_i$) is a key factor in the modulation of uterine contraction. The objective of this study is to investigate the effects of CRH on $[Ca^{2+}]_i$ in human pregnant myometrium cells, and compare these effects in labouring and nonlabouring myometrium. The myometrial tissues were obtained from pregnant women who were undergoing labour or not undergoing labour at term. The myometrial cells isolated and cultured. Radioimmunoassay was used to measure the CRH content in the culture media. $[Ca^{2+}]_i$ transient was determined by real-time confocal imaging. It was found that cultured human pregnant myometrial cells secreted CRH. CRH (10^{-10} - 10^{-7} mol/L) produced dose-dependent increase in $[Ca^{2+}]_i$ in myometrium cells from women underwent labour. These effects were reversed by CRH receptor type 1 (CRHR1) antagonist antalarmin, but not by CRH receptor type 2 (CRHR2) antagonist astressin 2b. For the nonlabouring myometrium, CRH did not induce $[Ca^{2+}]_i$ transient in myometium cells. Antalarmin(10^{-9} - 10^{-6} mol/L) dose-dependently increased $[Ca^{2+}]_i$ in the cells, whereas astressin 2b did not. The effects of antalarmin could be blocked by exogenous CRH, suggesting that endogenous CRH has a tonic inhibitory effect on $[Ca^{2+}]_i$. The possible signaling pathways involved in the CRH regulation of $[Ca^{2+}]_i$ were also investigated. For the labouring myometrium, blocking phospholipase C (PLC) activity with U73122 and application of IP3 receptor antagonist blocked CRH-evoked $[Ca^{2+}]_i$ transient. CRH (10^{-10} - 10^{-7} mol/L) dose-dependently induced phosphorylated PLC- β 3 expression in labouring myometrium cells. For nonlabouring myometrium, CRH (10^{-10} - 10^{-7} mol/L) dose-dependently increased cAMP production in the cells. Forskolin, the adenylate cyclase activator, reversed antalarmin-induced $[Ca^{2+}]_i$ transient. SQ 22536, an adenylate cyclase (AC) inhibitor, induced $[Ca^{2+}]_i$ transient. Our results indicate that CRH act on CRHR1 to induce $[Ca^{2+}]_i$ transient in labouring myometrium, but inhibit $[Ca^{2+}]_i$ in nonlabouring myometrium. Before labour onset, CRHR1 may coupled with Gs protein and CRH activates AC-PKA signaling pathway to decrease $[Ca^{2+}]_i$ in myometrium cells. During labour, CRHR1 may couple with Gq protein and CRH activates PLC/IP3 signaling pathway to increase $[Ca^{2+}]_i$ in myometrium cells.

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Nothing to Disclose: XN, XJY, LG, NH, HG

P3-349

Altering Coregulator Concentration by Conditional Genetic Modification: Gene Dosage of REA Is Critical for Fertility and Uterine Function.

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Estrogen and progesterone and their receptors are important regulators of successful female reproduction. Although the effectiveness of hormone-receptor complexes is known to depend on coregulator partner proteins, little is known about the crucial roles of coregulators in early stages of pregnancy and implantation, and in uterine development. We previously identified a coregulator, repressor of estrogen receptor activity (REA), and found that its conventional knockout was embryonic lethal, suggesting an essential role of REA in early embryonic development. In order to define the roles of REA in early stages of pregnancy, and in post-embryonic stages, we generated REA conditional knockout mice (REA^{fl/fl}PR^{Cre/+}) by cre-loxP recombination in which REA function was abrogated only in progesterone receptor (PR)-expressing tissues. Conditional homozygous mutants were found to develop to adulthood, but female homozygous mutant mice were completely infertile. Their ovaries were found to be normal, demonstrated by ovulation and corpora lutea formation. However, uterine development and function were severely compromised, and no implantation occurred in the uteri of mice lacking REA. Histological evaluation of the uterine tissues of homozygous REA mutant mice showed abnormal growth and maturation, with this altered phenotype developing between 10-14 days of age. Functional maturation and proliferation of uterine cells was arrested, resulting in a hypoplastic uterus showing poor response to estradiol. These phenotypic features indicate that REA is necessary for normal growth and maturation of the uterus.

Interestingly, female mice heterozygous for REA (REA^{fl/+}PR^{Cre/+}) had a uterine phenotype very different from that of homozygous knockout mice. While the uterine morphology of conditional REA heterozygous females was similar to that of the wild type mice, treatment with estradiol resulted in abnormally large uteri. Taken together, our findings reveal that REA is essential for normal uterine function and for successful implantation and fertility. They also demonstrate that correct REA gene dosage is critical for optimal uterine development and for successful reproduction.

Sources of Research Support: SCCPIR NICHD U54 HD055787.

Nothing to Disclose: SHP, SY, YZ, LL, ZL, JX, JPL, FJD, BWO, MKB, BSK

P3-350

Global Expression Analysis of Nuclear Receptors and Co-Regulators Reveals DNA Acetylation and Methylation and Select Gene and Signaling Pathway Dysregulation in Eutopic Endometrium of Women with Endometriosis.

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Introduction: Endometriosis is a sex hormone-dependent disorder characterized by endometrial tissue outside the uterus. Recently we studied the involvement of the PR co-activator Hic-5 in its pathogenesis. Herein, we investigated global expression of nuclear receptors and co-regulators in eutopic endometrium throughout the menstrual cycle in women with and without severe disease.

Materials & Methods: Endometrial tissue was collected in the proliferative (P, n=4/group), early secretory (ES, n=4/group), and mid secretory (MS, n=4 and n=5 respectively) phases of the menstrual cycle and processed for total RNA. Nuclear receptor and co-regulator PCR arrays were used that included 84 genes. Significance for ≥ 1.5 fold differences was $p \leq 0.05$. Ingenuity Pathway Analysis (IPA) evaluated the participating networks and canonical pathways.

Results: IPA identified DNA methylation and transcriptional repression signaling as the most affected pathway in endometrium, regardless of cycle phase in women with vs. without disease. Specifically, HDAC1 and HDAC2, which interact directly with proteins recruited to steroid hormone receptors, were up-regulated and MTA1, a potent ER co-repressor, was down-regulated. In ES, cell signaling, gene expression and drug metabolism networks were regulated (NCOA1 and NCOR1, involved in regulation of thyroid hormone receptor (THR) and vitamin D receptor (VDR) pathways). In MS only one gene (retinoic acid receptor beta (RARβ)) was up-regulated. IPA of endometriosis samples throughout the menstrual cycle revealed regulation of thyroid cancer signaling in ES vs. P; DNA methylation and transcriptional repression signaling (decrease in HDAC2) in MS vs. P, and thyroid cancer signaling and RA-mediated apoptosis signaling (decrease in RARβ) in MS vs. ES. In the absence of disease, up-regulation of PPAR signaling (NCOA1) in ES vs. P was noted. In MS vs. P, THRA and ESR1 transcripts were down-regulated, and NCOR1 mRNA was up-regulated.

Conclusions: Differences in endometrial nuclear receptor/co-regulator transcripts in women with vs. without endometriosis underscore DNA methylation and transcriptional repression as the most regulated canonical pathway and the importance of THRA ESR, and RAR dysregulation in endometrium in women with endometriosis.

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Nothing to Disclose: ZZ, LA, JCI, LCG

P3-351

Statins Decrease First Trimester Placental Function by Altering IGF1R Glycosylation and Trafficking to the Plasma Membrane.

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The rapid rise in obesity, metabolic syndrome and type 2 diabetes is one of the major healthcare problems of the Western world. Affected individuals are often treated with statins (HMG CoA reductase inhibitors) to reduce circulating cholesterol levels and the risk of developing cardiovascular disease; given the evolving demographic profile of these conditions, such drugs are increasingly prescribed to women of reproductive age. Recently we demonstrated that treatment of pregnant women with statins (pravastatin (PV) and cerivastatin (CV)) could have a detrimental effect on fetal growth by inhibiting the action of insulin-like growth factor (IGF)-I and -II which are key regulators of trophoblast proliferation and placental development. IGF signalling through the type 1 IGF receptor (IGF1R) is dependent on appropriate receptor presentation at the surface of trophoblast. Glycosylation influences intracellular trafficking, and N-linked glycosylation is disrupted by statin treatment. Here we compare the effects on the placental IGF response of treatment with statin or inhibitors of glycosylation.

First trimester villous placental explants were exposed to statins (PV (250nM) or CV (50nM)) or glycosylation inhibitors (tunicamycin (TM; 1ug/ml), deoxymannojirimycin (DMJ; 0.5mM) or castanospermine (CS; 5ug/ml) for 24h, IGF-I or IGF-II (10nM) was added and 24h later, proliferation was assessed by immunohistochemical (IHC) analysis of Ki67. The inhibition of IGF-induced proliferation by statins ($p < 0.05$, $n=5$) was similar to that observed in the presence of glycosylation inhibitors (TM, $P < 0.01$; CS, $P < 0.05$; DMJ, $P < 0.05$). IHC analysis of IGF1R localisation revealed decreased membrane expression following exposure of tissue to either statins or glycosylation inhibitors. Dot blots of IGF1R immunoprecipitates using four lectins (Con A, PSA, e-PHA and L-PHA) that recognise N-linked glycans confirmed reduced receptor N-glycosylation after statin exposure.

These data suggest that N-glycosylation of IGF1R in syncytiotrophoblast controls receptor delivery to the maternal-facing microvillous membrane and the tissue response to maternal IGF.

Nothing to Disclose: KF, VKS, JMG, JDA, MW

P3-352

Decidual PTEN Is Required for Trophoblast Invasion.

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During implantation, fetal trophoblast cells invade the uterine tissue to anchor the placenta to the uterus and to remodel the maternal vasculature, thereby assuring an adequate placental blood flow. Trophoblast invasion likely depends on complex crosstalk between the fetal and maternal tissues and may involve the modulation of PI3K/AKT signaling activity in maternal decidual cells. In this report, we aimed to study the effects of chronic derepression of the PI3K/AKT pathway during implantation in *Pten*^{tm1Hwu/tm1Hwu};*Amhr2*^{tm3(cre)Bhr/+} mice, which lack the PI3K signaling antagonist gene *Pten* in the myometrium and in stromal/decidual cells. Primiparous *Pten*^{tm1Hwu/tm1Hwu};*Amhr2*^{tm3(cre)Bhr/+} mice were found to be subfertile due to increased fetal mortality at 11.5dpc. Histopathologic analyses revealed a failure of decidual regression in these mice, accompanied by reduced or absent invasion of fetal trophoblast glycogen cells and giant cells, abnormal development of the placental labyrinth, and variable degrees of intrauterine fetal growth restriction. TUNEL and BrdU incorporation analyses associated the lack of decidual regression with decreased apoptosis and increased proliferation of *Pten*^{tm1Hwu/tm1Hwu};*Amhr2*^{tm3(cre)Bhr/+} decidual cells. Remodeling of the maternal vasculature was delayed in *Pten*^{tm1Hwu/tm1Hwu};*Amhr2*^{tm3(cre)Bhr/+} uteri at 11.5dpc, as evidenced by persistence of vascular smooth muscle and decreased infiltration of uterine natural killer cells. In addition, hypertrophy and disorganization of the muscle fibers was observed in the myometrium before and throughout gestation. Almost all *Pten*^{tm1Hwu/tm1Hwu};*Amhr2*^{tm3(cre)Bhr/+} failed to carry a second litter to term, apparently due to endometrial hyperplasia and uterine infections. Surprisingly, western blotting and immunohistochemistry analyses revealed that the deletion of *Pten* did not alter uterine AKT or phospho-AKT expression, nor the expression or activity of AKT downstream effectors in *Pten*^{tm1Hwu/tm1Hwu};*Amhr2*^{tm3(cre)Bhr/+} 11.5dpc uteri. This may indicate that PTEN has biological functions other than the modulation of PI3K/AKT signaling in maternal decidual cells. Together, these data demonstrate novel roles of PTEN in the mammalian uterus, and its requirement for proper trophoblast invasion.

Nothing to Disclose: MNL, JD, MP, AB, JSR, SLA, DSB

P3-353

miRNAs Differentially Expressed during Human Trophoblast Differentiation May Modulate Aromatase/*hCYP19A1* Gene Expression.

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To define mechanisms for human trophoblast differentiation and induction of aromatase/*hCYP19A1* gene expression, we have utilized a model system wherein mononuclear cytotrophoblasts (CYT) isolated from mid-gestation human placenta spontaneously fuse in culture to form syncytiotrophoblast (SYN) with a concomitant induction in *hCYP19* expression. This is associated with enhanced binding of endogenous estrogen receptor α (ER α) (1) and estrogen-related receptor γ (ERR γ) to response elements in the *hCYP19* placenta-specific promoter (1.1). ERR γ also serves a critical role in O₂-dependent upregulation of *hCYP19* expression in cultured trophoblast cells. MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate expression of specific mRNA targets and have been implicated in cell proliferation, differentiation and transformation. To define the roles of miRNAs in regulation of human trophoblast differentiation and induction of *hCYP19* expression, we performed miRNA microarray analysis of RNA from human CYT before (0 h) and after 24 and 48 h of culture (SYN). Analyses of the microarray data revealed 16 miRNAs that were significantly ($p < 0.05$) down-regulated and 7 miRNAs that were upregulated >2 -fold at both 24 and 48 h, as compared to 0 h. Downregulated miRNAs included several members of the polycistronic miR-17-92 cluster (miR-17, 19b, 20a). Using TargetScan analysis, we searched for predicted conserved mRNA targets of these miRNAs that may be of importance in trophoblast differentiation. Interesting targets include CYP19A1, GCM1, a transcription factor critical for labyrinthine trophoblast development, HIF-1 α , ER α and its coactivator SRC-3/AIB1. Other downregulated miRNAs included 519c-3p, 518f, 519e, 515-3p and 518e, known to be placenta-specific (2). Three members of this family, miR-517, miR-518b and miR-519e, have been reported to be upregulated in severe preeclamptic placentas (3), suggesting possible induction by hypoxia. Differential expression of these miRNAs in freshly isolated cytotrophoblasts vs. differentiated syncytiotrophoblasts was confirmed using quantitative real-time SYBR[®] Green and TaqMan-based qRT-PCR. Importantly, overexpression of miR-19b in cultured trophoblast cells caused a pronounced decrease in aromatase protein expression, identifying *hCYP19A1* as a target of miR-19b. Studies are in progress to elucidate the functional roles for these differentially expressed miRNAs during human placental differentiation.

1.Kumar P and Mendelson C.R., 2009, Mol Endo, 23,784-793.

2.Luo S., et al., Biology of Reproduction, 2009. 81, 717-729.

3.Zhu, X et al., American Journal of Obstetrics & Gynecology, 2009, 661.e7.

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Nothing to Disclose: PK, CRM

P3-354

NF- κ B Mediated Pro-Survival Signaling Occurs in Response to Elevated Uterine Caspase-3 Activity.

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Activation of caspase-3 is normally associated with the onset of apoptotic cell death, however in the pregnant uterus we have identified elevated levels of non-apoptotic uterine caspase-3 activity (1). The aim of this study is to identify the mechanism by which the pregnant uterus protects itself from the apoptotic action of caspase-3. Utilizing a stretch activated uterine caspase-3 model we test the hypothesis that the pregnant uterus maintains a non-apoptotic status by hosting an NF- κ B mediated pro-survival anti-apoptotic response.

Uteri were isolated and flash frozen from unilaterally pregnant rats from gestation day (gd) 6 to 23 and unilaterally pregnant rats at gd 12 undergoing artificial uterine stretch for 24 hours using an intra-uterine expandable tube in the empty uterine horn. mRNA, cytoplasmic, nuclear and mitochondrial proteins were isolated from the flash frozen samples. These model systems provide us a unique opportunity to examine the molecular response in vivo to stretch activated caspase-3 from gestationally matched, stretched and non-stretched uteri.

In the unilaterally pregnant rat, we confirm that caspase-3 is activated solely in the pregnant uterine horn at gd15 and decreases as term approaches. In contrast the non pregnant horn exhibits barely detectable levels of caspase-3 activity. Similarly in the artificially stretched uteri, caspase-3 activation was isolated to the stretched uterine horn. In response to elevated levels of uterine caspase-3 activity NF- κ B activation was also limited to the stretched uteri and increased towards term. The non pregnant uterine horn demonstrated barely detectable levels of NF- κ B activation at any gestational time point. XIAP and Mcl-1, both anti-apoptotic, pro-survival factors previously identified to be regulated directly by NF- κ B activation were both increasingly elevated in the stretched uterine horn towards term. These data lead us to conclude that increased mechanical stretch as a result of expanding fetal dimensions towards term has the capacity to not only trigger caspase-3 activity but also to activate a pro-survival uterine NF- κ B mediated response pathway. Activation of an NF- κ B mediated anti-apoptotic response through elevated XIAP and Mcl-1 ultimately limits the apoptotic actions and the levels of uterine caspase-3 as term approaches thereby regulating the timing of labor.

Jeyasuria P, Biol Reprod. 2009 May;80(5):928-34.

Sources of Research Support: March of Dimes #21-FY08-555.

Nothing to Disclose: AS, PJ, JCC

P3-355

Uterine Quiescence during Pregnancy Is Maintained by Myometrial Caspase Activation Triggered by the Endoplasmic Reticulum Stress Response.

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We have recently described that uterine caspase-3 activation during pregnancy facilitates the maintenance of uterine quiescence by disruption of the myometrial contractile architecture in a non-apoptotic and reversible manner (1). In the pregnant mouse uterus caspase-3 levels barely detectable at gd 12, reach maximal levels at gd 15 and decline to undetectable levels as term approaches. In order to successfully harness the action of uterine caspase-3 as an anti-contractional agent we must first understand its mechanism of regulation. This current study is focused on defining the mechanism of non-apoptotic uterine caspase-3 activation during pregnancy.

Continuing to utilize the pregnant mouse uterus as our model, myometrial tissue was isolated from gd 12 to 19. Cytoplasmic, nuclear and mitochondrial proteins were extracted and examined by western blot analysis for markers of the three recognized caspase activation pathways. We first examined the extrinsic pathway, which begins outside the cell through occupation of pro-apoptotic receptors by pro-apoptotic ligands. However we found no evidence of Bid phosphorylation and caspase-8 cleavage which act as markers of extrinsic pathway activation. Secondly we examined the intrinsic pathway which is initiated from within the cell and involves the release of pro-apoptotic proteins from the mitochondria which activate the caspase enzymes. Markers of intrinsic pathway activation such as mitochondrial cytochrome C release and Bid cleavage were also absent in the pregnant uterus across gestation. However we have discovered an alternative and novel pathway recently identified in the cardiac myocyte (2) that is utilized by the pregnant uterus to activate uterine caspase-3 in a non-apoptotic manner. We have identified that an elevated endoplasmic reticulum (ER) stress response mediates activation of the myometrial caspase cascade. Markers of the ER stress response such as Chop and BiP are barely detectable at gd 12 increase over a 100 fold to gd 13-14 and decline to undetectable levels as term approaches. The ER stress response which is activated by mechanical stress and myocyte hypertrophy leads to activation of caspase-12, caspase-9 and ultimately caspase-3. Thus we provide evidence of a mechanism of caspase-3 activation that permits the pregnant uterus to avoid the mitochondrial apoptotic pathway while maintaining uterine quiescence in a non-apoptotic and reversible manner.

(1) Jeyasuria P et al Biol Reprod. 2009 May;80(5):928-34.

(2) Okada K et al Circulation. 2004 Aug 10;110(6):705-12. Epub 2004 Aug 2.

Sources of Research Support: March of Dimes #21-FY08-555.

Nothing to Disclose: PJ, KS, AS, JCC

P3-356

***LHR* and *ERb* Polymorphism and the Risk of Infertility and Endometriosis.**

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Background: Clinically, one of the primary concerns regarding endometriosis is its propensity to cause infertility. It is currently estimated that 25% to 50% of women with endometriosis are infertile and that 25% to 30% of all infertile women have endometriotic lesions as the only identifiable cause for infertility. The association between endometriosis and infertility is well established, but the mechanisms responsible for these effects are unknown. Many aspects of female reproductive function are strongly influenced by genetic factors, and numerous studies have attempted to identify susceptibility genes for disorders affecting female fertility. The present study aimed to determine the frequency of PROGINS, *ERb* G+1730A and *LHR* G1502A polymorphism in infertile women with and without endometriosis and in a control group.

Material and Methods: Case-control study that included 119 infertile women with endometriosis, 84 infertile women without endometriosis and 179 fertile women as control group from Human Reproduction Service of ABC School of Medicine. PROGINS was identified by PCR (polymerase chain reaction) and *ERb* G+1730A and *LHR* G1502A were identified by PCR-RFLP (Restriction Fragment Length Polymorphism).

Results: The frequencies of P1P1, P1P2 and P2P2 genotypes of the PROGINS polymorphism in patients with endometriosis were 89.9%, 10.1% and 0.0% ($p=0.5041$), 90.4%, 9.6% and 0% in infertile women without endometriosis ($p=0.5954$) and 88.3%, 10.6% 1.1% (2/179) in control group. Regarding the *ERb* G+1730A polymorphism, the frequencies of genotypes GG, GA and AA in patients with endometriosis were 55.5%, 41.2% and 3.3% ($p=0.0022$), 52.4%, 38.1% and 9.5% in infertile women without endometriosis ($p=0.0002$) and 74.9%, 23.4% and 1.7% in control group. The frequencies of genotypes GG, GA and AA of *LHR* G1502A polymorphism in patients with endometriosis were 59.7%, 34.4% and 5.9% ($p=0.004$); 52.4%, 38.1% and 9.5% ($p=0.0123$) in infertile women without endometriosis and 75.1%, 18.2% and 6.7% in control group.

Conclusions: The presence of *ERb* G+1730A and *LHR* G1502A polymorphisms are associated with infertility and endometriosis-associated infertility.

Nothing to Disclose: BB, DMC, FAM, AMBS, ITNV, CPB

P3-357

Wnt Antagonist DKK1 Is a Target of Krüppel-Like Factor 9 (KLF9) in Endometrial Stromal Cells: Implications for Uterine Receptivity.

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A significant underlying cause of pregnancy loss in mammals is the inability of the uterine epithelium to enter a "state of receptivity" for embryo implantation, due partly to the dysfunctional response of endometrial cells to progesterone (P). We previously showed that mice null for the Sp1-related transcription factor KLF9 exhibit reduced numbers of implantation sites and display aberrant uterine growth-proliferative response and partial P resistance. We also showed that KLF9 regulates the expression and activity of the progesterone receptor (PR) *in vivo* and *in vitro*. To examine the contribution of KLF9 to P-responsive transcriptional programs in stromal cells for luminal epithelial receptivity, the human endometrial stromal cell line HESC was treated with a differentiation cocktail to mimic the peri-implantation hormonal environment, and in the presence of scrambled (control) or *KLF9* siRNAs were analyzed by gene microarray. A number of Wnt signaling pathway components including the Wnt antagonist *DKK1* were identified as KLF9-regulated. The association between KLF9 and *DKK1* was evaluated in HESC and in uteri of early pregnant WT and *Klf9* null mice. Time-course kinetics showed a parallel decrease in KLF9 protein and *DKK1* transcript levels in decidualizing (HESC) stroma. *Dkk1* expression in whole uteri was highest at day post-coitum (dpc) 3.5 relative to dpc 2.5 and 4.5 (vaginal plug=dpc0.5). However, uterine *Dkk1* expression was significantly attenuated at dpc3.5 with *Klf9* null mutation. In eutopic endometrium of women without endometriosis, highest levels of *KLF9* and *DKK1* transcripts were demonstrated at the mid-secretory phase, relative to the other menstrual cycle phases. In women with endometriosis, *KLF9* and *DKK1* expression in eutopic endometrium was coordinately reduced at the mid-secretory phase. HESC co-transfected with the human *DKK1* promoter/5'regulatory region (-2238 to +112 nt) linked to a Luciferase gene-reporter and control or *KLF9* siRNAs had significantly lower (>50%) *DKK1* promoter activity with *KLF9* knockdown, compared to control siRNA-treated cells. Given that *DKK1* is a P/PR-regulated gene and that appropriate Wnt signaling is critical for uterine receptivity, our studies demonstrating *DKK1* as a potential KLF9 target in stromal cells provide a novel mechanism by which the KLF/PR transcriptional network may transduce P-regulated paracrine signal(s) to neighboring epithelial cells, for successful embryo implantation.

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Nothing to Disclose: JMPP, FAS, MAN, DZ, LCG, RCMS

P3-358

Insulin-Induced Selective Impairment of the PI3Kinase-Akt Pathway with Enhanced MAPK Signaling in Endometrial Adenocarcinoma Cell Line.

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Women with the Polycystic Ovary Syndrome (PCOS) or Type 2 diabetes (T2DM) have a high incidence of endometrial dysfunction. In particular, T2DM is an independent risk factor for the development of endometrial hyperplasia and carcinoma. The endometrium is an insulin sensitive organ, with insulin receptors that are cyclically regulated, as well as demonstration of glucose uptake via GLUT 4. While chronic anovulation with unopposed estrogen stimulation is considered the classical pathophysiological mechanism for endometrial hyperplasia and carcinoma, the role of insulin in this process is not fully understood.

To study a potential mechanism for this association, Ishikawa cells cultured in MEM were stimulated with 0.3nM to 100nM insulin. Ishikawa cells are a well-characterized endometrial epithelial cell line with an intact estrogen receptor but an inactive mutant phosphatase and tensin homolog (PTEN) protein.

Using phospho-specific Akt antibodies, we found robust Akt phosphorylation at baseline, consistent with an inactive PTEN protein and uninhibited Akt activation. Not surprisingly, treatment with low concentrations of insulin (0.3nM-1nM) resulted in minimal changes in Akt phosphorylation from the basal state. However, treatment with higher concentrations of insulin (100nM) resulted in a significant decrease in Akt phosphorylation, indicating impairment of the PI3K-Akt pathway. In the presence of wortmannin, a PI3-kinase inhibitor, there was near complete inhibition of Akt phosphorylation at baseline and all doses of insulin. In contrast, insulin stimulation of wortmannin treated cells led to an increase in phosphorylated MAPK relative to the insulin response in cells without wortmannin treatment. We also identified an enhancement of MAPK phosphorylation when Akt blockade was induced by high doses of insulin alone. Our results confirm that the endometrium is sensitive to insulin, and also show for the first time that high levels of insulin induce a selective impairment of the PI3K-Akt pathway. This impairment is compensated by enhanced signaling through the MAPK pathway. Hyperinsulinemia is common to women with PCOS and T2DM, and may play a role in their endometrial dysfunction by altering intracellular signaling. Increased MAPK signaling has been associated with endometrial hyperplasia and carcinoma and here we demonstrate its induction by insulin.

Nothing to Disclose: CAF, JRL, HST

P3-359

Glucocorticoid Regulation of Gene Expression in Uterine Leiomyomas.

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Uterine leiomyomas, benign tumors originating as overgrowth of smooth muscle and connective tissue of the uterus, are the most frequently occurring tumor of the female reproductive tract, affecting at least 20-50% of women of reproductive age. Although leiomyomas rarely progress to malignancy, they are the leading cause of hysterectomies in the United States and are associated with pain and infertility. Estrogen and progesterone are key regulators that direct the growth and function of the uterus, and evidence supports an equally significant role for both hormones in the growth and development of uterine leiomyomas. Our lab and others have established glucocorticoids and their receptor as similarly important uterine regulators, able to block estrogen induced uterine growth. In the search for non-invasive treatments for leiomyomas, we employed microarray studies comparing dexamethasone- (Dex) and estrogen-treated (E₂) human uterine leiomyoma cells to those treated with both (Dex + E₂). Cells were treated for 6 hours with vehicle, 100nM Dex, 10nM E₂, or 100nM Dex + 10nM E₂ and harvested for RNA. In cells treated with Dex alone, 3128 genes were regulated (1578 up and 1550 down). In cells treated with only E₂, 2094 genes were regulated where down-regulation predominated (1384 down vs. 710 up). In cells treated with both Dex + E₂ 4626 genes were regulated (2110 up and 2516 down). The majority of genes co-regulated by Dex, E₂, and Dex + E₂ exhibited co-down regulation. Of interest was a small group of 17 genes that displayed antagonistic regulation by Dex and E₂. In this group, Dex reversed the E₂ effect with co-administration. Ingenuity Pathway Analysis was employed to analyze the functions of genes identified as regulated by Dex. Cell growth, development, and differentiation dominated the top biological pathways. E₂ regulated an entirely different subset of functions that primarily involved inflammatory pathways. Regardless of pathway involved, hepatocyte nuclear factor 4 alpha was identified as the key regulating gene. This transcription factor controls gene transcription, cell growth and differentiation. Our results demonstrate that glucocorticoids play a role in regulating gene expression that may involve uterine leiomyoma growth and development. Evidence that glucocorticoids and estrogen antagonistically regulate a small group of genes and target a common transcription factor may have implications for therapeutic development of uterine leiomyoma treatment.

Sources of Research Support: NIH intramural research program.

Nothing to Disclose: SDW, DD, JAC

P3-360

cAMP Induces Syncytialization of Cytotrophoblastic Cells Via a CRH Positive Feedback Pathway.

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In the placenta, the fusion of cytotrophoblasts to form syncytiotrophoblasts is critical for a successful pregnancy. Syncytiotrophoblasts are marked biochemically by the production of proteins such as human chorionic gonadotrophin (hCG) and syncytin, and morphologically as large multi-nucleated cells.

Placental corticotrophin releasing hormone (CRH) plays diverse roles at different stages of pregnancy and labour, through CRH receptors (CRH-R1 and CRH-R2). CRH production has been linked to the length of gestation because its secretion into maternal blood increases exponentially as pregnancy progresses towards labour onset. Although there is considerable evidence that placental CRH targets myometrial smooth muscle and controls myometrial contractility, there is little known of its role during trophoblast differentiation.

Using the well-established BeWo cell model of trophoblast syncytialization we investigated the effect of 8-Br-cAMP on viability and differentiation. We found that cAMP (50 μ M cAMP for 72h) inhibited BeWo cell viability by 29.7% (MTT assay), and increased apoptosis by 62.8% (FACS analysis), compared to control cells. DAPI staining showed nuclear fragments in cells treated with cAMP (50 μ M) for longer than 48h, and real-time RT-PCR showed that Fas ligand expression increased 6.8-fold by 48h. Meanwhile, hCG (ELISA assay) increased by 3.6-fold, and syncytin 1 (real-time RT-PCR) increased by 2.8-fold (48h). Importantly, by 72h the formation of syncytium could be seen (fluorescence microscopy; CellMask plasma membrane stain co-staining with Hoechst 33342 nuclear stain).

CRH mRNA increased (2-fold at 12h and 15-fold at 48h) following treatment of BeWo cells with cAMP. Interestingly, treatment of BeWo cells with CRH (100nM) increased cellular CRH mRNA expression (7.4-fold at 48h), as well as CRH-R1 (3.6-fold at 48h) but not CRH-R2 levels. CRH (1~100 nM, 48~72h) had no effect on BeWo cell viability, but did upregulate hCG and syncytin 1, and induced syncytium formation.

We show that 8-Br-cAMP is an inducer of syncytialization, and that syncytialization may be associated with apoptotic events. Furthermore, syncytialization may be mediated via a CRH signaling pathway and, although CRH is thought to be produced by differentiated trophoblasts, it may also play a positive feedback role in trophoblast differentiation, promoting cells to differentiate into syncytiotrophoblasts, and provide pregnancy hormones to help maintain a pregnancy to term.

Sources of Research Support: NHMRC Australia-China Exchange Fellowship (ID 510815); Early Career Researcher Pilot Grants (the University of Newcastle, 2009).

Nothing to Disclose: YXC, MA, CA-S, RCN

P3-361

Pathogenesis of Endometriosis through Matrix Metalloproteinase -9 Mediated Steroid Receptor Coactivators-1 Isoform Formation.

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Endometriosis is typically observed in reproductive-aged women, and roughly 5-10% of women (approximately 5 million American women) suffer from symptoms of endometriosis. Unfortunately, however, the detailed molecular mechanisms that modulate endometriosis progression is unknown. Previous studies revealed that steroid receptor coactivators (SRCs) have an essential role in endometrial cell function as well as in development of endometrial carcinomas. However, there is no direct evidence whether SRCs play a role in endometriosis progression. To investigate possible functional correlations of SRCs with endometriosis progression, expression profiles of endometrial SRCs were analyzed with in both a mouse model of surgically induced endometriosis and in primary human endometriotic stromal cells. Interestingly, a novel 75 kDa of SRC-1 isoform, but not isoforms of the other SRCs, is specifically detected in eutopic endometrium in both cases as compared with normal endometrium. In addition to this specific isoform, SRC-1 appears to have an essential role in endometriosis progression because the size and proliferative activity of SRC-1 null ectopic endometrium is smaller than normal ectopic endometrium in mouse model of endometriosis. Northern blot analysis revealed that the endometriotic SRC-1 isoform might be generated by a proteolytic cleavage process due to lack of detection of alternate RNA transcripts for the SRC-1 isoform in endometriotic endometrium. Among various proteases, MMP9 activity was highly elevated in eutopic endometrium as compared with normal endometrium in mouse model of endometriosis. Finally, inhibition of MMP9 activity by MMP2/MMP9 inhibitor III clearly promoted regression of the growth of ectopic endometrium as compared with vehicle; the inhibitor also blocked generation of the SRC-1 isoform in eutopic endometrium. Therefore, we hypothesize that activated MMP9 activity in endometriotic endometrium is involved in endometriotic SRC-1 isoform formation. Collectively, the evidence supports that the MMP9-mediated SRC-1 isoform may play an essential role in endometriosis progression and could provide a novel therapeutic target for endometriosis patients.

Sources of Research Support: NIH grant U54 HD07495 awarded to BWO.

Nothing to Disclose: SJH, FJD, BWO

P3-362

Endometrial Stromal Cell Decidualization Depends on Glucose Utilization Via the Pentose Phosphate Pathway.

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While embryo implantation is a highly synchronized event between the blastocysts and the endometrium, the endometrial receptivity develops independently of the embryo. Therefore, even when the embryonic development proceeds normally, implantation failure and pregnancy loss can occur due to an endometrial dysfunction. Endometrial stromal cells (ESCs) play a major role during embryo invasion of the uterine wall and undergo a drastic functional and morphological change, specifically decidualization, to form decidual cells, which are believed to provide the implanting embryo with numerous metabolic factors required for its development. In the present study, we analyzed regulation of glucose metabolism in the murine and human ESCs and its effect on the process and extent of decidualization. Using a primary culture system, we showed that that ESC decidualization in vitro is glucose dependent and that GLUT1 is a glucose transporter that plays a critical role in the decidualization process. Knockdown of GLUT1 in the stroma using shRNA prevents these cells from decidualizing in vitro. Importantly, GLUT1 expression is upregulated by progesterone during ESC decidualization in early pregnancy, and estrogen is capable of reversing this and downregulating GLUT1 expression, consequently lowering glucose uptake. Based on these results, we hypothesize that an imbalance between these two hormones, which is seen in a number of disease states, may have a drastic impact on glucose utilization by lowering the levels of GLUT1 in the endometrial stroma. In addition, we analyzed the metabolic fate of glucose in the decidualizing ESCs. Using several pathway-specific inhibitors, we demonstrated that the decidualization process is highly dependent on the activity of the pentose phosphate pathway (PPP), both in vitro and in vivo. Importantly, levels of serum DHEA, a hormone which acts as a potent inhibitor of PPP, are often elevated in women with PCOS who suffer from recurrent miscarriages. Thus, the results of our study suggest that the elevated levels of DHEA seen in patients with PCOS may inhibit the proper glucose metabolism required for ESC decidualization by inhibiting PPP. The outcomes of this study highlight two distinct steps of glucose utilization which are both necessary for proper ESC decidualization. First, at the level of GLUT1 expression and subsequent glucose uptake, and second, at the flux of internalized glucose into the pentose phosphate pathway.

Sources of Research Support: NIH F30DK083224.

Nothing to Disclose: AF, KO, MC, KHM

P3-363

Spatio-Temporal Expression of Anti-Müllerian Hormone and Its Role in Pregnant Mouse Uterus.

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Uterine endometrial differentiation during periimplantation is a critical event to successful maintaining of pregnancy in mammals. It is triggered by adhesion of blastocyst and is accomplished by the physiological or anatomical changes of uterine endometrium. The proliferation and differentiation of epithelial cells and stroma cells are prerequisite for progress of pregnancy but the control pathways of them are largely unknown. This delicate modification is regulated by hormonal and non-hormonal molecules originated from embryonic or maternal tissues. The members of transforming growth factor- β (TGF- β) family are renowned for their pleiotropic role in various developmental and physiological processes. One of the members of this family searched for the novel molecules is anti-Müllerian hormone (AMH). AMH specific mRNA was strongly expressed from day 4 and continued until day 9 of gestation. Its specific receptor AMHRII mRNA was also expressed from day 4 to day 9 of gestation in a same manner with AMH mRNA. AMH protein was mainly localized in primary decidua zone and AMHRII protein was localized in deep stroma cell layer on day 7 of gestation. The profiles of expression of AMH and its receptor AMHRII are matched with the decidual response. In addition, AMH works as a proliferation regulator in various tissues including uterine cancer cell lines. Put together, it is suggested that AMH may work as a paracrine factor in decidua for proper regulation of uterine modulation.

Nothing to Disclose: MO, YPC

P3-364

Knockdown of Syndecan-4 Expression Reduced Leiomyomal Cell Proliferation and Spreading.

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Leiomyoma is a benign uterine smooth muscle tumor of women at reproductive age. It is the most common gynecology problem to cause hysterectomy. Compared with matched myometrium, leiomyoma has higher cell proliferation rate and abundant extracellular matrix. The adhesion to extracellular matrix affects cell proliferation and survival. Syndecan-4 (SDC4), a transmembrane heparan sulfate proteoglycans, can cooperate with integrin to support cell adhesion to extracellular matrix. This study was to investigate whether the knockdown of SDC4 affects leiomyomal cell proliferation, survival or attachment. Cells were isolated from leiomyoma tissue obtained from the patients who underwent hysterectomy. Cells were cultured in 10% FCS and incubated with different titers of SDC4 shRNA lentivirus. LacZ shRNA was used as control. Treatment with SDC4 shRNA for two to three days reduced the expression of SDC4 in leiomyomal cells and decreased cell number. Cell rounding assay by trypsin-EDTA further showed that knockdown of SDC4 increased cell detachment rate. Knockdown of SDC4 also induced cell shape changes to increase elongated cells. The results of phalloidin staining showed that the average cell spreading area was reduced in SDC4 shRNA group compared to control cells (Figure1). All of these results suggest that SDC4 plays a role in leiomyomal cells attachment, survival and proliferation.

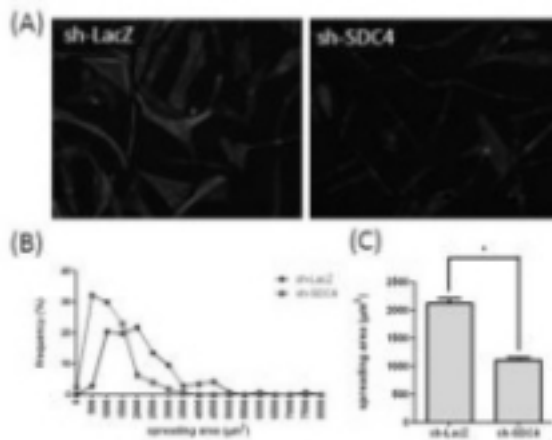


Figure 1. The morphological change induced by SDC4 knockdown in leiomyomal cells. The cells were infected with lentivirus carrying shRNA SDC4 or shRNALacZ and stained with phalloidin after infection for five days. (A) Phalloidin staining of infected leiomyomal cells. (B) Staining area was measured by Image-pro Plus. Percentage of cells with different spreading area was analyzed. Spreading area of sh-SDC4 cells shifted to the left compared to sh-LacZ cells. (C) The average spreading area of sh-SDC4 cell was reduced. * indicated the significant difference between sh-LacZ and sh-SDC4 (P<0.05).

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Nothing to Disclose: HMC, IHL, YMC, LYCW

P3-365

PLAC1 (Placenta-Specific-1) Interacts with ZBTB4 in the Syncytiotrophoblast of the Human Placenta.

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PLAC1 is a recently identified, placenta-specific gene that maps to a region of the X chromosome important in placental development. Additionally, PLAC1 is membrane-associated and shares 30% homology with the zona pellucida 3 protein suggesting an important role at the maternal-fetal interface. Its role in placental development has not been determined. The objective of this study was to identify proteins that interact with PLAC1 in the trophoblast as a prerequisite to understanding its function. A yeast-2-hybrid screen was therefore used to identify PLAC1 binding partners. The PLAC1 ORF was modified to remove the N-terminal transmembrane domain and served as bait. This fragment, representing 80% of the protein, was screened against a human placental library. Positive interactions with prey proteins were identified and sequenced. Each identified interaction was assigned a Predicted Biological Score (PBS) to assess its reliability using a validated methodology. Based on the strength of the PBS score, selected interactions were tested using a HTRF (homogeneous time-resolved fluorescence) interaction assay to confirm direct protein-protein interactions. The cellular localization of PLAC1 and candidate binding proteins were assessed using immunofluorescence microscopy. Positive interactions were detected between PLAC1 and 53 prey fragments, representing 18 distinct gene products. Of the identified prey clones, 16% were considered likely to represent true interactions based on their PBS scores. ZBTB4, a transcriptional regulator was identified as a likely binding partner. Its ability to interact directly with PLAC1 was tested in the HTRF interaction assay. A robust, specific increase (up to 1800%) in signal was consistently observed using a variety of experimental conditions. Additionally, immunofluorescence microscopy demonstrated colocalization of PLAC1 and ZBTB4 in the cytoplasmic compartment of the human syncytiotrophoblast. ZBTB4 has been shown in other tissues to repress the transcription of P21CIP1 and control the cellular response to p53 activation. Additionally, it can bind to methylated DNA to repress transcription. Its role in the placenta, however, has not been studied. These data demonstrate that ZBTB4 and PLAC1 colocalize in the syncytiotrophoblast and are capable of direct interactions. The significance of this interaction is not known but suggests PLAC1 may influence transcriptional events by interacting with known transcriptional regulators.

Nothing to Disclose: MEF

P3-366

Autocrine Factors Inhibit Human Uterine Decidualization In Vitro Via FOXO1 and ETS1-Dependent Pathways.

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Earlier studies from our laboratory identified several factors expressed by human endometrial stromal (ESC) cells and human uterine fibroblast (HUF) cells during in vitro decidualization that feedback in an autocrine manner to inhibit the differentiation process. These factors include prolactin, PTHrP, lefty2 and endocannabinoids, all of which are abundantly expressed by ESC cells and HUF cells 2-3 days following in vitro decidualization of the cells with medroxyprogesterone acetate (MPA), estradiol and PGE2. In this study, we examined whether the inhibition of decidualization by these autocrine factors is mediated, at least in part, by repressing the expression of the transcription factors FOXO1 and ETS1, both of which are upregulated shortly after induction of decidualization, prior to the upregulation of the autocrine factors. In initial experiments, FOXO1 and ETS1 mRNA levels (real-time PCR) were determined in HUF cells decidualized in vitro in the absence or presence of exogenous prolactin, PTHrP, lefty2 or the cannabinoid receptor 1 (CNR1) agonist WIN (R(+)-WIN 55,212-2 mesylate. The exogenous factors were added to the cultures at the start of decidualization when the endogenous levels of the factors are very low or undetectable. In each instance, early exposure of the HUF cells to the exogenous autocrine factors inhibited FOXO1 and ETS1 mRNA levels by 45-62% and blocked the expression of other decidualization marker genes (IGFBP1, laminin). In subsequent studies, repression of lefty2 and PTHrP mRNA expression during the decidualization period by specific siRNAs upregulated FOXO1 and ETS1 mRNA levels and decidualization-specific marker genes. Likewise, repression of CNR1 mRNA levels by the CNR1 antagonist AM 252 and inhibition of prolactin action with the prolactin receptor antagonist delta 1-9-G129R-PRL (gift from Dr. V. Goffin) upregulated FOXO1, ETS1 and other marker mRNA levels. Taken together, these investigations suggest that the transcription factors FOXO1 and ETS1 are pivotal components of the genetic pathway that regulates human uterine decidualization. FOXO1 and ETS1 are induced during the initial stages of decidualization. Later, as the differentiation processes, feedback inhibition of FOXO1 and ETS1 expression by autocrine factors serves to limit the extent of the decidualization process.

Sources of Research Support: HD15201.

Nothing to Disclose: SH, JKS, CAK

P3-367

Novel Role of the Canonical Wnt Pathway in Human Villous Cytotrophoblast Differentiation.

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The canonical Wnt signaling pathway is involved in the differentiation of human extravillous cytotrophoblast (CTB) cells, but its role in the differentiation of villous CTB cells is unknown. In this study, we used an in vitro model of human villous CTB cell differentiation to address the following questions: (1) What components of the Wnt signaling are regulated during CTB differentiation, and (2) what are the effects of Wnt pathway activation and repression on CTB differentiation and the expression of the transcription factor AP-2 α (activator protein 2 α , TFAP2A). AP-2 α expression was of particular interest since the transcription factor is critical for terminal differentiation of human villous CTB cells. Genome-wide DNA microarray analysis of highly purified villous CTB cells undergoing differentiation revealed that 70 genes of the Wnt/ β -catenin pathway were induced during 6 days of differentiation to a syncytiotrophoblast (STB) cell phenotype. Twenty genes were induced by >2.0-fold at four or more days, and another fifteen were induced for three days. The induced genes included β -catenin, Wnt7A and Wnt11, Frizzled1 and Frizzled10 (essential components of the Wnt7 receptor), and the secreted Wnt antagonists DACT1 (dapper) and SFRP1 (SECRETED FRIZZLED-RELATED PROTEIN 1). Activation of the Wnt signaling pathway in cultured CTB cells by Wnt3a inhibited AP-2 α mRNA levels and the mRNA levels for the STB cell markers hPL and hCG β . In contrast, DKK1 (dickkopf homolog 1), an inhibitor of the canonical Wnt pathway that binds to the LRP5/6 Wnt co-receptor and prevents formation of an active Wnt-Frizzled-LRP5/6 receptor complex, significantly stimulated AP-2 α and STB cell marker gene mRNA levels. Taken together, these findings suggest a novel role for the Wnt- β -catenin pathway in villous CTB differentiation. In undifferentiated CTB cells, Wnt agonists repress AP-2 α expression and cell differentiation and promote cell proliferation. Activation of CTB cell differentiation results in the induction of Wnt inhibitors that represses Wnt signaling and enhance AP-2 α expression and differentiation. This interaction between the canonical Wnt pathway and AP-2 α may play a critical role in the regulation of villous CTB differentiation.

Sources of Research Support: NIH07447.

Nothing to Disclose: SH, MAH, SLS

P3-368

Placental Corticotropin Releasing Hormone: Biological Actions on Trophoblast Proliferation and Differentiation.

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Human pregnancy is associated with increased production of corticotrophin releasing hormone (CRH) from the placenta. CRH, through paracrine or autocrine actions, appears to influence important placental processes such as trophoblast cell growth and invasion and tissue remodelling, endocrine capacity and vascular tone. To characterize CRH/CRH receptor mediated biological effects in the trophoblast, we used the choriocarcinoma cell line BeWo, a model of trophoblast differentiation.

BeWo trophoblast cells express functional CRH-R that upon CRH activation stimulate both ERK1/2 and p38MAPK signaling cascades in EGF-R dependent but PI3-K-independent manner. Treatment of cells with CRH (100nM) over a 48h period exerted a stimulatory effect on cell proliferation by 20-30%, as assessed by the MTS assay (Promega Inc). In contrast, CRH (100nM/24h) did not affect BeWo human chorionic gonadotropin (hCG) secretion (a hormonal marker of cell differentiation), despite a significant increase in syncytin-1 and GCMa mRNA levels by 2 fold. Furthermore, BeWo cell growth was sensitive to activation of the TLR4 signalling pathway since lipopolysaccharide (LPS) treatment for 24h significantly increased signaling events associated with the early apoptotic process such as cleavage of caspase-3 and poly(ADP-ribose)polymerase (PARP), increased p53 expression as well as phosphorylation.

Studies employing quantitative RT-PCR showed that the CRH-R1 receptors were significantly up-regulated (by 4-5 fold) during the BeWo trophoblast differentiation by forskolin treatment for 24h. The presence of CRH (100nM) during forskolin-induced syncytialization significantly augmented forskolin-effects on syncytin-1 but not -2 and GCMa mRNA expression by 2 fold respectively, as well as hCG release by 25%. These results raise the possibility that CRH might play an important role in accelerating trophoblast differentiation. Interestingly, CRH actions were sensitive to TLR4 activation, since LPS selectively prevented CRH potentiation of forskolin actions. These results provide novel evidence for a complex role of CRH in BeWo trophoblast proliferation and differentiation and identify the innate immune system as an important regulator of placental CRH biological actions.

Nothing to Disclose: MD, MG, MV, DG

P3-369

The Transcine Decidualization of Human Endometrial Stromal Cells.

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Decidualization during the female reproductive cycle is a process of differentiation of uterine stromal cells to enable implantation of the blastocyst. Estrogens and progestins stimulate decidualization. We hypothesize that decidualization could be accelerated and intensified in a transcine manner, whereby secreted male factors are transferred by seminal plasma (SMP) to the female at mating. The present study was undertaken to elucidate mechanisms and functions of molecular markers for accelerated and complete decidualization by transcine factors.

A human endometrial stromal cell (EnSC) differentiation model was used. EnSC were starved in 1% ctFBS RPMI for 72 hr and treated with progesterone (P4) (50 ng/ml), 0.5% of a dialysed pool of SMP or both (SMP+P4). The extent of decidualization was determined by measuring the levels of the marker prolactin (PRL). We examined whether the cAMP signal transduction pathway is associated with SMP treatment of EnSC using cAMP antagonists and real-time qPCR. Early (24 hr) and late (27 day) decidual EnSC were characterized by microarray to identify candidate genes and pathways involved in decidualization and verified using real-time qPCR. In addition, to identify novel effectors present in SMP HPLC was employed.

SMP+P4 treated EnSC exhibited accelerated and more complete decidualization. Compared to 24 hr P4 treated EnSC SMP+P4 induced PRL gene expression 3.3-fold. Day 12 P4 EnSC secreted equivalent PRL as day 6 SMP+P4. Day 27 SMP+P4 PRL gene expression was 34-fold increased and PRL secretion 32-fold. Measurement of prostaglandin E2, a SMP constituent and stimulator of cAMP, and treatment with an equivalent dose (4.6µg/ml) did not result in commensurate PRL production. Antagonism of cAMP PKA had no significant effect on SMP+P4 treatment, but sustained increase in cAMP (Rolipram 10 µM and 100 µM) induced a 4.6-fold and 3.6-fold respective increase. STC1 and HSD11B1, identified by microarray in late decidual EnSC, are induced via the cAMP pathway. Treatment of EnSC with fractionated SMP indicates a number of fractions are mediating the response.

The investigation of mechanisms underlying decidualization of the endometrium facilitates the study of a number of disease states, such as endometriosis, pre-eclampsia and possibly endometrial cancer. Foremost, it is hoped that the identification of constituents present in SMP eliciting a decidual response will yield an improved decidualizing agent for use in assisted conception.

Sources of Research Support: National Research Network (NRN) Austria.

Nothing to Disclose: UD, PS, PB

P3-370

Role of Cholesterol Sulfate in Human Endometrial Decidualization.

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Objective Cholesterol sulfate (CS) is widely distributed in human tissues, and has recognized as a regulator of steroid synthesis, enzyme activities and membrane stabilization. We previously demonstrated that the amount of CS increased in the endometrium of rabbit during implantation window, suggesting that CS plays an important role in reproduction. To explore the role of CS, we examined the function of CS in decidualization with the human uterine endometrium, and as progesterone receptor (PR) levels determined the onset of the decidualization response, we investigated the CS influence to the PR in decidualization.

Materials and Methods Endometrial tissue was obtained from women undergoing hysterectomy for benign disease under informed consent. The endometrial stromal cells (ESCs) were cultured in the presence of estradiol (E2) 10^{-8} M, progesterone (P4) 10^{-6} M to induce the decidualization, CS 10^{-6} M was added with E2 and P4 at the same time for 12 days, and prolactin (PRL) expression was examined as a marker of decidualization by quantitative RT-PCR. CS was added with E2 and P4 after 6 days incubation for decidualization, PRL mRNA expression and protein in medium were measured. To verify the effect of CS on PR, ESCs were cultured in the presence of CS alone, PR expression was measured by RT-PCR. PR mRNA expression in decidualized ESCs treated CS after E2 and P4 administration for 6 days was examined by RT-PCR. Additionally, we investigated the effect on promoter activity of PR by luciferase assay.

Results Quantitative RT-PCR revealed that the expression PRL mRNA was significantly induced at 9 and 12 days in decidualized ESCs treated with E2, P4 and CS more than E2 and P4. The amount of PRL mRNA and protein in decidualized ESCs treated with CS after incubation of E2 and P4 increased at 72 h after CS treatment respectively. The expression of PR mRNA was induced by single CS treatment, PR mRNA increased at 72 h after CS administration in the decidualized ESCs treated with CS after incubation of E2 and P4. We didn't verify the promoter activity of PR by luciferase assay. **Conclusion** These results indicate that CS promote decidualization by not up regulating PR promoter activity, but increasing the amount of PR mRNA. CS seems to play important roles by promoting decidualization during implantation window.

Nothing to Disclose: YH, HH, MM, MI, RT, MK, HN, TY, YT

P3-371

Further Studies on the Mode of Estrogen Receptor α Expression in Endometriotic Cells.

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Objective: Up-regulation of aromatase gene leading to a high estrogen environment in endometriotic tissues might be ascribed to the demethylation of aromatase gene (1). The clinical issue of importance to be clarified is the pathophysiological role of high estrogen. In contrast to the pattern of estrogen receptor expression in endometrial tissues, a higher estrogen receptor β (ER β) and a lower estrogen receptor α (ER α) expression have been well documented in endometriotic tissues. However, the molecular mechanisms of ER expression are not fully understood. In the present study, we further evaluate the ER α expression in stromal cells from chocolate cysts of patients with endometriosis. **Patients:** The chocolate cyst lining of the ovaries of patients with endometriosis was used as the source of endometriotic tissue. Endometrial tissues were obtained from uteri of cycling premenopausal women who underwent hysterectomy for uterine leiomyoma. These patients had received no hormonal treatment before surgery. We obtained informed consent from all patients. **Methods:** Stromal cells were purified from endometriotic and endometrial tissues. ER α expression was evaluated by RT-PCR and Western blot analysis. To verify the specificity of ER α antibody (Santa Cruz, MC20), knockdown experiments of ER α gene (ESR1) expression was performed using MISSION siRNA (Sigma). **Results:** ESR1 mRNA was detected both in endometriotic and endometrial cells. The ESR1 mRNA expression in endometrial cells was not always higher than that in endometriotic cells. When compared the ESR1 mRNA expression in endometriotic and endometrial cells from the identical patients, the expression level varied depending on patients. A single cDNA sequence, which includes an open reading frame (8 exons) predicting a 66KDa ER α , was exclusively amplified from cDNAs prepared from endometriotic and endometrial cells. Western blot analysis, however, demonstrated a molecule of 55KDa. Knockdown experiments using ESR1 siRNAs suppressed the 55KDa signal, suggesting an alternative translation of ER α . **Conclusion:** We further evaluated the expression level of ER α in stromal cells from endometriotic and endometrial tissues. Although ER α expression in endometriotic cells seemed higher than that in endometrial cells, the expression level varied significantly depending on patients. Interestingly, a novel ER α isoform seemed to be expressed in these stromal cells. Further studies are underway to come to an ultimate conclusion.

(1) Izawa M, Harada T, Ohama Y, Takenaka Y, Taniguchi F and Terakawa N. (2008) *Fertil Steril* 89, 1390

Nothing to Disclose: MI, FT, NT, TH

P3-372

Up-Regulation of Hydroxysteroid (17beta) Dehydrogenase (HSD17B) 6 and Down-Regulation of HSD17B2 in Peritoneal, Deep and Ovarian Endometriosis.

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Endometriosis is a common and chronic benign gynecological disease leading to pelvic pain and/or subfertility. The disease is characterized by the presence of endometrial-like glands and stroma outside the uterine cavity, primarily on the pelvic peritoneum, ovaries and infiltrating organs within the peritoneal cavity. Estrogens and progesterone regulate the cellular proliferation and differentiation of the endometrium while in endometriosis the hormone action may be dysregulated, leading to the increased proliferation of endometriosis cells. However, the data on the expression of estrogen metabolizing enzymes e.g. hydroxysteroid (17beta) dehydrogenases (HSD17Bs) in different types of endometriosis lesions is inconclusive. In this study, we have evaluated the expression of all presently described HSD17Bs (types 1-14) in peritoneal, deep and ovarian endometriosis as well as in eutopic endometrium of endometriosis patients and healthy controls. The data shows that the expression of HSD17B2 and HSD17B6 present the most marked differences between the healthy endometrium and endometriosis tissue. The level of HSD17B2 mRNA in the secretory phase peritoneal, ovarian and deep endometriosis was 0.13-0.15 fold ($p < 0.05$) as compared to the healthy endometrium. No differences were detected in the proliferative phase. Interestingly, the expression of HSD17B6 was highly increased in endometriosis, particularly in deep lesions (6.7 and 8.2 -fold in proliferative and secretory phase, respectively). In the endometrium of healthy controls, the mRNA expression of HSD17B2 and HSD17B6 correlated with serum progesterone concentration, while in the patient endometrium the correlations were diminished and totally lost in endometriosis lesions. As HSD17B2 and HSD17B6 metabolize a variety of steroidal substrates, the aberrant expression of these enzymes in the different types of endometriosis may be involved in several pathophysiological mechanisms of the disease.

Nothing to Disclose: KH, AS, HK, PS, MS, PH, JJ, JF, AP, MP

P3-373

Serum Concentrations of Carboxylated Osteocalcin Are Increased and Associated with the Components of the Polycystic Ovarian Syndrome.

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Objective: Intriguing studies suggest that osteocalcin (OC) and its carboxylated (Gla) / uncarboxylated form are involved in the regulation of insulin secretion and action. Additionally, advanced glycated end products (AGEs), directly regulate the secretion of these osteoblast derived molecules. In PCOS among the pathophysiological aberrations, deregulation of insulin secretion and action as well as elevated AGEs levels have been demonstrated. In this study, we evaluated the serum levels of OC and Gla and their possible associations with PCOS metabolic, hormonal and ultrasonographic components.

Research Design and Methods: 97 women were studied, 50 PCOS and 47 controls, age and BMI-matched. In each subject the levels of bone metabolism markers have been evaluated and metabolic, hormonal profiles as well as ovarian ultrasound were carried out.

Results: Osteocalcin (4.30 ± 1.74 vs. 6.20 ± 1.78 ng/ml, $p < 0.0005$) values were significantly lower, whereas Gla (37.93 ± 6.87 vs. 9.64 ± 8.21 ng/ml, $p < 0.0005$) and RANKL (0.54 ± 0.26 vs. 0.16 ± 0.15 pmol/l, $p < 0.0005$) values were significantly higher, in PCOS subjects compared to control group, independently of obesity. A significant association was disclosed between osteocalcin and Gla with androgens, insulin resistance, AGEs and ovarian morphology. ROC analysis revealed that Gla, [AUC 0.975 (95% CI 0.93-1,00)], as well as AGEs are significant prognostic factor of PCOS existence [AUC 0.986 (95% CI 0.97-1,00)].

Conclusions: Lower osteocalcin and elevated serum levels of its carboxylated form are displayed in PCOS subjects and are associated with several PCOS components. These findings suggest a potential interaction between bone derived markers and metabolic / hormonal abnormalities observed in PCOS. However, the pathophysiological mechanisms and more over the possible clinical implications require further investigation.

Nothing to Disclose: ED-K, SL, IK, CP, AM, AGP, DP

P3-374

Predictors of Spontaneous Versus Assisted Conception in Women with Polycystic Ovarian Syndrome.

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BACKGROUND: Polycystic ovary syndrome (PCOS) is associated with a number of reproductive disorders including infertility. PCOS patients often receive therapeutic intervention (FERTRx) to assist with conception. However, a significant number conceive without intervention. In a large, community-based sample of PCOS patients achieving pregnancy and delivery, we compared baseline characteristics of PCOS patients conceiving spontaneously with those who required FERTRx to identify predictors of spontaneous pregnancy. **METHODS:** Electronic databases were used to identify pregnant women clinically diagnosed with PCOS who delivered at a Kaiser Permanente of Northern California hospital during 2002-2006. The study subset included non-diabetic patients with a clinical diagnosis of PCOS meeting Rotterdam criteria verified by chart review. Method of conception, race/ethnicity, BMI and family history were ascertained using electronic patient and pharmacy records and chart review. FERTRx included ovulation induction, ovarian drilling and in vitro fertilization. Data were compared using chi-square or t-tests. **RESULTS:** Among 1021 pregnant PCOS women, 68.0% received FERTRx to assist with conception. East Asian and South Asian women were more likely to require FERTRx for conception (81.9% vs. other race/ethnic groups (62.9%, $p < 0.01$)). In contrast, Hispanic PCOS women were most likely to have conceived spontaneously (37.4%). Prior parity ($p < 0.01$) was associated with spontaneous conception compared with nulliparity. There was no association with BMI and spontaneous conception overall and across all ethnic groups. Similarly, there was no association between method of conception and family history of metabolic disorders. **CONCLUSION:** In our large community-based subset of PCOS women who achieved pregnancy and delivery, East and South Asian and nulliparous women were more likely to have required FERTRx compared with other groups. These results confirm and extend prior studies demonstrating lower fertility among Asian women undergoing assisted reproduction. Armed with this knowledge, health care providers may more confidently identify infertile PCOS women who might benefit from earlier referral for fertility care.

Sources of Research Support: NIH / NICHD Grant R01 HD052966 awarded to JCL.

Nothing to Disclose: GAL, SLF, MPY, SLJ, JRG, JY, JCL

P3-375

Lean-Mass Correlates with Insulin Resistance in Women with Polycystic Ovary Syndrome (PCOS).

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Women with PCOS are more insulin resistant than the weight matched control women with normal ovarian function. An intrinsic defect in insulin signaling in the muscle contributes to insulin resistance in PCOS. Muscle is the major tissue responsible for glucose utilization, while adipose tissue secretes several cytokines that cause insulin resistance. Therefore, we anticipated lean mass to correlate with insulin sensitivity and fat mass to correlate with insulin resistance. In thirty-nine women with PCOS (age: 29.9±1.0 years; BMI: 33.8±1.2 kg/m²) body composition was measured using DEXA, and the surrogate measures of hepatic and peripheral insulin resistance (HOMA and Matsuda index, respectively) were calculated from oral glucose tolerance tests.

Results: While total fat mass correlated only with Matsuda's index ($r=-0.353$), total lean mass correlated with fasting glucose ($r = 0.444$), fasting insulin ($r=0.445$), HOMA ($r= 0.453$) and Matsuda index (-0.393). Similarly, segmental lean mass (arms, legs and trunk) showed stronger correlations with insulin resistance than the corresponding fat mass segments. Sixteen pairs of individuals were matched for average lean mass (47.8±1.8 and 47.9±1.9 kg) but had discordant fat mass values (42.3±3.3 vs. 29.3±2.6 kg). The group with higher fat-mass was not significantly more insulin resistant than the lower fat-mass group (HOMA: 4.6±0.4 vs. 4.2±0.4; Matsuda Index: 2.4±0.2 vs. 3.1±0.5). Eleven pairs of individuals were matched for the fat mass (36.8±0.8 and 36.8±0.9 kg) but were discordant for the lean mass (52.8±1.8 vs. 44.9±1.8 kg). The higher lean-mass group tended to be more insulin resistant (HOMA: 4.7±0.4 vs. 3.7±0.4; Matsuda index: 2.2±0.3 vs. 3.1±0.5, $p = 0.1$ for both).

Conclusion: Total and segmental lean mass, measured by DEXA, correlated with insulin resistance in PCOS more strongly than the corresponding fat mass. Studies to identify the potential mechanisms of this unexpected finding are in progress.

Sources of Research Support: NCCAM: AT002280 and AT003401; The ALSAM Foundation, Los Angeles, CA; UC Davis Clinical and Translational Science Center Grant (RR 024146).

Nothing to Disclose: KC, LMV, RA, SEK

P3-376

In Women with PCOS, Growing Embryos to Day 3 or Blastocyst Stage Prior to Cryopreservation Leads to Fewer Frozen Embryos Stored but Does Not Compromise Pregnancy Rates.

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Polycystic ovarian syndrome is one of the most common endocrine disorders in women of childbearing age. In women with infertility, its prevalence may be as high as 20% and accounts for a significant proportion of women seeking in-vitro fertilization with embryo transfer treatment. PCOS has been linked to negative effects on ovulation induction, IVF-embryo transfer outcomes and has also been associated with poor embryo quality. Here, we examine if the poor embryo quality associated with PCOS impacts their ability to survive and implant after cryopreservation. A retrospective chart review of all patients who had previously frozen embryos transferred to their uteri from 2002-2008 at the IVF Program at Northwestern was conducted. Survival rates refer to the number of zygotes, D3 embryos or blastocysts that appeared viable after thaw. Implantation is defined as observing fetal heart motion on ultrasound. The average patient age was 36.5 for cycles using frozen zygotes, 37.4 for cycles using D3 embryos, 37.2 for cycles using blastocysts. 1991 zygotes, 2880 cleavage-stage (D3) embryos and 503 blastocysts were frozen 1, 3 and 5 days post IVF, respectively, thawed and transferred. Of this, there were 367 zygotes, 312 D3 embryos and 105 blastocysts from women with PCOS. For diagnoses other than PCOS, the survival rate was 65% for zygotes, 85% for D3 embryos, and 86% for blastocysts. For women with PCOS, the thaw survival rate was 85% for zygotes, 82% for D3 embryos, and 95% for blastocysts. Significantly more zygotes from women with PCOS survived the thawing process ($p < 0.05$). When examining women with PCOS, significantly more D3 embryos and blastocysts survived the thawing process compared to zygotes ($p < 0.05$). The average number of thawed embryos transferred was 2.7 and did not differ between zygotes, D3 embryos, and blastocysts. The overall implantation rate per number transferred was 17% for zygotes, 17% for D3 embryos and 19% for blastocysts and did not significantly differ for those with PCOS. A diagnosis of PCOS does not appear to affect the embryo's ability to survive the thaw process or impact subsequent implantation. Embryos from women with PCOS which are grown to day 3 or blastocyst stage prior to freezing do survive the thaw process better than zygotes. Therefore, in women with PCOS undergoing IVF, growing embryos beyond zygote stage prior to cryopreservation may lead to fewer frozen embryos but does not appear to compromise pregnancy rates.

Nothing to Disclose: MEP, RK, RB, EC, MM, JZ

P3-377

Association between Ischemia-Modified Albumin, Oxidative Stress Markers and Carotid Intima-Media Thickness in Patients with Polycystic Ovarian Syndrome.

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Objective: Polycystic ovarian syndrome is a disorder of ovarian dysfunction. Oxidative stress, inflammation and endothelial dysfunction are thought to play concomitant roles in atherosclerosis. The aim of this study was to evaluate the association between ischemia-modified albumin (IMA), oxidative stress markers (total antioxidant status, TAS, total oxidant status, TOS), carotid intima-media thickness (CIMT), and endocrine parameters in patients with polycystic ovarian syndrome (PCOS).

Research design and methods: We studied 52 PCOS patients (mean age 23.26 ± 6.27 body mass index (BMI) 24.48 ± 3.92 kg/m²) and 36 healthy control subjects (mean age 26.47 ± 6.52 and BMI 22.35 ± 4.89 kg/m²). PCOS was defined by the Rotterdam PCOS consensus criteria. We measured serum IMA, TAS, TOS, CIMT and endocrine parameters in 52 consecutive patients with PCOS and 36 age-matched control subjects.

Results: No statistically significant difference was found between mean IMA, TAS and TOS levels in PCOS patients and control group ($p > 0.05$). Mean CIMT was significantly higher in patients with PCOS than in control subjects (0.41 ± 0.06 mm, and 0.33 ± 0.04 mm, respectively) ($p < 0.0001$). There was statistically significant difference between HOMA-IR level in PCOS patients (3.82 ± 4.28) and in control subjects (2.20 ± 0.83) ($p < 0.017$). A significant positive correlation was found by Pearson's correlation test between BMI ($r = 0.377$, $p = 0.001$), waist to hip ratio ($r = 0.300$, $p = 0.009$) and CIMT levels.

Conclusion: Oxidative stress and insulin resistance are risk factors for cardiovascular diseases in polycystic ovarian syndrome. Elevated insulin resistance and increased CIMT were observed in PCOS patients. In our study, participants in study group were young and non-obese patients with PCOS, with these findings it is thought that total antioxidant status, total oxidant status, ischemia-modified albumin levels (as a pre-indicator of cardiovascular disease) can be associated with BMI and waist hip ratio but not only PCOS itself.

Nothing to Disclose: EC, MO, EO, NC, EC, MS, AG, Y, AM, IT, SD, TD

P3-378

Effect of Race on the Metabolic Dysfunction of Polycystic Ovary Syndrome (PCOS): Comparing African-American (AA) and Non-Hispanic White (NHW) Patients.

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Introduction: PCOS is one of the most common endocrine-metabolic abnormalities, affecting 7-10% of women. Our preliminary data suggest that the prevalence of PCOS in the US may be higher in African-American (AA) and Mexican-American than in Non-Hispanic White (NHW) populations (1,2), possibly from the higher prevalence of metabolic dysfunction in the former ethnic/racial groups. However, little is known regarding the impact of race/ethnicity on either the metabolic dysfunction or phenotype of PCOS. We hypothesized that the degree of metabolic dysfunction was greater in AA women with PCOS compared to NHW patients.

Methods: We studied 279 (41 AA, 238 NHW) women with PCOS by NIH 1990 criteria in a referral clinic and used the Homeostasis Model Assessment to determine insulin resistance by HOMA-IR and b-cell insulin secretion by HOMA-%B.

Results: AA and NHW women were similar in mean age and body mass index, severity of menstrual dysfunction, and distribution of PCOS phenotypes by NIH 1990 criteria. AA women tended to be more hirsute than NHW patients (modified Ferriman-Gallwey score: AA, 9.2±0.26; NHW, 7.6±8.9, p=0.07), although both races had similar mean levels of total and free testosterone and DHEAS. AA PCOS patients had a greater waist-to-hip ratio (AA, 0.87±0.07; NHW, 0.84±0.08, p<0.025), and tended to have higher fasting serum insulin (AA, 32.6±35.2; NHW, 22.2±18.0 mIU/mL, p=0.07) and glucose (AA, 96.3±26.4; NHW, 88.7±14.5 mg/dL, p=0.08) levels, and HOMA-IR (AA, 4.9±4.3; NHW, 9.6±16.0, p=0.71) values. There were no differences in HOMA-%B between races (AA, 106.4±87.2 %; NHW, 127.2±512.8 %, p=0.6).

Conclusions: AA women with PCOS might have a greater degree of insulin resistance than NHW patients, possibly related to their higher abdominal obesity. Although few other phenotypic differences exist, AA patients with PCOS also tended to have a greater degree of facial hirsutism despite similar androgen levels, either due to intrinsic difference in skin response to androgens and/or the anabolic actions of insulin. Further studies are needed to ascertain the mechanisms underlying these racial differences, whether such differences increase the risk for diabetes and cardiovascular disease in AA women with PCOS, and whether differences also exist in other minority populations (e.g. Mexican-Americans).

(1) Azziz et al., J Clin Endocrinol Metab 2004; 89:2745

(2) Goodarzi et al., Fertil Steril 2005; 84:766

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Nothing to Disclose: RA, UE, MP, DAD, MOG

P3-379

The Prevalence of Hypertension and Associated Factors in the Classic Phenotype of Polycystic Ovary Syndrome (PCOS).

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PCOS is the most prevalent endocrine disorder in women of reproductive age and in its classical phenotype is characterized by hyperandrogenism and ovulatory dysfunction. Women with PCOS are also at substantial risk for development of metabolic and cardiovascular abnormalities, having insulin resistance as a central pathogenic feature. However the association of hypertension with PCOS is still controversial, since obesity may explain the alterations of blood pressure frequently seen in this syndrome. We performed a cross-sectional study comparing 73 hypertensive and 109 PCOS patients with normal blood pressure regarding to clinical, hormonal and metabolic variables. Classical PCOS phenotype was defined as the presence of anovulatory cycles, clinical and/or laboratorial hyperandrogenism with or without polycystic ovary in ultrasonography. Hypertension was defined as blood pressure levels of $\geq 130/85$ mm Hg. While 90% of the sample were younger than 30 years old, hypertensive PCOS (H-PCOS) patients were older (24 ± 7 vs 21 ± 6 years, $p=0.006$) and more obese (BMI 35.2 ± 2 vs 28.2 ± 7.4 , $p < 0.001$) than normotensive PCOS (N-PCOS) group. Ferriman-Galwey score for hirsutism were similar between groups [12.5 (10-19) vs. 13 (9-17), respectively], as the androgen levels [total testosterone (ng/ml): H-PCOS: 1.1 (0.78-1.36) vs N-PCOS: 0.93 (0.71-1.2); DHEA-S ($\mu\text{g/dl}$) H-PCOS: 218.5 (137.5-297.6) vs N-PCOS: 185 (134-267.6), $p=0.3$). The prevalence of metabolic syndrome (67.1% vs 8.9%, $p < 0.001$) and type 2 diabetes (13.2% vs 0.9%, $p < 0.001$), as well as total cholesterol levels [H-PCOS: 193.1 ± 46.8 vs N-PCOS: 174.4 ± 38.4 ; $p = 0.005$) and LDL-c levels [H-PCOS: 118.1 ± 40.6 vs N-PCOS: 104.4 ± 33.5 ; $p = 0.016$], were higher in H-PCOS. However, after adjustment for BMI, only triglycerides levels [H-PCOS: 119.5 (88-182) vs N-PCOS: 76 (64-119); $p=0.01$], insulin resistance measured by HOMA index [H-PCOS: 6.13 (3.58-10.23) vs N-PCOS: 3.36 (1.96-5.91); $p=0.008$) and stimulated glucose levels (120' post-glucose load) [136.2 ± 57.2 vs 105.9 ± 26.5 ; $p=0.006$], remained significantly higher in hypertensive PCOS patients. In conclusion, our results suggest that hypertension in PCOS is associated with more severe metabolic disturbances, independent of obesity and androgen levels. These findings reinforce the hypothesis that insulin resistance is probably more important than androgen action to explain the abnormalities in the regulation of blood pressure in PCOS.

Sources of Research Support: INCT - Instituto Nacional de Hormônios e Saúde da Mulher.

Nothing to Disclose: DW, PMS

P3-380

Mental Disturbances Are Associated with Hormonal and Metabolic Parameters in Women with Polycystic Ovary Syndrome.

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INTRODUCTION: Women with PCOS are at risk of a wide range of significant psychological difficulties, including depression, anxiety, and spur eating disorders in comparison to normal women. However, the mechanisms regulating these disturbances intra-PCOS subjects have not been elucidated yet.

AIM OF THE STUDY: To observe the relationship between anxiety, depression and eating disorders with several distinct PCOS anthropometric, hormonal and metabolic parameters.

METHODS: 130 patients with PCOS, diagnosis based on Rotterdam criteria, with mean age 25,26±6,09years and BMI 26,60±7,01kg/m², were evaluated. Data considering the degree of occasional, characteristic anxiety as well as the presence of depression and eating disorders were obtained through the questionnaires STAI-1, STAI-2, BDI and EAT-26, respectively. Additionally, hormonal and metabolic profile was assessed.

RESULTS: Considering STAI-1 (occasional anxiety), 75 (57.6%) subjects displayed higher score than the upper limit of normal, and BMI (p:0.05) and HOMA-IR (p:0,015) were higher in the group with higher STAI-1 scores. Considering STAI-2 (characteristic anxiety), 85 subjects (65.3%) displayed higher score than the upper limit of normal, and BMI (p:006), HOMA-IR (p:0,038), and Ftesto (p:0,044) were higher in the group with higher STAI-2 scores. Consequently, the cohort was subdivided in 3 subgroups according to the results of either test. Specifically, when the cohort was divided into tertiles according STAI-1 score, it was found that the three subgroups did not differ in age and BMI, but a significant difference (p<0.05) in STAI-1 and both HOMA-IR and FAI was documented, between the subgroup with the higher degree of anxiety in comparison to the lower. When the same categorization was done based on STAI-2 scores, a significant difference (p<0.05) was noticed in STAI-2 and HOMA-IR, between the subgroup with the higher degree of anxiety, in comparison to the lower. When this type of analysis was carried out for BDI and EAT-26 no differences were observed between subgroups.

CONCLUSION: In PCOS women a significant association between the degree of anxiety and the characteristic hormonal abnormalities of the syndrome, such as hyperandrogenemia and insulin resistance, has been disclosed. However, the common etiologic link relating these morbidities remains to be elucidated.

Nothing to Disclose: SH, AK, GS, SL, FE, MC, ET, AM, ED-K

P3-381

The Effects of Metformin with Lifestyle Therapy on Polycystic Ovary Syndrome: A Randomized Double Blind Study.

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Objective: We hypothesized that the combination of lifestyle and metformin (MET) would be superior to placebo and lifestyle (Placebo) in improving reproductive and metabolic abnormalities in women with PCOS.

Research Design and Methods: A two-center, racially diverse, double-blind randomized 6 month trial of MET vs Placebo, powered to detect differences in ovulation rates and testosterone levels. Subjects collected urines daily for pregnanediol-glucuronide monitoring, had monthly monitoring, and determination at baseline and completion of body composition, glucose tolerance, and quality of life.

Results: We randomized 136 subjects, but noted high dropout rates (52% in MET and 66% in Placebo). There was no significant difference in ovulation rates between groups. Testosterone levels were significantly lower compared to baseline in MET at 3 mos (-12 ng/dl, 95% CI: (-19, -4), P = 0.03), but no difference at 24 wks. There were no differences in weight between the two treatment arms, though MET had a significant decline at 6 mos compared to baseline (-3.2 kg, 95% CI: (-4.8, -1.6)). Blood pressure improved significantly compared to baseline in both groups. We noted divergent and significant effects of MET compared to Placebo on OGTT indices of insulin sensitivity (improved) and secretion (worsened). Total bone mineral density increased significantly on MET (mean change = 0.02 g/cm²; 95% CI: (0.00, 0.03); P = 0.009) and compared to Placebo (P = 0.003), with no difference at the hip. There were no differences between quality of life measures between groups.

Conclusion: The addition of metformin to lifestyle had mixed benefit on glycemic parameters and little reproductive benefit in women with PCOS, but did result in increased bone mineral density.

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Nothing to Disclose: GL, WCD, SDS, AEA, ARK, LMD, NW, PJC, RSL

P3-382

Vitamin D in PCO Syndrome.

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PolyCystic Ovary Syndrome (PCOS) is the main cause of anovulatory and hirsutism in young women. About 30-70% of women with PCOS exhibit the characteristics of metabolic syndrome (MS). Recently low level of vitamin D have been associated with MS assuming that it could be correlated with insulin resistance (IR) exerting a positive effect on the mechanism of action of the insulin, stimulating the expression of its receptors or increasing the peripheral response of the liver or muscle tissue, or indirectly, affecting calcium homeostasis. Thus we aim to study the correlation of vitamin D with the features of MS in patients affected by PCOS. We enrolled 32 patients affected by PCOS, mean age 25,15 years ($\pm 4,65$), BMI 24,82 kg/m² ($\pm 4,82$). We evaluated lipid profile (triglycerides, total, LDL and HDL cholesterol), serum levels of vitamin D and hormonal pattern (testosterone, DHEAS, androstenedione, 17 OHP, SHBG, estradiol, and FSH and LH in basal condition and after GnRH stimulation). To evaluate glucose metabolism we performed oral glucose tolerance test (OGTT) and hyperinsulinemic euglycemic clamp (M value, glucose metabolized·kg⁻¹·min⁻¹). We found that vitamin D serum levels inversely correlated with BMI (R=-0.4883; p<0.05), homeostasis model assessment of IR (HOMA-IR)(R=-0.4558;p<0.05), and a directly with M value (R=0.4013;p<0,05) and with Matsuda index (R=-0.259;p=0.15); indirectly correlated with testosterone levels (R=-0,0929;p=n.s.) and directly with SHBG (R=0.0022;p=0.5225). M value showed a direct correlation with SHBG (R=0.3437;p=0.0541), an inverse correlation with testosterone (R=-0,5098; p<0.05). Dividing our population in normal (BMI<25kg/m²) and obese/overweight (BMI>25kg/m²), we found that: obese patients showed a significant correlation between BMI and Vitamin D (R=-0.6572;p<0.05); M value inversely correlated with testosterone in normal (R=-0.5736; p<0.05) and in overweight/obese (R=-0,3469;p=0,2959), directly with DHEAS (R=-0.4842;p<0.05) and with SHBG in overweight/obese patients (R=0.3896;p=0.2362). Our data suggest that lower vitamin D may contribute to worsening the IR, the metabolic feature of the PCOS patients. Further, the correlation between vitamin D and androgens (overall testosterone) and SHBG, allows us to suppose that low vitamin D level could play a role on increasing the hyperandrogenism in these patients.

Nothing to Disclose: CP, ES, AP, GM, GPS, APL, TM, AG, AP, SDC

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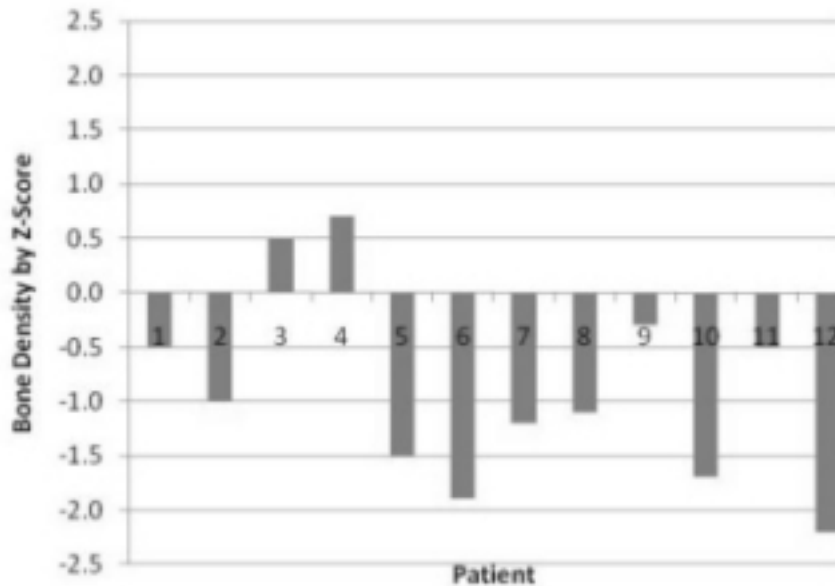
Lower Bone Density in Lean Women with Polycystic Ovary Syndrome (PCOS).

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Background: Both anovulation and low body weight are considered risk factors for decreased bone density in premenopausal women. Women with PCOS, despite being chronically anovulatory, have been thought to have protection from bone loss due to their elevated circulating androgen levels. Indeed, bone density has been reported to be increased in obese women with PCOS. **Aim:** To observe if lean women with PCOS have bone densities that differ from age and race comparable reproductively normal women. **Methods:** Twelve lean women who fulfilled NICHHD criteria for PCOS with hyperandrogenism and chronic anovulation had dual energy x-ray absorptiometry (DXA) scans. **Results:** The PCOS had a mean age of 26.7 years \pm (SD) 4.4 and a mean BMI of 21.0 kg/m² \pm 1.6. Seven of 12 PCOS (58.3%) had a Z-score of less than or equal to -1.0 at either the femoral neck, lumbar spine (L1-L4), or total hip (right and/or left) (Figure 1). Of those 7 PCOS, 2 had a Z-score between -1.0 and -2.0 and 1 had a Z-score of less than -2.0 at the lumbar spine. At the left femoral neck, 5 PCOS had a Z-score less than -1.0; 2 of those PCOS had a Z-score between -1.5 and -2.0, and 1 had a Z-score less than -2.0. At the left total hip, 3 PCOS had a Z-score less than -1.0. Based on the Gaussian distribution, the number of women who have a Z-score less than -1.0 should represent approximately 15% of the age and race comparable populations. **Conclusions:** Lean women with PCOS may have lower peak bone mass and/or bone density than age and race comparable reproductively normal women. Hyperandrogenemia may be insufficient to protect against the deleterious effects of anovulation in lean affected women.

Figure 1: Lowest Bone Density Z-score for each PCOS at a Primary Site.



Nothing to Disclose: AW, AH, MC, AD

P3-384

Effects of Metformin Therapy on Salivary Testosterone and Androstenedione in Women with Polycystic Ovary Syndrome.

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CONTEXT: Polycystic ovary syndrome (PCOS) is characterized by ovarian dysfunction and hyperandrogenism. It is associated with insulin resistance and increased cardiovascular risks. PCOS is one of the most common endocrine diseases in women. Metformin has been shown to improve endocrine and metabolic aspects of PCOS.

OBJECTIVE: The aim of the preliminary study was to check the value of monitoring of salivary androgens in women with PCOS during 12 weeks' metformin treatment.

DESIGN: 29 women with PCOS were included in a prospective trial. Blood samples for levels of total testosterone (T), free testosterone (fT), bioavailable testosterone (bioT), androstenedione (A), dehydroepiandrosterone sulfate (DHEAS), luteinizing hormone (LH), follicle-stimulating hormone (FSH), LH/FSH, prolactin and fasting blood sugar (FBS) as well as saliva samples for levels of salivary testosterone (salT) and salivary androstenedione (salA) were taken before starting metformin treatment (1). Metformin was given at a dose of 500 mg three times daily for 12 weeks, then the pretreatment study was repeated (2). Clinical symptoms of PCOS, including acne, hirsutism score and body mass index (BMI), were assessed before and after the treatment cycle.

SETTING: The study was conducted at an academic medical center.

PRIMARY OUTCOME: The changes of salivary testosterone and androstenedione during metformin treatment were measured.

RESULTS: 26 subjects completed the study. After 12 weeks of treatment, T levels significantly decreased by 19,4%, salT levels by 13,2%, bioT by 36,5%, freeT by 37,5% while A and salA decreased respectively by 23% and 20%. SalA correlates better with A than salT with T ($r_1=0,75$, $r_2=0,65$ vs $r_1=0,31$, $r_2=0,5$) and salT with bioT ($r_1=0,3$, $r_2=0,5$).

CONCLUSIONS: Our preliminary data suggest that testosterone and androstenedione's monitoring in saliva may be a good alternative to blood testing. The level of serum and salivary androstenedione correlates better with clinical symptoms of hyperandrogenism than testosterone's level. Metformin treatment in patients with PCOS is one of the known management options for hyperandrogenism treatment. Metformin therapy in subjects with PCOS results in a decrease of testosterone and androstenedione levels in serum and saliva and leads to improvement in the clinical manifestation of hyperandrogenism.

Nothing to Disclose: DS, ZB, AK, EB-A

P3-385

Clinical and Biological Features of Polycystic Ovarian Syndrome in Middle Eastern Women Living in Michigan.

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Poly cystic ovarian syndrome (PCOS) is common among reproductive age women. The biologic and clinical features of PCOS are not well characterized in US minorities. Since a large Middle Eastern (ME) population lives in Michigan we retrospectively compared clinical and biological features of PCOS (hyperandrogenism/hyperandrogenemia \pm oligomenorrhea) in ME patients (pts) to those of other ethnicities. We used serum levels of total testosterone (TT) >55 ng/dL, free testosterone (FT) >0.53 ng/dL or DHEA-S >380 μ g/dL as thresholds for hyperandrogenemia. Pts were excluded if prolactin > 30 ng/mL, TSH < 0.2 or > 4.8 μ U/mL, or age < 16 years. Out of 201 charts reviewed, 107 met our criteria: 60 were ME, 29 Caucasians, 15 African Americans & 3 others. At presentation, the age of ME pts was similar to that of the non ME group (24.9 year ± 1.0 vs. 26.7 ± 1.1 , $P=0.22$). However, their body weight (BW) and body mass index (BMI) were significantly lower than the non-ME group (BW: 190.1 ± 7.0 vs. 228.6 ± 9.2 lb, $P<0.001$; BMI: 34.3 ± 1.2 vs. 40.5 ± 1.6 , $p<0.005$). There was no significant differences in serum TT or FT levels (TT: 62.0 ± 2.7 vs. 70.6 ± 4.6 , $P=0.12$ and FT: 1.7 ± 0.2 vs. 1.8 ± 0.3 ng/dL, $P=0.78$). The concentrations of DHEA-S (ME: 283 ± 25 mcg/dl vs. Non-ME: 241 ± 44 mcg/dl) ($P < 0.40$), LH, or FSH were not significantly different between the two groups. Interestingly, the fasting insulin concentrations were significantly lower in the ME than in non-ME pts (13.7 ± 1.9 vs. 22.4 ± 2.5 , $P<0.008$) although there was no difference in serum concentrations of total, LDL and HDL cholesterol nor triglycerides. The fasting glucose over insulin ratio was at the limit of significance (ME: 9.9 ± 2.3 and Non-ME: 5.4 ± 0.7 , $p=0.08$). Among the PCOS clinical features amenorrhea was significantly less prevalent among ME pts (12 out of 58 vs. 22 out of 47, $P < 0.006$). Acanthosis nigricans and diabetes were more prevalent in non-ME pts than in ME patients (acanthosis nigricans: 11 out of 55 vs. 18 out of 45 patients, $P < 0.05$; diabetes: 0 out of 59 vs. 5 out of 47, $P < 0.05$). There were no differences among the groups in the prevalence hyperlipidemia, or in the family history of diabetes, hypertension, PCOS and thyroid disease. We therefore conclude that although PCOS women of ME descent have comparable levels of serum androgens to age-matched non-ME patients, however, they are less likely to present with amenorrhea and have lower BMI, lower insulin levels and lower prevalence of diabetes.

Nothing to Disclose: FAE, OA, PDV, RK, WT, ABA-S

P3-386

Baseline Insulin Sensitivity Does Not Predict Weight Loss Success in Obese Women with and without the Polycystic Ovary Syndrome.

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BACKGROUND: Previous reports suggest that presence of insulin resistance make it more difficult to lose weight. Women with the polycystic ovary syndrome (PCOS) are likely to be insulin resistant and therefore may have more difficulty losing weight. We evaluated the relationship between insulin sensitivity measures and successful weight loss in obese women with and without PCOS participating in a weight reduction study.

METHODS: Obese (BMI > 30 kg/m²) women with PCOS (n=13) and without PCOS (n=12) underwent a 75-gram oral glucose challenge, a frequently-sampled IV glucose tolerance test (FSIVGTT), and anthropometric measurements at baseline. Insulin sensitivity (Si), acute insulin response to glucose (AIRg) and DI (disposition index) were obtained from FSIVGTT. All participants received a 1-hr dietary education session provided by a registered dietician at baseline, followed by weekly weight check and follow-up dietary education for 8 weeks. All participants kept a daily food log. Self assessment and dietician assessment of adherence were recorded weekly. Weight loss success was defined as a minimum mean weight loss of 3.6 kg (8 pounds) over the 8 week period.

RESULTS: Mean weight loss during the study was 3.5±3.3 kg. In all women, baseline Si (p=0.9732), DI (p=0.2287), AUCglucose (p=0.9459) and AUCinsulin (p=0.5445) did not correlate with weight loss success. AIRg marginally predicted unsuccessful weight loss attempt: for every 100 μunits•L⁻¹•min increase in AIRg, odds of unsuccessful weight loss during the study increased by 1.22 (p=0.0993). These measures were not predictive of weight loss success when analyses were stratified by PCOS group status (p=NS for all parameters).

CONCLUSIONS: Baseline insulin sensitivity does not predict weight loss success in obese women with and without PCOS over 8 weeks. Elevated AIRg marginally predicts unsuccessful weight loss attempt. The mechanism by which increased AIRg may affect weight loss success (e.g. through subsequent hunger after a meal) will need to be investigated.

Sources of Research Support: National Institutes of Health Grants K23HD049454 (to K.I.C.) and K24HD40237 (to J.E.N.).

Nothing to Disclose: KIC, SK, SNS, JEN

P3-387

Polycystic Ovaries Appearance as a Marker of Clinical Features of Prolactinomas.

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Introduction: Hiperprolactinemia due to prolactinomas may present several phenotypes. Here we test whether sonographic polycystic ovarian appearance correlates to clinical and laboratorial features of women with prolactinoma. **Subjects and Methods:** Ninety-four women diagnosed with prolactinomas through MRI or CT and high levels of serum prolactin were included in the study. From all of them, 54 had the whole investigational protocol fulfilled, *i.e.*, clinical examination, laboratorial analysis and pelvic ultrasonography. Groups were compared using ANOVA and Tukey tests. **Results:** From the 94 subjects, 68(72.3%) presented microprolactinomas and 26(27.6%) macroprolactinomas. Nineteen (35.2%) patients had sonographic polycystic ovaries appearance (PCO); 33 (61.1%) had normal ovaries (NO), and 3.7% had ovarian cysts. In the PCO group, 14 (73.7%) presented amenorrhea, 5 (26.3%) oligomenorrhea. In the NO group, 25 (78.1%) had amenorrhea, 6 (18.7%), oligomenorrhea and 1 (3.2%), hypermenorrhea, respectively. There were no differences between groups regarding BMI (26.5 ± 6.3 vs 27.0 ± 6.1 , $p=0.78$). Prevalence of hirsutism was higher in PCO group than NO group (26.3% vs 6.1%), however, with a tendency to statistical significance ($p=0.08$). In the PCO group the levels of LH and testosterone were significantly higher than normal ovaries group ($p=0,002$ and $p=0,009$, respectively) but no differences in prolactin, FSH, estradiol, androstenedione, SDHEA, DHEA and 17OH progesterone levels. **Conclusion:** Subjects with prolactinomas presenting PCO appearance on sonography have higher levels of testosterone and LH and the prevalence of hirsutism is also higher. PCO appearance seems to characterize two distinct groups of women with prolactinoma.

Nothing to Disclose: SAYH, JAMM, GARM, CRGB, MPR, JMS, CMCL, ECB

P3-388

Clinical and Metabolic Heterogeneity in Obese and Lean Women with Polycystic Ovarian Disease.

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Aim

To study the differences in clinical, metabolic and sonographic profiles in obese and lean women having polycystic ovarian syndrome (PCOS).

Materials and Methods

Women attending the endocrine clinic (age group 16-35 years) and diagnosed to have PCOS by the European Society of Human Reproduction and Embryology and the American Society of Reproductive Medicine, Rotterdam 2003 criteria were included in the study. They were divided into two groups based on their body mass index (BMI): Group A (n = 50), overweight and obese with BMI >23 kg/m² and Group B (n = 50), normal weight and lean with BMI ≤23 kg/m², according to Asian Indian Criteria. All patients underwent detailed clinical history and physical examination, 75 gm Oral glucose tolerance test (OGTT) after overnight fasting, fasting lipid profile, measurements of fasting insulin (FI), serum testosterone (T), sex hormone binding globulin (SHBG), and 25-hydroxy-vitamin D (25-OH-vit D) levels. Ultrasound abdomen was done for ovarian volume (OV) and endometrial surface area (ESA).

Results:

Clinical parameters

| Clinical features | Group A | Group B | p |
|---|---------|---------|-------|
| Menstrual irregularities (%) | 70.2 | 49 | 0.05 |
| Hyperandrogenism (according to Ferrimen-Galloway scale) (%) | 78.2 | 59.6 | 0.05 |
| Central obesity (waist-hip ratio >0.85)(%) | 51.3 | 39.9 | 0.056 |
| Previously unidentified hypertension(%) | 23 | 17.5 | 0.211 |
| Impaired glucose tolerance (%) | 18 | 11 | 0.05 |
| Previously unidentified Type 2 diabetes (%) | 11.7 | 6 | 0.05 |

Biochemical parameters

| Biochemical parameters | Group A | Group B | p |
|---|------------------------------|-----------------------------|--------------------|
| Fasting Insulin (mU/ml) | 30.4 ± 4.5 | 19.8 ± 7.2 | <0.001 |
| SHBG (nmol/L) | 91.3 ± 14.2 | 70.9 ± 11.2 | <0.001 |
| Testosterone (ng/dL) | 448 ± 60 | 392 ± 42 | 0.05 |
| 25-OH-vit D (nmol/L) | 9.1 ± 2.1 | 13.2 ± 3.3 | <0.01 |
| Lipids (mg%)[Triglycerides, HDL, LDL] | 191 ± 12, 35 ± 3.1, 112 ± 14 | 175 ± 10, 38 ± 4, 109 ± 9.2 | 0.001,0.009, 0.025 |
| Ultrasonic features:[OV (ml),;ESA (cm ²)] | 15.1 ± 4.3; 11.1 ± 1.8 | 11.5 ± 3.2; 8.1 ± 2.3 | >0.5; <0.01 |

Conclusions:

Amongst the biochemical features, obese PCOS women (group A) had comparatively lower HDL & 25-OH-vit D and higher ESA on ultrasound as compared to lean PCOS women (group B).

Nothing to Disclose: MAS, MG, SKW, JA

P3-389

In Vitro Effect of Inositol on Inflammatory Status of Erythrocytes in Polycystic Ovary Syndrome.

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Patients with polycystic ovary syndrome (PCOS) have insulin resistance and hyperinsulinemia. Recent studies have also reported that women with PCOS do have an inflammatory status (increase of PCR and of aldosterone). Studies in PCOS have evidenced a deficiency of chiroinositol-containing phosphoglycan that mediates the action of insulin.

Administration of chiroinositol was able to increase the action of insulin and improve the ovulatory function. Aim of the study was to investigate in vitro a possible inflammatory role of erythrocytes in these patients both in the presence or absence of inositol. Oxidative assault can be highlighted by variation of the Tyr-phosphorylation level of erythrocyte band 3 after diamide treatment. Diamide is known to induce alteration in the erythrocyte membrane, which, in presence of pre-existing pathological conditions, triggers higher Tyr.phosphorylative response. We analyzed the effect of inositol on both basal and diamide-treatment conditions, on erythrocytes from patients suffering from PCOS. We have investigated 6 patients with PCOS mean age 21±3 BMI 24±2.3. Four control normal women, comparable for age and BMI, were also investigated. Diagnosis was done upon the presence of two of the three criteria (oligo anovulation, features of hyperandrogenism and ultrasound analysis). None of the patients were taking any drug nor had other inflammatory disease in the past three months. Blood was collected in fasting conditions and erythrocytes were washed and incubated in their autologous plasma at 20% hematocrit for 24 h at 35° C, in the presence or absence of 2 mM inositol. 1.5 mM diamide was then added. Western blotting and immunorevealed with anti-P-Tyr antibodies.

Results: Our findings show that, diamide induced higher band 3 Tyr-P level in erythrocytes from patients (185% ± 20% compared to healthy controls), indicating pre-existing oxidative assault in all cases of PCOS. Pre-treatment with inositol reduced sensitively diamide-induced band 3 Tyr-P level in patients, thus resembling control samples, whose Tyr-P was only lightly modified by inositol pretreatment.

Conclusion: the study has confirmed that women with PCOS do have an inflammatory status involving also circulating erythrocytes, and inositol treatment greatly improves erythrocyte response to oxidative assault. This effect is not genomic and maybe it is superimposed to the known genomic inflammatory parameters related to aldosterone or to other substances

Nestler JE et al., N Engl J Med 1999; 34:1314

Nothing to Disclose: GD, LB, CF, ET, CS, AD, GA, AA, FLG, GC, DA

P3-390

Hyperinsulinemia Was Associated with the Severity of Hirsutism in Polycystic Ovary Syndrome.

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Clinical or biochemical hyperandrogenism is one of the important phenotype for the diagnosis of polycystic ovary syndrome (PCOS). It is well known that androgens have significant effects on hair follicle growth, but the relationship of androgens to hirsutism is not completely understood and controversial. Recently, hyperinsulinemia has been reported to be associated with hirsutism in PCOS. We aimed to determine the relationship of hirsutism, hyperandrogenemia and hyperinsulinemia in women with PCOS.

We recruited 266 women with PCOS (age: 22±4 yrs, BMI: 22.9±4.2 kg/m²) diagnosed by NIH criteria and 306 controls (age: 25±5 yrs, BMI: 21.2±2.7 kg/m²). Hirsutism was defined by the modified Ferriman-Gallwey (mFG) score, a level of ≥ 8 signaling. Total testosterone (TT) was measured by chemiluminiscent immunoassay and free testosterone (FT) was calculated from TT, SHBG, and albumin. Basal hyperinsulinemia was assessed by fasting insulin and hyperinsulinemia of stressed state by area under the curve for insulin (AUCinsulin) during 75 gram oral glucose tolerance test. Eighty four women with PCOS (31.6%) were hirsute, and mFG score was 6±4 in women with PCOS. Age, TT, FT, and total cholesterol (TC) levels were significantly lower in hirsute women than non-hirsute women, and TT was still significant after age adjustment ($p < 0.05$). The mFG score was positively correlated with AUCinsulin ($r = 0.160$, $p < 0.05$), and negatively correlated with age ($r = -0.148$, $p < 0.05$), TT ($r = -0.192$, $p < 0.05$), and TC ($r = -0.125$, $p < 0.05$). Multiple linear regression analysis showed association of mFG score with TT ($\beta = -0.176$, $p < 0.05$) and AUCinsulin ($\beta = 0.172$, $p < 0.05$) in women with PCOS. In controls, mFG score was not related to AUCinsulin and only associated with age ($\beta = -0.146$, $p < 0.05$) and FT ($\beta = 0.166$, $p < 0.05$).

Hyperinsulinemia appears to have association with severity of hirsutism in women with PCOS, but not in controls. Contrast to other previous studies, hyperandrogenemia showed inverse relationship with hirsutism in women with PCOS.

Nothing to Disclose: HJL, EJS, J-YO, YSH, Y-AS, K-AJ, HWC

P3-391

Use of Metformin To Achieve Ongoing Pregnancy in Women with Polycystic Ovarian Syndrome.

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INTRODUCTION: Polycystic ovary syndrome (PCOS) is the most common endocrinopathy among reproductive-aged women. Prominent metabolic signs and symptoms include those associated with insulin resistance and infertility. Metformin (MET) is used as an alternative ovulation induction (OI) agent in clomiphene-resistant women based on early studies, wide margin of safety, and low cost. With more experience, it has become increasingly utilized in the fertility setting. In a large, diverse, community-based sample of PCOS patients achieving pregnancy, we investigated the frequency of MET use for OI, either alone or in combination with other OI agents for specific demographic and pregnancy outcome features. **METHODS:** Using electronic databases within the Kaiser Permanente Northern California health care system, we identified pregnant women with PCOS who had a 2nd or 3rd trimester delivery during 2002-2006. PCOS was defined according to Rotterdam criteria and verified by chart review. Patients with diabetes mellitus and those conceiving by in vitro fertilization were excluded. Method of conception was ascertained using both individual chart review and pharmacy records. Outcomes were compared across race/ethnicity, age, BMI, and parity using chi-squared tests. GA at delivery was compared using t-test.

RESULTS: Among 951 pregnant women with PCOS, 27% used preconception MET for OI. Among these 254 women, 30% conceived on MET alone and 70% conceived with MET and other OI agents. Unlike clomiphene or gonadotropins, no patient using MET alone experienced a multiple gestation. MET use increased with increasing pre-pregnancy BMI, ranging from 15% for BMI <25 to 38% for BMI ≥ 40 kg/m². There were no significant differences in rates of MET use by race/ethnicity or maternal age. However, a higher proportion of women of Asian descent received MET with adjunctive OI agent(s). PCOS women using MET to assist with conception had similar gestational age at delivery as women who achieved pregnancy spontaneously. **DISCUSSION:** In a large community-based cohort of PCOS women achieving ongoing pregnancy, MET was successfully used alone and in conjunction with other OI agents. In a little less than a decade following the first report of its use in PCOS women, MET has become an effective and commonly utilized primary or adjunctive OI agent for these women in current fertility practice.

Sources of Research Support: NIH / NICHD Grant R01 HD052966 awarded to JCL.

Nothing to Disclose: SYP, SLF, SLJ, MPY, MKH, RDN, JY, JCL

P3-392

Lipid Metabolism Response to 3 Weeks of Testosterone Treatment in Obese Premenopausal Women.

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Polycystic ovary syndrome is associated with insulin resistance and dyslipidaemia. It is thought that alterations in glucose and lipid metabolism are due to hyperandrogenemia. However, the effect of testosterone on substrate metabolism in women has never been evaluated. We therefore evaluated the effect of testosterone treatment (AndroGel®; 1250 mg/day for 3 weeks) on lipid metabolism (by using stable isotope labeled tracer methods) and lipoprotein subclass profile (by using proton nuclear magnetic resonance spectroscopy, NMR) in five obese (body mass index = 30-40 kg/m²) premenopausal women. Total and free testosterone concentrations in plasma increased from 27.6 ± 4.2 and 0.84 ± 0.12 to 305.4 ± 104.8 and 11.7 ± 4.9 ng/dl, respectively, mean ± standard error) in response to testosterone treatment. The concentrations of VLDL (particle and triglyceride [TG]) and LDL (total and subclasses) were not affected by testosterone treatment, but there was a trend (p = 0.06) for a decrease in HDL concentration (Table 1). Testosterone treatment had no effect on free fatty acid (FFA) rate of appearance (Ra) in plasma, VLDL-apoB-100 and VLDL-TG secretion rates and VLDL-TG plasma mean residence time (MRT); however, there was a trend (p = 0.07) for a decrease in the VLDL-apoB-100 MRT (Table 1).

Effect of testosterone treatment on lipid concentrations (determined by using 1H-NMR spectroscopy), particle size, and kinetics

| | Before | After |
|--|-----------|-----------|
| VLDL concentration (nM) | 37±13 | 34±15 |
| VLDL-TG concentration (µM) | 148±46 | 135±48 |
| VLDL mean particle size (nm) | 47±2 | 49±3 |
| LDL concentration (nM) | 786±119 | 757±121 |
| LDL mean particle size (nm) | 21.8±0.2 | 21.7±0.3 |
| HDL concentration (µM) | 30±1 | 29±1 |
| HDL mean particle size (nm) | 9.2±0.2 | 9.2±0.2 |
| VLDL-apoB-100 secretion rate (nmol/min/l plasma) | 0.18±0.06 | 0.20±0.11 |
| VLDL-apoB-100 MRT (min) | 186±15 | 133±26 |
| VLDL-TG secretion rate (µmol/min/l plasma) | 2.2±0.3 | 3.2±0.9 |
| VLDL-TG MRT (min) | 64±10 | 52±17 |
| FFA Ra (µmol/min) | 393±53 | 438±53 |

Our preliminary data suggested that a short-term increase in plasma testosterone concentration had no major effect on plasma lipid kinetics and the plasma lipoprotein profile in obese premenopausal women.

Sources of Research Support: NIH Grant P50 HD057796 awarded to BM; NIH Grant K99AG031297 awarded to XW.

Nothing to Disclose: XW, BWP, DNR, BM

P3-393

Tissue Glucose Utilization and Body Mass in Women with Polycystic Ovarian Syndrome.

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Insulin resistance plays a crucial role in the pathogenesis of Polycystic Ovarian Syndrome.

The aim of the study was to evaluate the tissue glucose utilization in women with Polycystic Ovary Syndrome (PCOS) depending on body weight.

41 women were diagnosed with PCOS according to Rotterdam criteria. 21 cases with normal body weight (NW) - BMI: 18,5-24,9 kg/m² and 20 overweight or obese (OW) - BMI > 30 kg/m². The oral glucose tolerance test (OGTT) was done simultaneously with the assessment of glucose and serum insulin levels at 0, 60 and 120 minute. The Matsuda index and homeostatic model assessment (HOMA) were calculated. The hyperinsulinemic euglycemic clamp was performed to assign the tissue glucose utilization.

There were significant differences between NW and OW group in serum glucose levels 60' NW:1,49 mmol/l, OW:1,73mmol/l (p=0.0001) and 120' NW:4,24 mmol/l, OW:5,93 mmol/l (p=0.0011) in OGTT. There were significant differences between NW and OW group in fastum serum insulin levels NW: 8,38 uU/ml, OW: 15,11 uU/ml (p=0.0008). There were significant differences between NW and OW group in Matsuda index values NW: 11,79, OW: 5,81 (p= 0.0278) and HOMA index values NW: 1,74, OW: 3,44 (p= 0.0090). Mean tissue glucose utilization tests were as follow: in NW: 8,49, in OW: 3,91 mg/kg/min (p=0.0000). The Spearman's Rank Tests revealed strong negative correlation between tissue glucose utilization and BMI (R= -0,76) .

Conclusion: Tissue glucose utilization refer to normal range in slim women with PCOS. Higher body mass index decreases tissue glucose utilization in women with PCOS.

Nothing to Disclose: MS, TM, JK, AZ, AH-D

P3-394

Ethinylestradiol-Chlormadinone Acetate Combination for the Treatment of Hirsutism and Hormonal Alterations of Normal-Weight Women with Polycystic Ovary Syndrome: Evaluation of Metabolic Impact.

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Polycystic ovary syndrome (PCOS) represents a common endocrine disorder affecting about 7-10% of women in reproductive age. A complex physiopathologic setting including inappropriate gonadotrophin secretion, impaired glyco-insulinemic metabolism, hyperandrogenism and chronic anovulation, is thought to produce the large spectrum of clinical and biochemical manifestations of PCOS. The therapeutic approach is often complex and variable in relation to the primary symptom of the patient. Estrogen-progestin combinations have been long considered a first line therapy for women with menstrual irregularities, hirsutism and acne. However, especially in cases of long term treatment, the use of such drugs has been associated with an increased risk of cardiovascular disease, due to their negative impact on lipid, glucose and insulin metabolism. This is the first study evaluating the clinical, metabolic and hormonal effects of the Ethinylestradiol/Chlormadinone acetate combination in hirsute women with PCOS. Twelve hirsute normal-weight women with PCOS (age range 18-27 years) were enrolled for this study. Ultrasonographic pelvic exams, hirsutism score, hormonal profile assays were performed at baseline and after 3 and 6 cycles of treatment with 30 mg EE and 2mg CMA. Oral glucose tolerance test, euglycaemic hyperinsulinaemic clamp and lipid profile were also performed. No significant modifications in mean body weight and in body fat distribution were observed throughout the treatment period. A significant improvement of hirsutism was obtained at the 6th cycle. Plasma levels of sex hormone binding globulin markedly raised, achieving a statistical significance from the 3th cycle on. Free androgen index, androstenedione and 17-hydroxy-progesterone significantly decreased after 6 cycles.[table I]The treatment did not affect glucose and insulin homeostasis. Total cholesterol, triglycerides and HDL plasma levels remained unvaried, whereas LDL plasma concentrations showed a significant reduction. A significant increase was seen in VLDL levels at the 6th cycle of therapy [table II]. Notwithstanding the small sample size and in the lack of a control group, our pilot study suggests that EE-CMA combination may ameliorate the clinical and hormonal features of PCOS women, with no detrimental impact on glycoinsulinemic and lipidic metabolism.

Nothing to Disclose: DR, LR, AB, SDC, VT, MG, AL

P3-395

Hyperandrogenemia in Obese Peripubertal Girls: Relationships with Insulin Resistance, Integrated Hyperinsulinemia, and Integrated LH Secretion.

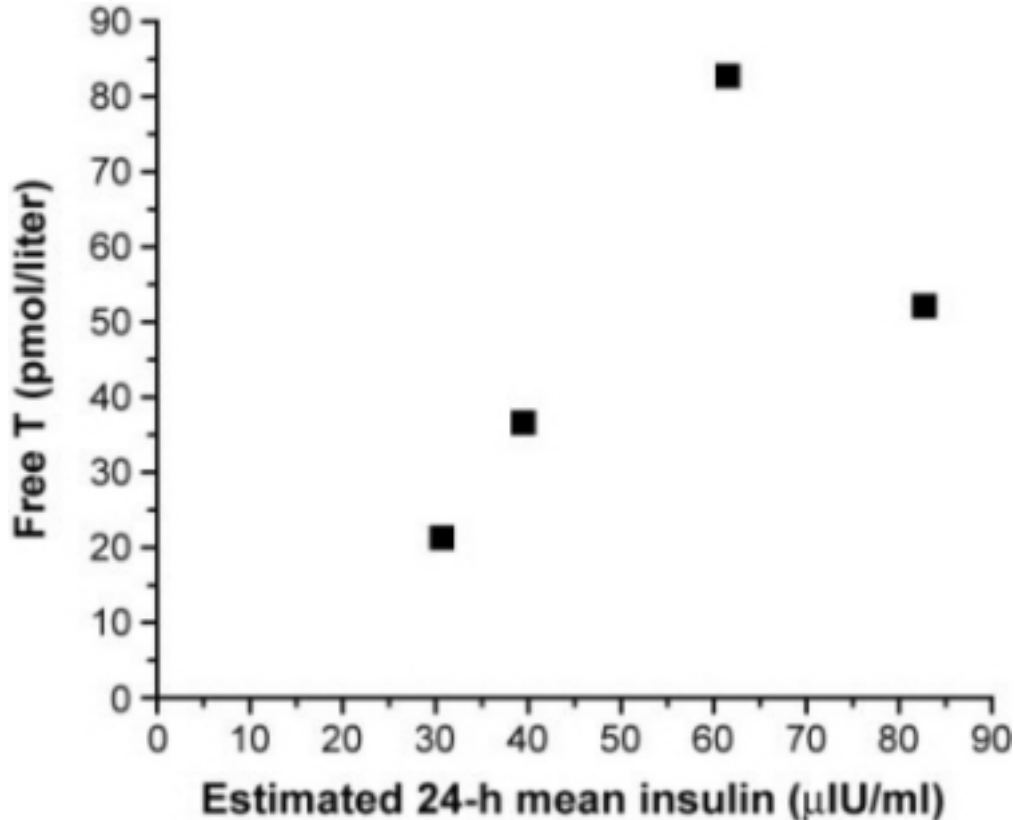
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Peripubertal hyperandrogenemia (HA) can represent a precursor to polycystic ovary syndrome (PCOS), but the etiology of peripubertal HA is unclear. A majority of obese peripubertal girls demonstrate elevated free testosterone (T) compared to their normal weight counterparts (1). However, not all obese adolescent girls have HA, and the proximate cause of HA in obese girls remains unclear. A previous analysis suggested that morning LH and fasting insulin (imprecise measures of overall LH secretion and insulin resistance/hyperinsulinemia, respectively) are independent predictors of free T in obese girls.

To assess these relationships, we are pursuing a study protocol that includes: (a) insulin every 30 min starting 1 h before and ending 2 h after a standardized mixed meal (ingested at 1900 h); (b) LH every 10 min and T every 60 min from 1800-0900 h; (c) fasting insulin every 30 min from 0700-0900 h; (d) SHBG at 0700 h; and (e) a 2-h hyperinsulinemic euglycemic clamp (insulin dose: 80 mU/m²/min). An estimate of 24-h insulin exposure was calculated as [(mean insulin from 1800-2100 h * 2) + mean insulin from 0700-0900 h] divided by 3.

To date, we have complete data on 4 obese (BMI-for-age percentile > 95) girls (one Tanner 3, three Tanner 5, all ≤ 2 y postmenarche). These girls exhibited variable free T values (between 21 and 83 pmol/L). Hyperinsulinemic euglycemic clamps revealed marked insulin resistance in all subjects (M values 94-128 mg/m²/min). 24-h integrated insulin values ranged from 31 to 83 μIU/ml and appeared to be directly correlated with free T (Figure). There was no obvious relationship between free T and mean LH.



Preliminary data in 4 obese girls—all of whom were highly insulin-resistant—suggest that achieved insulin concentrations correlate best with free T levels. This implies that variable beta cell secretory capacity may help explain the marked variability of free T concentrations in mid- to late pubertal obese girls.

(1) McCartney CR et al, J Clin Endocrinol Metab 2007; 92:430

Sources of Research Support: Eunice Kennedy Shriver NICHD/NIH through cooperative agreement [U54 HD 28934] as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research; General Clinical Research Center Grants M01 RR00847; R01 HD058671 (C.R.M.); and T32 HD007382 (K.L.K.).

Nothing to Disclose: KLK, JSPC, JCM, CRM

P3-396

Hyperandrogenemia and Its Relationship with Insulin Sensitivity in Chilean Adolescents.

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Introduction: Hiperandrogenism is a prevalent condition in age reproductive woman. It's a frequent manifestation of the ovary polycystic syndrome and it's associated with insulin resistance and metabolic syndrome. Although this conditions begin at puberty, information about adolescents is scarce.

Objective: To evaluate the clinical expression and biochemical parameters related with insulin sensitivity in adolescents with biochemical hyperandrogenism.

Material and Methods: Records of the ambulatory Adolescence Care Unit at Clinica Alemana of Santiago de Chile from years 2007-2009 were evaluated. The eligible patients should be post menarchial, 13-20 years old, consulting because of : irregular menses, acne or hirsutism, and should have an androgenic index (FAI) >4, 5 measured in follicular phase. They were excluded if they had eating disorder, were on diet and exercise prescription or were taking medications that could alter the measurements, like oral contraceptives, insulin sensitizers, steroids, antipsychotics and mood stabilizers. Secondary etiologies for hyperandrogenemia were excluded. All patients should have basal 17OH progesterone level <2,5ng/ml.

Results: From 1046 clinical records 110 patients were selected. The clinical presentation was (mean \pm SD): age 16, 7 \pm 1, 9; BMI 22,3 \pm 3,0; irregular menses 72%; acne 64%; hirsutism 46%. nutritional status: 6% low weight, 59% eutrophic and 35% overweight. Biochemical parameters: total testosterone 0,68ng/ml \pm 0,2; SHBG 30,3nmol/l \pm 12; FAI 9,0 \pm 4; basal glicemia 86mg/dl \pm 6,7; basal insulin 9,3uU/l \pm 4,4; HOMA-IR 2,1 \pm 1,0; Insulin 120min post 75g oral glucose load 79uU/l \pm 72. The eutrophic adolescents had FAI 8,4 \pm 3,2 and the overweight group 10,1 \pm 5,0 (T test p= 0.04) .The correlation analysis (Pearson) found significant results between BMI vs. FAI (r= 0,22 p=0.02), BMI vs. SHBG (r= -0,35 p=0.00), BMI vs. basal insulin (r= 0,36 p=0.001), BMI vs. HOMA (r= 0,22 p=0.03), and FAI vs. Insulin 120min (r= 0,36 p=0.02).

Conclusions:

- Hyperandrogenic adolescents present significant relationship between nutritional status, androgens measurements and insulin sensitivity. This results support the hypothesis that hyperinsulinemia must be a pathogenic mechanism in hyperandrogenemia and strengthens the importance of the management of overweigh in the therapy of this pathology.
- The determination of insulin at 120min post oral glucose load seems to be a sensitivity test to detect insulin resistance in these adolescents.

Sources of Research Support: Clinica Alemana of Santiago, Chile.

Nothing to Disclose: PR, VM

P3-397**Results of Long Term Follow up on Women with Central Hypogonadism after Neurosurgical Treatment of CNS Tumors.**

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Women (16-45 y.o., n=53, group1) with central hypogonadism after neurosurgical treatment of CNS tumors (mostly craniopharyngiomas) were observed initially and after 5.2(3.7;7.2) y. of treatment with 17 β -estradiol 2mg and dydrogesterone 10 mg in sequent manner (HRT). Except hypogonadism, women had hypothyroidism (n=53), hypocortisolism (n=26) which were compensated before HRT started; and GH deficiency (n=15) without GH replacement. Healthy women (20-38 y.o.) were included in control group2 (n=45). Initially patients had not only estrogen but also androgen deficit. After HRT statistically significant decrease of total and free T levels was not observed, DHEA-S levels even increased.

| | group1 | group1(HRT) | group2 | p 1vs2;1vs1(HRT) |
|------------------|--------------|---------------|-----------------|------------------|
| Estradiol pmol/l | 39(30;55) | 395(262;627) | 156(116;218) | <0.001; 0.005 |
| Total T nmol/l | 0.1(0.1;0.2) | 0.1(0.08;0.3) | 1.05(0.8;1.4) | <0.001; 0.6 |
| Free T pmol/l | 1.4(0.6;2.1) | 0.75(0.4;1.6) | 10.4(7.2;16.1) | <0.001; 0.1 |
| DHEA-S nmol/l | 128(73;850) | 270(94;1357) | 5590(4030;6630) | <0.001; 0.046 |

Total cholesterol and triglycerides levels as well as concentrations of Ca⁺⁺ and alkaline phosphatase (AP) were considerably higher in group1 compared to group2. After HRT statistically significant decrease of these parameters was found. Differences in HDL, LDL and phosphorus levels between 1 and 2, before and after HRT were not revealed.

| | group1 | group1(HRT) | group2 | p 1vs2; 1vs1(HRT) |
|---------------------------|-----------------|-----------------|-----------------|-------------------|
| Cholesterol(mmol/l) | 5.85(5.3;7.8) | 4.8(3.95;5.1) | 4.63(4.15;5.15) | 0.004; 0.041 |
| Triglycerides(mmol/l) | 1.42(1.3;2.6) | 0.65(0.6;0.9) | 0.76(0.6;0.85) | <0.001; 0.044 |
| Ca ⁺⁺ (mmol/l) | 1.16(1.11;1.21) | 1.08(1.04;1.12) | 1.05(1.03;1.09) | <0.001; 0.004 |
| AP(U/l) | 160(142;255) | 145(127;229) | 141(119;151) | 0.006; 0.017 |

Quality of life (according to GHQ-28) before HRT was significantly impaired in group1 compared to group2 (p<0.001) and improved after HRT (before vs after HRT p=0.006). During this survey it was the only one case of tumor growth in patient with residual tissue of craniopharyngioma.

Thus, significant improvement of lipid and mineral homeostasis as well as quality of life was found in this cohort of patients after HRT even in women without GH-treatment.

Nothing to Disclose: IAI, VYZ, NAM, DSM, AVI, NPG, GAM, IID

P3-398

ACTH & Cortisol Response to Dex/CRH Testing in Women with and without Premenstrual Dysphoria during GnRH Agonist-Induced Hypogonadism and Ovarian Steroid Replacement.

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In rodents, estradiol stimulates hypothalamic-pituitary-adrenal (HPA) axis function and in part accounts for the observed sex-differences in HPA axis response (females > males). In healthy normal-cycling women, menstrual cycle phase-related differences in HPA axis function have been attributed to changes in estradiol (E), although a role for progesterone (P) also has been suggested. We (Roca et al., 2003) have shown luteal-phase enhancement of HPA axis responsivity during exercise and that these changes are mediated by P. Further, the HPA axis and behavioral responses to physiologic levels of E and P differ in women with severe premenstrual dysphoria (PMD) compared with asymptomatic controls (ACs). To better characterize these differences, we examined in detail the effects of ovarian steroids on the HPA axis in women with PMD and ACs. Women with PMD (n=14) and ACs (n=19) received depot leuprolide (3.75 mg IM Q1 month) for 6 months. After 3 months of induced hypogonadism (L), all women received 5 weeks each of E (100 mcg patch daily) or P (suppositories 200 mg BID) in a randomized, double-blind, crossover design. Combined dexamethasone-suppression/CRH-stimulation (dex/CRH) tests and 24-hour urinary free cortisol (UFC) collections were conducted during L, E, and P. Dex/CRH tests were selected over CRH-stimulation tests for their sensitivity to dysregulated HPA axis activity and widespread use in the study of affective disorders. Symptoms were measured by standardized PMD rating scales. Results were analyzed with ANOVA-R and Bonferroni t-tests. Results show an enhanced cortisol response during P in all women compared with E: [baseline cortisol ($t_{56}=4.0$, $p<0.01$), area under the curve (AUC) ($t_{56}=2.8$, $p<0.05$), delta-max (the difference between peak and baseline) ($t_{56}=3.3$, $p<0.01$), and UFC ($t_{50}=3.2$, $p<0.01$)]. Results also show an enhanced HPA axis response during P in all women compared with L: [ACTH AUC ($t_{56}=2.8$, $p<0.05$) and UFC ($t_{50}=3.1$, $p<0.05$)]. Overall, no significant differences were seen across diagnostic groups, though there was a trend for ACTH AUC during P (PMD > AC). Plasma E and P levels did not differ between diagnostic groups. These data are in contrast to pre-clinical studies and extend in another paradigm the findings of Roca et al. that in women, P, not E, modulates the HPA axis response.

Nothing to Disclose: EEL, LKN, PEM, VLH, DRR, PJS

P3-399

Reproductive Hormone Profiles in Patients Affected by Neurocysticercosis.

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Neurocysticercosis is endemic in developing countries, its incidence is growing in developed ones because of immigration and travel facilities. It is due to localization of larval forms (cysticerci) of *Taenia solium* in the human central nervous system (CNS). Clinical presentations are heterogeneous and depend on factors as the host age and gender, immune response intensity, and location and number of cysticerci. Cysticerci and taenias have steroidogenic capabilities that include synthesis of androgens and estrogens from steroid precursors, are influenced by sex steroids, and have key steroidogenic enzymes (1, 2). Interestingly, a polycystic ovarian syndrome have been reported in a female patient with extensive neurocysticercosis who consulted for secondary oligomenorrhea (3). We have investigated the hormone reproductive blood profile in 50 patients with NC (21 females, 18 to 61 years old; 29 men, 21 to 65 years old, and 30 healthy controls (16 females, 23 to 52 year-old and 14 men, 30 to 60 year-old). The patients did not receive cysticidal nor antiinflammatory treatments before blood sampling. Diagnosis was made based on clinical story and radiological studies. All patients included fulfilled a special questionnaire directed to evaluate reproductive data. Clinical severe patients were those presenting intracranial hypertension defined by headache, nausea or vomits and papilledema at eye examination. Radiologic severe patients were those presenting multiple vesicular (viable) parasites at the subarachnoid space of skull base. Hormones were measured by RIA.

Male patients had lower 17 β -estradiol (21 ± 10 vs 28 ± 10 , pg/mL, $P < 0.05$) and DHEA serum concentrations (132 ± 107 vs 219 ± 131 μ g/dL, $P < 0.03$) and higher LH concentrations (6.5 ± 11 vs 2.5 ± 2.1 mIU/mL, $P < 0.04$) than controls. Female patients had decreased 17 β -estradiol (47.2 ± 63 vs 98.7 ± 80 , $P < 0.03$ pg/mL) and DHEA serum concentrations (95.4 ± 99 vs 168.8 ± 88 , μ g/dL, $P < 0.025$).

Clinical male severe patients had significantly lower testosterone and 17 β -estradiol but higher FSH serum concentrations compared to the non-sever cases. Alterations on female progesterone serum concentrations were also related with the severity of the disease.

These results indicate that the presence of cysticerci in the CNS deeply affects the hypothalamic-pituitary-gonadal axis function in men and women. Thereby, the alterations caused by the infection may have important reproductive implications.

(1)Romano MC et al., Current Topics in Medicinal Chemistry. 2008, 8, 408

(2)Fernández-Presas AM et al.,Parasitol Res 2008;103:847

(3)Choudhry S et al. Endocrine Society Meeting Abstract, Washington, U.S.A. 2009. http://127.0.0.1:9080/ENDO09/view.y?nu=ENDO09L_P3-55.

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Nothing to Disclose: AF, GC, RAV, MCR

P3-400

Circulating Dehydroepiandrosterone Sulfate (DS) Is Associated with an Increase in Circulating Androstenediol.

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A positive inflection of dehydroepiandrosterone sulfate (DS) during the initial phases of the menopausal transition (MT) in most women represents a dynamic change in steroid hormone production in middle-aged women. It has been reported that women with higher circulating DS during the MT have better cognition retention and maintain superior administrative function compared to matched women with lower DS levels. Thus, the rise in circulating DS production may provide substrate for peripheral conversion to generate bioactive steroids that can reduce menopausal symptoms and improve health trajectories. Alternatively, androstenediol (Adiol) which is secreted in parallel to DS may contribute to circulating estrogenicity by contributing to the total estrogen receptor ligand load (ERLL) and this alternative hypothesis was examined. Serum samples from 264 middle-aged women during the early and late perimenopausal transition were evaluated for estradiol (E2), Adiol, DS, androstenedione (Adione), testosterone (T) and circulating estrogen receptor ligand load (ERLL). In an initial analysis, concentrations of Adiol, Adione and T were correlated to DS to determine which of these steroids contribute to the positive effects attributed to increased circulating DS concentrations. In a second study, ERLL was compared to E2 in groups of middle-aged women with higher and lower circulating DS concentrations to determine the contribution of estrogenic bioactivity in association with higher and lower circulating DS. The results of the first study revealed that the circulating concentrations of all androgens were positively correlated, and that Adiol was more closely associated with DS levels than either Adione or T. In the second study, the ratio of E2 to ERLL was similar in all groups where DS levels were higher, independent of the E2 concentrations. In contrast, the contribution of E2 to total ERLL was significantly lower in the group of women in which both E2 and DS were lowest. These data demonstrate that circulating Adiol concentrations are parallel to circulating DS concentrations during the menopausal transition, and that Adiol may complement the estrogenic effects of E2 in women with the lowest E2 circulating concentrations. Taken together, these data suggest that circulating DS concentrations may be a surrogate for circulating Adiol concentrations and the benefits that have been attributed to DS may actually be estrogenic contributions of Adiol.

Sources of Research Support: The Study of Women's Health Across the Nation (SWAN) has grant support from the National Institutes of Health (NIH), DHHS, through the National Institute on Aging (NIA), the National Institute of Nursing Research (NINR) and the NIH Office of Research on Women's Health (ORWH) (Grants NR004061, AG012505, AG012535, AG012531, AG012539, AG012546, AG012553, AG012554, AG012495). The content of this abstract is solely the responsibility of the authors and does not necessarily represent the official views of NIA, NINR, ORWH or NIH.

Nothing to Disclose: DSM, JC, NAG, FZS, NS, JR, MFS, EG, BLL

P3-401

ARCHITECT® 2nd Generation Testosterone Assay, a Direct Immunoassay Capable of Accurate and Precise Measurements for Both Male and Female Specimens.

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Introduction

There is a need for a direct testosterone immunoassay that is both sensitive and specific enough to measure male and female specimens. We report the results from the ARCHITECT® 2nd Generation testosterone immunoassay and its comparison to LC/MS/MS(1) and the ID-GCMS reference method(2).

Methods

The ARCHITECT® 2nd Generation Testosterone Assay is a delayed one step chemiluminescent microparticle immunoassay. 50µL sample is automatically diluted with 100µL specimen diluent (phosphate buffered saline with preservative). Then assay specific diluent and magnetizable particles coated with ovine monoclonal antibody are added and incubated prior to the addition of the testosterone-acridinium tracer. Following washing, addition of pretrigger and trigger solutions the light output is measured. Results are interpolated from a calibration curve derived from 6 calibrators (0 to 30nM). Options to measure very low concentration specimens undiluted and high concentration specimens automatically diluted 1 in 4 are offered on the instrument for the approximately 3% of specimens that would require this.

LC/MS/MS measurements were performed using the method of Gallagher et al at the Wythenshawe Hospital, Manchester, UK(1). The ID-GCMS measurements were performed at the University of Ghent, Belgium(2).

Results

ID-GCMS reference method study:

2nd Gen Testosterone = 0.99 ID-GCMS - 0.04; R² = 0.99; for 10 male and 28 female specimens. For female specimens R² = 0.95

LC/MS/MS study:

2nd Gen Testosterone = 1.02 LC/MS/MS - 0.2; R² = 0.99; for 75 male and 120 female specimens. For female specimens R² = 0.90

2nd Gen Testosterone functional sensitivity < 0.06nM and LOQ < 0.07nM.

Precision < 10% from 0.4 - 35nM.

Dilution linearity was demonstrated for both male and female specimens.

Bilirubin up to 20mg/dL, Haemoglobin up to 100mg/dL and triglycerides up to 1000mg/dL caused less than 10% interference in male and female specimens from 0.5-25nM.

Very low cross reactivities were observed for endogenous steroids including DHEAS (0.0009%), DHT and Androstenedione. Nandrolone by contrast showed a strong cross reactivity.

Conclusion

The ARCHITECT® 2nd Generation Testosterone Assay is a direct immunoassay capable of measuring both male and female samples accurately and precisely. This assay offers an accurate, automated and more widely available alternative to LCMS.

(1)Gallagher LM, Owen LJ, Keevil BG. Simultaneous determination of androstenedione and testosterone in human serum by liquid chromatography-tandem mass spectrometry. *Ann Clin Biochem* 2007; 44:48-56

(2)Thienpont LM, De Brabandere VI, Stöckl D, De Leenheer AP. Use of cyclodextrins for prepurification of progesterone and testosterone from human serum prior to determination with isotope dilution gas chromatography/mass spectrometry. *Anal Chem* 1994;66:4116-9

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Disclosures: SB: Employee, Abbott Laboratories. EJD: Employee, Abbott Laboratories. AW: Employee, Abbott Laboratories. JMR: Employee, Abbott Laboratories. RCD: Employee, Abbott Laboratories.

Nothing to Disclose: BGK, P-YW, PMS

P3-402

Interleucin-6 and Leptin Levels in Serum and Peritoneal Fluid of Patients with Endometriosis.

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Endometriosis is a chronic inflammatory condition characterized by implantation and growth of endometrial tissue outside the uterine cavity. Interleucin-6 (IL6) and leptin are cytokines with immunoregulatory and angiogenic properties and might have a role in the pathogenesis of endometriosis. The aim of the present study was to assess IL6 and leptin levels in serum and peritoneal fluid (PF) of patients with pelvic endometriosis and to evaluate the associations between these cytokines with menstrual phases and severity of endometriosis. Forty five women that went to laparoscopy surgery because of infertility, chronic pelvic pain and/or tubal ligation agreed to participate in the study. Twenty five patients presented endometriosis and 20 had normal pelvis at the laparoscopy and were included in the control group. The severity of endometriosis was graded according to the revised 4-stage American Fertility Society-revised (1997) scoring system. Blood was collected immediately before laparoscopy and PF immediately after starting the procedure and stored in aliquots at - 80°C. IL6 levels were determined by the Human IL-6 Enzime Immunometric Assay Kit, Assay Designs and leptin levels by Human Leptin ELISA kit, Linco Research. Age and BMI were similar either between the groups as among the different stages of endometriosis. No differences were observed between cases and controls in serum and PF leptin levels. When only cases were analyzed serum and PF leptin levels did not vary in the proliferative and secretory phases of menstrual cycle. There was a significant positive correlation between serum leptin and endometriosis score (rs: 0.45 p=0.025). IL6 levels were below the limit of detection of the kit in both cases and controls. In contrast, IL6 levels in the PF was found to be significantly higher in endometriosis group than in controls [49.1 (34.2 and 96.2) and 21 (13.2 and 36.2), p=0.004]. In addition, IL6 levels in PF was significantly higher in patients with endometriosis III and IV in comparison to I and II [76.9 (48.8 and 134.7) and 41.5 (13.8 and 45.3), p=0.003]. IL6 levels were similar in proliferative and secretory menstrual phases. In conclusion, our study suggests that IL-6 and leptin may be associated with the presence of pelvic endometriosis and its severity. Studies evaluating gene and protein expression in topic and ectopic endometrium are needed to better elucidate the role of these cytokines in the pathogenesis of endometriosis.

Sources of Research Support: National Institute of Hormones and Women's Health, CNPq, Brazil and Fundo de incentivo a Pesquisa HCPA (FIPE).

Nothing to Disclose: AN, SL, DMM, PMS

P3-403

Effect of Dienogest Administration on Dynamics of Plasma Gonadotropin and Estradiol Levels; Application for Treatment of Endometriosis.

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Objective: Dienogest, a new generation of synthetic progestin, is using as an oral contraceptive (OC) in combination with ethinylestradiol. The present study was performed to elucidate the clinical effect of dienogest for the treatment of endometriosis and the dynamics of the plasma various hormone levels during the administration of dienogest.

Subjects & Methods: Thirty-one cases of the endometriosis patients were subjected under the enough informed consent. All of the patients had taken the laparoscopic operation, and dienogest (2mg/day, continuous administration) was administered for 6 months. Then, the dynamics of plasma gonadotropin and estradiol levels, recurrent rate of endometriosis (evaluated by the MRI) were examined.

Results: Plasma levels of gonadotropin decreased gradually by the administration of dienogest (FSH: $6.2 \pm 2.1 \rightarrow 3.5 \pm 1.7$, LH: $4.5 \pm 2.2 \rightarrow 3.0 \pm 1.1$ mIU/ml, $p < 0.01$). Plasma estradiol levels also decreased significantly ($86.5 \pm 32.9 \rightarrow 29.9 \pm 15.4$ pg/ml, $p < 0.01$). Four cases were diagnosed as recurrence out of 31 subjects (12.9%), and this was as same as the result of the OC.

Conclusion: The plasma levels of gonadotropin and estradiol were decreased adequately by the administration of dienogest. From these results, it was suggested that the continuous administration of dienogest is useful for the treatment of endometriosis.

Nothing to Disclose: HH, OH, KA, YI, SO, HM

P3-404

The Relationship between Estrogen Withdrawal and Muscle Weakness in Female Patients with Chronic Kidney Disease.

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Background; It has been reported that female patients with chronic kidney disease suffer from ovarian insufficiency and reach menopause early, compared with normal subjects. We measured muscle volume in these patients as estrogen withdrawal plays an important role in muscle weakness.

Methods; One hundred and twelve patients (Female/Male; 43/69) receiving maintenance hemodialysis (HD) were enrolled in this study. The patients were classified into the three groups; Group A1: The patients reached menopause after the initiation of HD treatment. Group A2: The patients have not reached menopause, but are now receiving HD treatment. Group B: The patients reached menopause before the initiation of HD treatment. The age at menopause in female, arm-muscle area (AMA) and body mass index (BMI) were calculated. AMA was calculated by arm-muscle circumference (AMC), which was measured by multifrequency bioelectrical impedance analysis. AMA and BMI were corrected using the following formula. Corrected AMA and BMI were calculated as follows; the measured AMA and BMI values were divided by the mean of AMA and BMI values of subjects with same age, which was obtained from Japanese Anthropometric Reference Data 2001. Additionally, we examined the relationship between duration of HD and AMA.

Results; The number of patients was 21 in Group A1, 14 in Group A2 and 8 in Group B. The mean age at menopause of Group A1 was lower than Group B (47.5 ± 3.8 vs. 49.4 ± 3.5 years). The 92.9 % of all patients had an irregular menstrual cycle in Group A2. The corrected AMA was significantly lower in Group A (A1 and A2) than in Group B (0.82 ± 0.14 vs. 0.98 ± 0.17 , $P = 0.035$), however, the corrected BMI was not different between Group A and Group B (0.94 ± 0.22 vs. 1.00 ± 0.14). The duration of HD in Group A was longer than in Group B (20.1 ± 8.2 vs. 4.7 ± 4.6 years, $P < 0.001$). The duration of HD was negatively associated with the AMA in female patients ($r = -0.509$, $P < 0.001$), however, there was no significant relationship between the duration of HD and the AMA in male patients.

Conclusion; The ovarian insufficiency and the early menopause in female HD patients may lead to the reduction of muscle mass.

Nothing to Disclose: YA, NK, SO, AY, TA, KN

P3-405

Thyroid Autoimmunity and Recurrent Miscarriages in a Greek Population.

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Aim: Determination of the association of thyroid autoimmunity with recurrent miscarriages in a sample of the Greek population.

Material and Methods: 474 non-pregnant women with an age below 40 years old with a history of recurrent miscarriages (defined as two or more consecutive pregnancy losses) who were attended at our Recurrent Miscarriage Unit during the last five years (2003-2008) were studied for the presence of thyroid autoantibodies (thyroglobulin and thyroid peroxidase antibodies). All women had been investigated for anatomic uterine defects, vaginal and cervical positive cultures, endocrine abnormalities, antiphospholipid syndrome and thrombophilia defects. Cases with positive results in any of the above examinations were excluded.

Results: The mean age of patients was 34.0 ± 0.9 in the study group with 2.8 ± 0.2 miscarriages. 172 women from the study group fulfilled our inclusion criteria. High titles of thyroid antibodies were found in 36 out of 172 women (21%). The mean percentage of the presence of at least one of thyroid antibodies in white non-hispanic disease-free population in the USA has been found to be about 12% in the ages between 20 and 39 years old (2002). These percentages are similar to those found in Greek population studies. The difference in the occurrence between our study group and the general disease-free population is statistically significant ($p < 0.05$).

Conclusions: The data of our study revealed a high prevalence of thyroid antibodies in women with recurrent miscarriages compared to the general disease-free population. These findings suggest a possible role of thyroid autoimmunity as a causal factor of recurrent miscarriage.

Nothing to Disclose: AAV, PD, EKK, KS, DM, EA, EZ, CT, DL, AA

P3-406

Nonalcoholic Fatty Liver Disease (NAFLD) in Lean, Obese and Gestational Diabetic Pregnant Women.

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Introduction:

NAFLD has become the leading cause of liver disease in the Western world and has a strong association with obesity and diabetes. Little is known about NAFLD in pregnant women.

Methods:

Fifty seven (57) pregnant volunteers in the first or third trimester were recruited and evaluated for the presence of NAFLD using abdominal ultrasound scans using accepted criteria. Liver biopsies were not performed since this invasive procedure would present undue risks in otherwise healthy pregnant subjects. A total of 57 pregnant women were enrolled in this preliminary study. Fasting liver enzymes (AST and ALT), triglycerides, adiponectin, leptin, glucose, and insulin levels were drawn. Gestational diabetes was defined in third trimester subjects using criteria developed by Carpenter and Coustan. Subjects were subsequently subdivided into lean, obese, and gestational diabetics.

Results:

Half of the subjects enrolled had ultrasound evidence of NAFLD. NAFLD was observed equally among those presenting in the first or third trimesters. Additionally, the prevalence of NAFLD was similar between those with normal BMI versus obese women. No liver enzymes elevations were encountered. Serum adiponectin levels were significantly higher in the lean subjects irrespective of gestational age while obese and gestational diabetic subjects had similar adiponectin concentrations. Serum leptin levels in the first trimester were significantly lower in lean subjects compared to the obese and GDM women. In the third trimester, levels were elevated (compared to first trimester levels) in the lean and obese, and gestational diabetic groups. No relationship with between adipokine levels and the presence of NAFLD was detected.

Conclusions:

Pregnant women with NAFLD have normal AST and ALT levels probably due to physiologic hemodilution associated with pregnancy, and hence, liver enzymes are not a reliable diagnostic tests to distinguish the presence or absence of NAFLD in pregnancy. Distribution of NAFLD is comparable in lean, obese and gestational diabetic subjects.

Sources of Research Support: Laura Bush Women's Health Research Institute.

Nothing to Disclose: GD, BM, CM, RPK, VDC

P3-407

Role of Antioxidants Manganese Superoxide Dismutase (MnSOD) and Catalase (CAT) in Preventing Adipogenic and Promoting Osteogenic Differentiation of Human Bone Marrow Derived Mesenchymal Stem Cells (hMSC) in Hyperglycemia.

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Background: Pluripotent MSCs are essential for tissue regeneration, and can differentiate into adipocytes, osteocytes or chondrocytes. Exposure to high glucose (25mM) may alter survival and differentiation of MSCs compared to normal glucose (5.5mM) **Methods:** We exposed MSCs to high glucose (HG) for 4 days and noted differentiation by gene expression using RT-PCR. We also stained for adipogenic differentiation by Oil Red O stain. We undertook FACS analysis using DCF-DA dye to detect reactive oxygen species (ROS) accumulation and propidium iodide-annexin V incorporation for cell apoptosis assays. We transduced hMSCs with Adenovirus containing eGFP (green fluorescent protein) as control or MnSOD or Catalase (CAT) at 100 MOI each before differentiating the cells in normal or high glucose, to note if intracellular upregulation of antioxidants will affect hMSC survival and differentiation in HG. **Results:** Glucose increased adipogenic differentiation (oil red-O stain positive) and reduced osteogenic differentiation of MSCs in HG. We noted that there is up-regulation of mRNAs in HG for markers of white adipose tissue (WAT), such as, Leptin (LEP)11-fold, Perilipin (PLIN) 4-fold, and PPARG-25 fold while the bone formation markers Alkaline Phosphatase (ALPL) and osteocalcin (BGLAP) mRNA were reduced by 4.5 and 0.4 fold respectively. There was increased ROS accumulation and apoptosis in HG by FACS. When MSCs were transduced with AdMnSOD or AdCAT, during differentiation in HG, both CAT and MnSOD over-expression separately improved MSC survival (22% and 33.3% compared to controls, respectively) in HG. The over-expression prevented adipogenesis and promoted osteogenesis. Of the two antioxidants, over-expressed separately, MnSOD gives relatively better suppression of WAT markers, compared to CAT over-expression. This finding emphasizes the importance of reducing ROS accumulation in high glucose, by either mitochondrial (MnSOD) or cytosolic (CAT) antioxidant activity and thereby prevent adverse differentiation of hMSCs in HG. **Conclusion:** hMSCs undergo adipogenic differentiation in high glucose and over-expression of either MnSOD or Catalase in MSCs prevents adipogenic and promotes osteogenic differentiation. Our findings emphasize the protective role of anti-oxidants in hMSC differentiation with implications in management of obesity and osteoporosis in a clinical setting of diabetes.

Sources of Research Support: Collaborative Research Grant, 2009-BMC and UMass, Amherst.

Nothing to Disclose: SS, MY, YCK, JES

P3-408

Aldo-Keto Reductase 1C2 (AKR1C2) Drives Glucocorticoid-Induced Androgen Inactivation in Human Preadipocytes.

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Adipogenesis and lipid storage in human adipose tissue are inhibited by androgens such as 5 α -dihydrotestosterone (DHT). Aldo-keto reductase 1C2 (AKR1C2), which inactivates DHT to 5 α -androstane-3 α ,17 β -diol (3 α -diol), has been suggested to regulate the exposure of adipose cells to active androgens. Moreover, dexamethasone was recently shown to stimulate DHT inactivation in human preadipocytes. The aim of the study was to characterize glucocorticoid-induced androgen inactivation in human preadipocytes and establish the role of AKR1C2 in this reaction. Experiments were performed in subcutaneous (SC) and omental (OM) primary preadipocyte cultures established from fat samples obtained in men and women undergoing elective surgeries. Inactivation of DHT to 3 α / β -diol over a 24h period was measured in dexamethasone- or vehicle-treated cells. Specific downregulation of AKR1C family members in human preadipocytes was achieved using RNAi and confirmed by quantitative RT-PCR analysis. The maximal dexamethasone stimulation (1 μ M) of DHT inactivation was higher in OM fat from obese men compared to SC fat from obese men as well as to SC and OM fat from women (P<0.05). A significant positive correlation was observed between body mass index and maximal dexamethasone-induced DHT inactivation rates in SC and OM adipose tissue of men and women (P<0.05). AKR1C2 siRNA transfection in human preadipocytes reduced AKR1C2 mRNA expression levels by at least 80%, while AKR1C1 and AKR1C3 mRNA expression levels were unaffected. As a consequence of AKR1C2 downregulation in human preadipocytes, DHT inactivation rates were reduced by half (P<0.05). Moreover, the responsiveness of DHT inactivation to dexamethasone was completely abolished by AKR1C2 siRNA transfection. Progesterone inactivation to 20 α -OH-progesterone, which is mainly performed by AKR1C1, was not altered by AKR1C2 downregulation. On the other hand, the AKR1C1 siRNA blocked progesterone inactivation but failed to significantly alter basal and dexamethasone-stimulated DHT inactivation. Preadipocyte differentiation experiments suggest that downregulation of AKR1C2 increases the sensitivity of cells to the anti-adipogenic effects of DHT. In conclusion, glucocorticoids are particularly effective in inducing DHT inactivation to 3 α / β -diol in OM preadipocytes of obese men, and AKR1C2 is the enzyme responsible for this reaction. Modulation of AKR1C2 by active glucocorticoids may locally modify exposure of adipose cells to androgens.

Nothing to Disclose: AV, KB, MN, PM, PYL, VL-T, AT

P3-409

Role of Poly(ADP-Ribose) Polymerases 1 and 2 in Adipogenesis.

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Adipogenesis is tightly controlled by the sequential regulation of thousands of genes through the interplay of transcription factors and coregulators, some of which have chromatin-modifying activities. Despite identification of the major transcription cascades and protein factors, our understanding of the precise molecular mechanisms of adipogenesis, particularly during the early stages of differentiation, are incomplete. Previous studies have suggested a role of poly(ADP-ribosyl)ation mediated by the nuclear poly(ADP-ribose) polymerases 1 and 2 (PARP-1 and PARP-2) in adipogenesis. PARP-1 and PARP-2 are nuclear proteins that interact with chromatin and regulate various nuclear processes, including transcription.

To determine the transcriptional regulatory functions of PARP-1 and PARP-2 in adipogenesis, we are using a well-established model of adipogenesis: the murine 3T3L1 preadipocyte cell line. Using a variety of cell-based and molecular assays, we have found that: (1) PARP-1 and PARP-2 protein and poly(ADP-ribosyl)ation activity fluctuate during adipogenesis, (2) inhibition of PARP-1 and PARP-2 catalytic activity with the PARP inhibitor PJ34 blocks the differentiation of 3T3L1 cells, (3) RNAi-mediated depletion of PARP-1 or PARP-2 alters adipogenesis-related gene expression and modulates the 3T3L1 cell differentiation program, and (4) PARP-1 and PARP-2 bind to the genomic regions of regulated adipogenic target genes in a pattern that partially overlaps with the peroxisome proliferator activated receptor (PPAR), a key transcriptional regulator of adipogenesis. We are now conducting additional experiments to elucidate the exact role and regulatory mechanisms of PARP-1 and PARP-2 in adipogenesis. Collectively, these studies will help to elucidate the adipogenic regulatory network, as well as shed light on the mechanisms by which PARP-1 and PARP-2 regulate transcription to affect cell proliferation and differentiation. These studies have the potential to reveal new aspects of the pathogenesis of obesity and the potential therapeutic benefits of PARP inhibitors.

Sources of Research Support: Grants from the NIH/NIDDK to W.L.K. and a predoctoral training award from the DOD/BCRP to X.L.

Nothing to Disclose: XL, WLK

P3-410

Effect of Insulin like Growth Factor-1 on Adipocyte Differentiation in Preadipocytes Derived from Lean and Obese Adults.

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Background: Obesity is characterized by increased adipose tissue mass. Expansion of adipose tissue can occur due to enlargement of existing adipocytes as well as increases in the number of new adipocytes (adipogenesis) from adipocyte precursor cells, termed preadipocytes. Many studies have shown that insulin like growth factor-1(IGF-1) and insulin act in concert to stimulate adipogenesis in 3T3-L1 and human preadipocytes. Subcutaneous abdominal preadipocytes derived from obese individuals demonstrate poor differentiation in the presence of insulin. In vitro differentiation of subcutaneous abdominal preadipocytes has been found to inversely correlate with degree of central obesity. However, the impact of obesity on differentiation of these cells in the presence of IGF-1 alone is unclear. We undertook the following study to examine the effect of IGF-1 on differentiation of subcutaneous abdominal preadipocytes derived from lean and obese adults.

Methods: Subcutaneous abdominal adipose tissues were obtained from 9 lean adults (BMI < 25) and 8 obese adults (BMI >25) at the time of elective abdominal surgeries. Preadipocytes were isolated, cultured and exposed to standard differentiation media (without insulin) in the presence or absence of IGF-1 (10 ng/mL) for 10 days. Adipogenesis was measured both by assessing an increase in adiponectin mRNA gene expression and by quantification of the degree of Oil red O staining of mature lipid-laden adipocytes.

Results: Preadipocytes from lean adults had a greater increase in adiponectin mRNA expression (mean 2641.6 fold, range: 2.6- 15742.7) in response to IGF-1 treatment than cells from obese adults (mean 32.5 fold, range 0.92- 121, p= 0.01). Oil red O staining with digital quantification supported these results.

Conclusion: Subcutaneous abdominal preadipocytes from obese individuals demonstrate blunted response to the proadipogenic effects of IGF-1. Better understanding of this response may give insight into cellular mechanisms involved in body weight homeostasis. Future studies examining the impact of weight loss or weight gain on differentiation potential of preadipocytes will clarify whether impaired differentiation might be a compensatory response to prevent further weight gain in obese individuals.

Nothing to Disclose: RLA, SK, MJC, SAD, RSB

P3-411

Growth Differentiation Factor (GDF3) Effects on Adipogenesis.

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Growth differentiation factor 3 (GDF3) is a member of TGF-beta superfamily whose expression in white adipose tissues is induced by high fat diet (HFD). Body weight and adipose tissue mass normally increase under HFD conditions, and these processes are augmented when GDF3 is overexpressed systemically by adenovirus-mediated gene transfer. In contrast, GDF3 knockout mice have less adipose tissue mass than wild type mice under HFD and exhibit higher basal metabolic rates. These results suggest that GDF3 may act as an adipogenic factor under conditions of caloric excess, and that GDF3 deficiency results in the dysregulation of energy expenditure. However, the signaling pathway(s) that mediate these effects are unknown. Recent reports indicate that GDF3 bind BMP(s) and inhibits their signaling pathway. Several BMP(s) play important roles in adipogenesis, and BMP7 is sufficient to initiate brown adipose differentiation. Interestingly, the brown adipose markers, PGC1-alpha, CPT1b and UCP1 are overexpressed selectively in the white adipose tissues of GDF3 knockout mice on HFD, suggesting that GDF3 may modulate the activity of BMP signaling on fate decisions during adipocyte differentiation. Recently, myostatin (GDF8) has been shown to inhibit adipocyte differentiation through the selective inactivation of BMP7 signaling. Similarly, we are studying the potential molecular mechanisms underlying the effects of recombinant human GDF3 on BMP-dependent adipogenesis using pre-adipocyte cell lines, C3H10T1/2 and 3T3L1, BMP-reporter assays, and quantitative PCR. In addition, we are developing transgenic mouse models of GDF3 overexpression under spatiotemporal control. Ultimately, these models will help us to better understand the metabolic role of GDF3 in obesity and energy expenditure.

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Nothing to Disclose: JCB, NZ, CWB

P3-412

SUMO-1 Function in Regulation of Energy Metabolism In Vivo.

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SUMOylation is a dynamic process involving the covalent attachment of SUMO to target proteins. The SUMO family consists of four related proteins. We have previously generated a Sumo1 null mouse whose phenotype under normal conditions was indistinguishable from that of wild-type (WT) littermates, at least in part, due to compensation by other SUMO paralogues for the lack of SUMO-1 (1). Since distinct SUMO paralogues have been implicated in the regulation of several nuclear receptors, including PPARs, we have studied the role of Sumo1 in adipocyte differentiation and fat metabolism.

Sumo1 null mouse embryonic fibroblasts (MEFs) differentiated into adipocytes less efficiently than WT cells after 12 days, as judged by Oil Red O staining and decreased expression of mRNAs encoding adipocyte markers aP2 and Pparg in Sumo1 null cells. In addition, expression of Fsp27, a gene important in the regulation of lipid droplets and fat storage, was attenuated during differentiation of Sumo1 null MEFs to adipocytes.

Feeding of mice with high fat food (60% of total calories from fat) for 22 weeks resulted in a significantly decreased weight gain in Sumo1 null mice compared to their WT littermates, whereas food consumption normalized to the body weight of the animals was the same in the two groups. Histological analysis revealed that Sumo1 null mice did not develop hepatosteatosis on high fat diet, and their white adipose tissue consists of smaller adipocytes than that of WT mice. Despite their decreased adiposity, Sumo1 null mice have no difference in insulin sensitivity or glucose tolerance compared to WT mice. Gene expression profiling of liver, white and brown adipose tissue, and skeletal muscle identified a transmembrane protein having acyltransferase activity as heavily down-regulated in all Sumo1 null tissues studied. Experiments are underway to identify the role of this protein in the lean phenotype of Sumo1 null mice on high fat diet. Additional experiments have demonstrated that Sumo1 null mice have disturbances in responses to general inflammation induced by lipopolysaccharide as well as in tumor incidence seen after treatment with a carcinogen, indicating that, in spite of their apparently normal phenotype, Sumo1 null mice have clear differences in their responses to stress.

(1) Zhang F-P et al., Mol Cell Biol 2008; 28: 5381

Sources of Research Support: CRESCENDO; Sigrid Juselius Foundation.

Nothing to Disclose: LM, PP, JH, OAJ

P3-413

C-Type Natriuretic Peptide as a New Regulator of Food Intake and Energy Expenditure.

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Objectives: The physiological significance of C-type natriuretic peptide (CNP) in food intake and energy expenditure remains unclear, due to markedly short stature in CNP null mice. To elucidate the significance of CNP without influences of skeletal abnormality, we generated CNP null mice with chondrocyte-targeted CNP expression (CNP-Tg/*Nppc*^{-/-} mice) and analyzed their phenotypes. **Methods:** Growth curves of male mice were analyzed in naso-anal length and body weight. Food intake, rectal temperature, urinary catecholamine excretion, and oxygen consumption were measured, and insulin sensitivity was assessed by insulin tolerance tests in male mice at age of 17-20 weeks. White adipose tissues, hypothalami, and interscapular brown adipose tissues (BAT) were obtained from 20-week-old male mice. Fat pad weight and plasma leptin concentrations were measured. CNP, uncoupling protein (UCP)-1, peroxisome proliferator-activated receptor (PPAR) γ , and PPAR γ -coactivator (PGC)-1 α mRNA levels were assessed by a real-time RT-PCR. **Results:** The naso-anal length of CNP-Tg/*Nppc*^{-/-} mice was smaller than that of CNP-Tg/*Nppc*^{+/+} mice, but it surpassed that of wild-type (WT) mice. In CNP-Tg/*Nppc*^{-/-} mice, the body fat weight and serum leptin concentrations were decreased by 50% and 80%, respectively, and insulin sensitivity was augmented, as compared with CNP-Tg/*Nppc*^{+/+} mice. In CNP-Tg/*Nppc*^{-/-} mice, rectal temperature and urinary noradrenalin excretion were increased by 1.1 C and 28%, respectively, and oxygen consumption was significantly greater, than those in CNP-Tg/*Nppc*^{+/+} mice. In CNP-Tg/*Nppc*^{-/-} mice, UCP-1, PPAR γ , and PGC-1 α mRNA levels in BAT were more than 2-fold increased than those in CNP-Tg/*Nppc*^{+/+} mice. In WT and CNP-Tg/*Nppc*^{+/+} mice, CNP mRNA was detected in hypothalami, but not detectable in BAT. Food intake of CNP-Tg/*Nppc*^{-/-} mice upon *ad libitum* feeding and after 48-hour starvation was smaller by 21% and 61%, respectively, than that of CNP-Tg/*Nppc*^{+/+} mice. **Conclusions:** This study proposed that CNP is a new regulator of food intake and energy expenditure. It was suggested that CNP suppresses energy expenditure in BAT by attenuating the sympathetic nervous system activity possibly under the control of the hypothalamus. Further analyses on precise mechanisms of CNP actions would lead to the better understanding of the significance of the CNP/guanylyl cyclase-B system in food intake and energy expenditure.

Nothing to Disclose: MI, NT, NY, GK, NO, KO, MS, YF, DT, TS, KK, KH, YT, EK, TF, TN, MS, MM, NK, AY, HA, KN

P3-414

Lipocalin 2 in Adiposity and Metabolism in Female Mice: An Important Regulator of Estrogen Production and Signaling.

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Lipocalin 2 (LCN2), a novel adipose-derived cytokine, belongs to the lipocalin subfamily of low molecular mass secreted proteins that bind small hydrophobic molecules. Increased LCN2 is associated with obesity and insulin resistance in both human and rodent models of obesity. Decreased endogenous estrogen has been linked to postmenopausal obesity and increased cardiovascular risk in women. Previous studies have shown that LCN2 is a putative target gene of estrogen receptor. However, the role of LCN2 in modulating estrogen action and the underlying mechanism by which it influences obesity and insulin resistance remains unclear. In the present study, we explored the effects of LCN2 on estrogen receptor signaling in high-fat-diet induced obesity in female LCN2 deficient mice. Female wild-type (WT) and LCN2 ^{-/-} mice were fed either high fat diet (HFD) or regular chow diet (RCD) at 4 weeks of age. After 22-week HFD feeding, systemic insulin sensitivity, metabolic and lipid profile, serum estrogen and estrogen receptor signaling in different tissues and cells were assessed in LCN2 deficient mice. Our data demonstrated that upon the challenge of HFD, LCN2^{-/-} female mice exhibited increased body weight and body fat mass along with systemic insulin resistance when compared with WT mice. Strikingly, LCN2^{-/-} mice had significantly lower levels of serum 17 β -estradiol than WT controls, particularly under the HFD condition. Serum levels of total cholesterol and LDL-cholesterol were significantly elevated while HDL-cholesterol levels were reduced in LCN2 ^{-/-} mice compared to WT mice. LCN2^{-/-} mice also displayed down-regulated expression of estrogen receptor alpha in adipose tissue, muscle, and liver as well as primary adipocytes and S-V cells in comparison with WT controls. Interestingly, the expression levels of the enzymes regulating the biosynthesis of estrogen including aromatase and 17 β hydroxysteroid dehydrogenase-1 were decreased in the liver of LCN2^{-/-} mice as compared with WT mice. Collectively, our findings demonstrate that LCN2 deficiency led to decreased production and action of estrogen and worsened HFD-induced obesity, insulin resistance, and hypercholesterolemia in female mice. Thus, LCN2 may play a protective role in obesity and cardiovascular risk in females partially through regulating estrogen biosynthesis and signaling.

Nothing to Disclose: HG, DAB, XLC

P3-415

Mineralocorticoid Receptor Activation Mediates Thermogenesis in White Adipocytes.

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Obesity is at the heart of the metabolic syndrome and raises the risk for high blood pressure, arteriosclerosis and insulin resistance. Whereas white adipocytes are responsible for energy storage, brown adipose tissue dissipates energy by uncoupling oxidative phosphorylation from ATP production, mediated by the mitochondrial uncoupling protein-1 (UCP-1). We investigated the role of the mineralocorticoid receptor (MR) in mediating mitochondrial biogenesis and thermogenesis in newly generated immortalized murine white wildtype control and MR knock-out (KO) adipocytes. UCP-1 mRNA expression was strongly diminished in MR KO adipose cells. Furthermore, GR knock-down by small interfering RNA and subsequent stimulation of the remaining MR with corticosterone enhanced the gene expression of UCP-1 and PGC-1 alpha in differentiated white adipocytes. Chronic selective MR stimulation with 10nM aldosterone during the 7-day course of differentiation did not affect lipid accumulation in differentiating wildtype control adipocytes (assessed by oil red o staining). Yet, mRNA-expression of UCP-1 and PGC-1 alpha was increased by more than 50% in aldosterone-stimulated cells as compared to non-treated cells. This effect was entirely abolished in the presence of the MR antagonist eplerenone. Moreover, the expression of the mitochondrial markers cytochrome c and NRF-1 was significantly elevated by 40% in adipocytes treated with 10nM aldosterone during the differentiation course. This effect was reversed in the presence of the MR antagonist eplerenone. Finally, oxygen consumption significantly increased by 25% in response to chronic aldosterone stimulation. Again, this effect was abolished by eplerenone. In conclusion, our results demonstrate a role for the mineralocorticoid receptor in shifting white adipocytes towards a mitochondrial-rich, thermogenic brown adipose cell phenotype. Selective corticosteroid receptor modulation which dissects the previously demonstrated pro-inflammatory from these pro-thermogenic properties of the MR may present a novel therapeutic approach against obesity.

Nothing to Disclose: II, JH, NP, HL, JK

P3-416

Corticotropin-Releasing Hormone (CRH) Signalling and Biological Actions in Brown Adipocytes.

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In addition to a well established role in coordinating mammalian adaptive responses to stressful stimuli, “stress” peptides such as corticotropin releasing hormone (CRH) and urocortins (Ucns), are emerging as important regulators of the homeostatic mechanisms regulating energy balance and metabolism. These peptides, acting through specific G-protein coupled receptors, CRH-R1 and R2, exert well documented central anorectic and thermogenic actions by controlling food uptake, and can also target multiple peripheral tissues such as skeletal muscle, pancreas and white adipose tissue to influence important metabolic pathways. Functional CRH-Rs as well as CRH, Ucn1 and Ucn2 are expressed in brown adipose tissue. Using as an experimental paradigm the differentiated T37i, a cell line derived from a hibernoma (malignant brown fat tumour), we have previously identified functional CRH-Rs that regulate lipolysis and adaptive thermogenesis in an autocrine manner. In this study we further investigated the signalling pathways mediating CRH actions in T37i cells.

CRH at low (nanomolar) but not high (submicromolar) concentrations induced adipocyte glycerol release through a cAMP/PKA-dependent pathway and downstream phosphorylation of HSL at Ser563, Ser565 and Ser660. CRH also elevated AMPK phosphorylation at Thr 172 (by 10-14 fold), a signalling event required for CRH-induced phosphorylation of HSL Ser565, as shown by the use of the specific AMPK inhibitor compound C. Interestingly, inhibitors of the cAMP/PKA pathway (PKAi and Ht-31) demonstrated that phosphorylation of AMPK (at Thr172) and HSL (at Ser563, Ser660 but not Ser565), required intact PKA activity and PKA-AKAP interactions. Quantitative RT-PCR studies showed that exposure of mature T37i cells to low (1 nM) but not high (100nM) concentrations of CRH for 24h induced UCP1 mRNA upregulation by 4-6 fold, whereas high concentrations of CRH and Ucn2 induced PGC-1 α and 11 β -HSD1 mRNA levels by 3-4 fold, implicating the involvement of CRH-R2 receptors. Finally, the CRH system seems to demonstrate a certain degree of plasticity in response to food deprivation since starvation of B6 CB F1 mice for 16h significantly increased by 3-7 fold CRH, Ucn1 and Ucn2 mRNA levels. In conclusion our results suggest that CRH (and Ucns) stimulate multiple signaling cascades to activate specific lipases important for tissue lipolysis and regulate gene expression involved in energy balance and adaptive thermogenesis.

Nothing to Disclose: BL, DM, JP, HL, DG

P3-417

Hormonal and Nutritional Regulation of PTRF in Adipose Tissue: Role of PTRF in Regulating Lipolysis.

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Mobilization of energy from lipids stored in adipose tissue is crucial during periods of energy deficit. Dysregulation of fat breakdown may contribute to metabolic diseases.

Polymerase I and transcript release factor (PTRF) is a protein expressed at high levels in adipose tissue where it is a major protein component in caveolae microdomains of the plasma membrane. Recently, it has been demonstrated that PTRF is required for the formation of caveolae, suggesting that PTRF may serve as a caveolar coat protein. However, PTRF localizes specifically to TG-metabolizing caveolae subclass and translocates upon insulin treatment to the cytosol where it associates with HSL. These data indicate that PTRF may also have a role in lipid metabolism. Therefore we investigated the involvement of PTRF in regulating lipolysis.

We have found that in mice PTRF expression in adipose tissues is regulated by nutritional status. PTRF expression levels increased by eight fold during fasting and decreased after re-feeding in white adipose tissues. No difference was seen in the expression levels of PTRF brown adipose tissues, suggesting that PTRF in white adipose tissue has a role in mediating fat mobilization. Intraperitoneal injection of mice with insulin resulted in a two fold decrease of PTRF expression, while injection with catecholamine isoproterenol increased PTRF expression by two fold in white adipose tissue, indicating a hormonal regulation of PTRF expression during fasting/feeding.

Fasting and isoproterenol treatment resulted also in PKA-dependent-phosphorylation of PTRF on multiple serine sites in adipose tissue. Mutation of two of those sites resulted in a reduction in lipolytic activity in 3T3-L1 adipocytes. Together, these results indicate a pivotal role for PTRF in regulating lipolysis.

Sources of Research Support: Grants from the NIH to EMF and JSF and the Swedish Wenner-Gren Foundation to NA.

Nothing to Disclose: NA, EM-F, JSF

P3-418

The PI3K/Akt/PDE4 Pathway Mediates the Antilipolytic Effects of QRFPs through GPR103b Receptor.

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Background: The orexigenic RFamide peptides QRFP-43 and -26 have been shown to bind to GPR103a and b receptors in hypothalamic regions involved in feeding regulation. We have shown that adipocytes from epididymal fat tissue as well as 3T3-L1 differentiated cells express both GPR103b and QRFPs, and that GPR103b is responsible for mediating the anti-lipolytic effects of QRFPs through, as of yet, unknown mechanisms. **Objectives:** 1) to determine the pathway(s) involved in QRFP-elicited GPR103b signaling and 2) to determine the mechanism(s) of the antilipolytic effects of QRFPs in differentiated 3T3-L1 adipocytes. **Methods:** Differentiated 3T3-L1 cells were preincubated with QRFPs in presence of LY294002 (10 mM), SH-5 (5 mM), cilostamide (250 nM) or rolipram (250 nM) for 30 min. Isoproterenol (ISO, 300 nM) was then added for 30 min at 37C. After the incubation period, incubation medium was collected for free glycerol measurement and lipolysis index (% of lipolysis induced by ISO) was calculated. For western blot detection, differentiated 3T3-L1 cells were starved 1 h and then preincubated with QRFP-43 (10 nM) in presence of LY294002 or SH-5 for 30 min. ISO was then added for 5 min at 37C. **Results:** QRFP-43, alone or combined with ISO, elicited a 32 and 55% (P<0.01 vs untreated cells) increase in Akt phosphorylation, respectively. In contrast, QRFP-43 inhibited ISO-elicited phosphorylation of HSL and perilipin by 22 and 32% (P<0.01), respectively. Inhibitors of PI3K (LY294002) and Akt (SH-5) completely abrogated QRFP-43-antilipolytic effects. Further investigation with rolipram, a specific phosphodiesterase 4 (PDE4) inhibitor, completely reversed the inhibitory effect of QRFPs on ISO-induced glycerol release. In contrast, cilostamide, a specific PDE3 inhibitor, did not exert significant inhibitory effects on QRFPs-mediated anti-lipolysis. **Conclusion:** Our results support that the antilipolytic effects of QRFPs may be coupled to GPR103b signaling through PI3K/Akt and PDE4 activation, the latter being responsible for the inhibition of ISO-induced lipolysis.

Sources of Research Support: Educational grant of Aeterna Zentaris Inc.

Nothing to Disclose: MM, CJ, MGS, SM, HO

P3-419

A Comparative Analysis of Adipose Tissue Secretome in a Model System of Lean and Fat Chickens.

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In mammals, the adipose tissue holds a central role in the control of energy homeostasis. The adipose-secreted proteins signal the amount of fat stored to the brain and other tissues, thereby affecting appetite, energy expenditure, fat and glucose metabolism, insulin sensitivity, vascular haemostasis, reproduction and so on. In mammals, a prominent player in these activities is leptin; however, a growing number of newly identified adipose-secreted proteins are also being implicated in these regulatory circuits.

While it is logical to assume that in chickens, adipose tissue has a role similar to that in mammals, we failed to identify the chicken orthologue of the mammalian leptin. Using a leptin bioassay (1), which is based on expression of the chicken leptin receptor in cultured cells, no leptin activity was detected in blood samples of fat or lean chickens or in wild birds such as Adelie penguins and Bar-tailed Godwit.

Assuming that other adipose-secreted proteins should take the role of leptin in avian species, we performed a comparative analysis of the adipose tissue secretome in two strains of chickens with lean and fat phenotypes. Significant difference in the secreted-protein profiles of these strains was observed. Some of the differentially expressed proteins were also validated by real-time PCR. Differentially expressed adipose secreted proteins such as retinol binding proteins, destrin and others were characterized. The possible physiological implications of these differences in the specific phenotypes of the chicken strains will be discussed.

Hen G, Yosefi S, Ronin A, Einat P, Rosenblum CI, Denver RJ & Friedman-Einat M 2008 Monitoring leptin activity using the chicken leptin receptor. *J Endocrinol* 197 325-333.

Sources of Research Support: Chief Scientist of the Ministry of Agriculture, Israel.

Nothing to Disclose: MF-E, AR, SY, DC, FC, SB

P3-420

Effects of AT1 Receptor Antagonist on Adiponectin Expression and Morphology of the Adipocytes in Metabolic Syndrome Rats.

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Angiotensin II plays important roles in the regulation of differentiation and function of adipocytes and insulin resistance. The aim of the present study was to investigate the effects of ARB on the function and morphology of adipocytes in metabolic syndrome. Ten-week-old male SHR/NDmcr-cp (ND) rats with gene mutation of leptin receptor were used as a model of metabolic syndrome. Rats were divided into three groups: control/ND group treated with vehicle, ARB/ND group (olmesartan, 5mg/kg/day, p.o., 8 weeks), and hydralazine (Hyd)/ND group (7.5mg/kg/day, p.o., 8 weeks), respectively. Plasma levels of leptin (p-Lep), adiponectin (p-Adip), and mRNA expression levels (RT-PCR) of leptin, adiponectin and PPAR-gamma in adipose tissues were determined. The morphology of adipocytes was microscopically investigated in sections of hematoxylin-eosin staining and the area of adipocytes was measured using an analytical program for personal computer. Results were compared between the 3 groups and with those in SHR/Izm without mutation (non-ND group). In the control/ND group, body weight, p-Lep and leptin mRNA levels in the adipose tissues were significantly elevated, while mRNA levels of adiponectin and PPAR-gamma in the adipose tissues were significantly decreased compared to those in the non-ND group. In the ARB/ND and Hyd/ND groups, while blood pressure was significantly decreased, there was no significant difference in body weight, p-Lep and leptin mRNA levels compared to those in the control/ND group. While adiponectin mRNA levels was significantly elevated in both ARB/ND and Hyd/ND groups compared to that in the control/ND group, p-Adip and PPAR-gamma mRNA levels in the adipose tissues were significantly elevated only in the ARB/ND group. In addition, the adipocytes in mesenteric fat but not in subcutaneous fat tissues became irregular in shape and smaller in size in the ARB/ND group but not in the Hyd/ND group. These results suggest that ARB improves adipocyte function and adipocyte remodeling through the effects on the expression of adiponectin and PPAR-gamma.

Nothing to Disclose: AT, MT, AT, HK, MN, KT

P3-421

Total and High Molecular Weight (HMW) Adiponectin Levels in Mice with Altered GH Signaling.

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Mice with altered growth hormone (GH) signaling represent an interesting paradox in terms of adiposity and insulin sensitivity. That is, mice with a targeted gene disruption of the GH receptor/GH binding protein gene (GHR^{-/-} mice) are characterized by a significant increase in adiposity, most notably in the subcutaneous region, and yet are extremely insulin sensitive and long-lived. In contrast, bovine GH transgenic (bGH) mice are lean, but insulin resistant and short-lived. In comparison, mice transgenic for a GH antagonist (GHA mice) are obese with relatively normal insulin sensitivity and lifespan. Adiponectin, an insulin sensitizing and anti-inflammatory adipokine, has been shown in numerous systems to be negatively correlated to both fat mass and insulin resistance and positively correlated with longevity. Previous studies have shown that total circulating adiponectin levels are higher in the obese GHR^{-/-} and GHA mice yet decreased in lean bGH mice (1,2), suggesting an interesting disconnect between adiponectin and adiposity in this system. However, it appears that high molecular weight (HMW) adiponectin is a better indicator of adiponectin activity than total levels. Thus, the purpose of this study was to evaluate the proportion of HMW versus total adiponectin and to also evaluate the contribution of four specific adipose depots to the production of adiponectin in these mice. Plasma and tissue adiponectin concentrations were determined using 6 month old male and female bGH, GHR^{-/-} mice, GHA and WT littermate controls. Results show that total adiponectin is increased in GHR^{-/-} and GHA mice and decreased in bGH mice in comparison to WT controls, as reported previously. However, HMW adiponectin and the ratio of HMW/total adiponectin were only significantly increased in long-lived GHR^{-/-} mice but were not significantly different between GHA or bGH and WT male mice. Female mice had higher circulating levels, suggesting a gender-specific effect. While all adipose depots contributed to the circulating adiponectin concentrations, the subcutaneous depot appeared to be a more significant contributor for the male and female GHR^{-/-} mice. The increase in HMW adiponectin observed in the obese GHR^{-/-} mice, but not the obese GHA mice, and its depot-dependent expression in GHR^{-/-} mice raises the intriguing possibility that elevated adiponectin concentrations could contribute significantly to health benefits and to the increased lifespan observed in these mice.

(1) Berryman DE et al., Growth Horm IGF Res 2004 14:309-18.

(2) Nilsson L et al., Biochem Biophys Res Commun 2005 331:1120-6.

Sources of Research Support: In part by the State of Ohio's Eminent Scholar Program that includes a gift from Milton and Lawrence Goll, by NIH Grants DK075436-01, AG019899-06, and 1P01AG031736-01A1, by the Diabetes Research Initiative at Ohio University, and by AMVETS.

Nothing to Disclose: DEB, EL, EOL, JJK

P3-422

Effect of Rimonabant on Body Weight and Composition of Adults with Prader Willi Syndrome.

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Background: Prader Willi Syndrome (PWS) is associated with marked obesity, leading to significant morbidity and mortality. Subjects also have lower Insulin Like Growth factor 1 (IGF-1), which contributes to increased adiposity. Currently, there are no effective treatments for this condition, therefore we conducted this pilot study to evaluate the effect of rimonabant, a cannabinoid receptor CB1 antagonist, on body weight and composition of adults with PWS. **Study design:** This was a double blind randomized placebo controlled study of 6 months duration. Body weight, total fat mass (TFM), fasting ghrelin, leptin and Insulin Like Growth factor 1 (IGF1) were collected from 10 adult subjects at the baseline, and after 90 and 180 days of treatment with placebo or 20 mg of rimonabant. **Results:** Administration of rimonabant was associated with high rate of psychiatric adverse events leading to 50% drop out in the rimonabant group. The study was then terminated early due to safety concerns over the psychiatric effects. With early termination of study, numbers at each time point were small and statistical significance was not expected. However, we observed a non-significant trend for weight loss, lower TFM and higher IGF1 level at the end of the study in rimonabant group; The median body weight in placebo group was 98.64 kg (ranged 84.37-114.30) at baseline and increased to 99.56 kg (ranged 81.19-118.73) after 6 months, whereas in the rimonabant group was 95.5 kg (ranged 71.7-115.7) at the baseline and decreased to 77.11 kg (ranged 73.02-112.80) at the end of study. In the placebo group the TFM was 54951.5 g/cm² (ranged 39487.0- 59116.0) at the first visit, and 56401.0 g/cm² (ranged 42976.0-64852.0) by the end of 6 months. In subjects treated with rimonabant, TFM was 54951.5 g/cm² (range 31415.0-66527.0) at the baseline and decreased to 37259.0 g/cm² (range 33081.0-63869). Leptin followed the fat mass and decreased with fat loss in rimonabant group. Fasting ghrelin levels were higher in both groups at 6 months (50% higher in the rimonabant and 58% higher in the placebo group). Despite a trend for weight loss, the IGF1 levels increased in rimonabant group. **Conclusion:** The current CB1 antagonist, rimonabant, is associated with high risk of psychiatric side effects and should not be used in subjects with PWS who are prone to psychiatric disorders. However, future compounds with less psychiatric side effects may prove to be promising in reversal of obesity in PWS.

Sources of Research Support: Grant from Prader Willi Association (USA), and Clinical Translational Science Center of Weill Cornell Medical College (M01 RR00047 and UL1-RR024996).

Nothing to Disclose: RM, EGL, JEH, PJC, MG, MAA

P3-423

Metformin Maintains Weight Loss and Reduction in Alanine Aminotransferase and Glucose in Obese Patients with Impaired Fasting Glucose Pre-Treated with Rimonabant.

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Objectives

Maintenance of weight reduction in the non surgical setting is challenging because after cessation of weight loss drugs the weight is often regained. However patients with polycystic ovary syndrome subsequently treated with metformin therapy after rimonabant maintained their weight loss[1]. We therefore sought to determine if metformin therapy after rimonabant would maintain improvement in weight and biochemical markers of the metabolic syndrome - alanine aminotransferase and fasting glucose in obese patients with impaired fasting glucose (IFG).

Design

16 patients who were obese with IFG were initially treated with either rimonabant (8 patients) or placebo (8 patients) for 6 months and then open labelled metformin therapy for 3 months.

Measurements

The primary end point was change in weight; secondary endpoints were a change in alanine aminotransferase (ALT) and glucose.

Results

The average weight loss of 10.7 kg associated with 6 months rimonabant was maintained by 3 months metformin treatment (average change -1.35kg P=0.10). This was compared to average weight gain in the placebo group of 1.6kg. The weight was maintained with subsequent metformin therapy (average change +1kg P=0.13). The average reduction of ALT in the rimonabant group was 10.8 U/L which was maintained with subsequent metformin treatment (average change 4.3 U/L compared to before metformin treatment P=0.10). The average reduction of glucose was 0.65 mmol/L when the patients were treated with rimonabant which was maintained with metformin therapy (average change -0.17 mmol/L compared to before rimonabant treatment P=0.35).

Clinical and biochemical parameters

| Parameter | Rimonabant | | | Placebo | | |
|------------------|---------------|--------------------|----------------|---------------|--------------------|----------------|
| | Baseline | After 6/12 Rim | After 3/12 Met | Baseline | After 6/12 Plac | After 3/12 Met |
| Weight (kg) | 104.41 (9.72) | 93.68 (12.11) * | 95.03 (11.84) | 96.51 (15.27) | 98.11 (15.40)** | 97.11 (15.27) |
| ALT (U/L) | 30.8 (8.9) | 20.0 (5.3)* | 15.7 (0.4) | 34.0 (0.9) | 33.3 (18.1) | 16.8 (5.4) *** |
| Glucose (mmol/L) | 6.5 (0.36) | 5.9 (0.67)* | 6.0 (0.79) | 6.2 (0.54) | 6.1 (0.63) | 5.7 (0.41) |

data are average (SD), *=P<0.05 after 6/12 Rim; **=P<0.05 after 6/12 placebo, ***P<0.05 after 3/12 metformin

Conclusion

In these obese patients with impaired fasting glucose, metformin maintained the weight loss and markers of the metabolic syndrome achieved with rimonabant.

1. Sathyapalan, T., et al., Metformin maintains the weight loss and metabolic benefits following rimonabant treatment in obese women with polycystic ovary syndrome (PCOS). Clin Endocrinol (Oxf), 2009. 70(1): p. 124-8.

Nothing to Disclose: AJD, TS, DDM, ESK, SLA

P3-424

Weight Reduction with a Novel Carbohydrate Modified Diet in 52 Study Subjects in the EMPOWIR Trial.

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EMPOWIR (NCT00618071) is a double blind, placebo controlled, randomized clinical trial to evaluate metformin (MF) & MF plus 2 mg rosiglitazone along with a unique carbohydrate modified diet in women with Syndrome **W**-normoglycemic, hyperinsulinemic **W**omen with midlife **W**eight gain, **W**aist gain, and **W**hite Coat hypertension.¹ The **primary study hypothesis** is that insulin sensitizing medications, in combination with alterations in carbohydrate intake, will reduce insulin levels and improve Metabolic Syndrome risk factors. **METHODS** We compared body weight (BW) at screening and randomization visits in 52 subjects meeting inclusion criteria (age 35-55; 20 lb wt gain after the 20's; normal GTT, hyperinsulinemia).

Table 1 Baseline Characteristics of 52 EMPOWIR Study subjects

| | mean (se) | median |
|------------------------------|------------|--------|
| age | 46.3(9.9) | 47.0 |
| BMI (kg/m ²) | 30.8(.4) | 30.5 |
| Weight (lbs) | 181.2(2.9) | 186.9 |
| Waist circumference (inches) | 37.2(.5) | 37.6 |
| Systolic BP (mmHg) | 117.5(1.9) | 118.0 |
| Diastolic BP (mm Hg) | 75.1(1.2) | 76.0 |
| Fasting glucose (mg/dL) | 90.5(1.5) | 90.5 |
| 2-hour glucose (mg/dL) | 105.9(3.4) | 107.5 |
| fasting insulin (μU/ml) | 10.5(.6) | 9.4 |
| AUC-insulin (μU/ml) | 157.2(8.3) | 144.0 |
| Hemoglobin A-1C (%) | 5.4(.4) | 5.3 |
| HDL cholesterol (mg/dL) | 58.1(3.0) | 56.0 |
| Triglycerides (mg/dL) | 91.9(9.3) | 82.5 |
| HOMA | 2.4(.2) | 2.1 |

Study subjects attended 4 weekly nutrition workshops (lead-in phase) at 2 study sites to introduce the **EMPOWIR dietary intervention**: a food exchange program (~40% carbohydrates-40% protein-20% fat) promoting increased intake of vegetables, low-glycemic index fruits, low-fat protein and dairy products, elimination of free sugars, and **restriction of 3 allowable additional carbohydrates (starches) to after 4PM.**²⁻³ Weights were assessed at randomization following a 7.2((se).4) week treatment free interval with paired t-tests & multivariate models (SPSS). **RESULTS** Mean wt loss was 3.9(.0)%. Mean BW decreased in 42 subjects (-9.2(1.0) [-1 to -23]lbs); increased in 8 (4.0(1.8)[1-16.0] lbs) & remained the same in 2. Despite differences in race (37.8 vs. 7.6% non-white) & baseline BMI (31.6(.6) vs 29.2(.6), BW changes were similar at the 2 sites (-4.5(.0) vs -3.3(.0)% P=.3). **CONCLUSIONS** Significant, sustainable weight loss was observed in diverse hyperinsulinemic midlife women with the EMPOWIR dietary intervention. These findings are consistent with studies demonstrating macronutrient based differences in *ad libitum* intake⁴ and gut peptide responses⁵⁻⁶ and merit additional study.

1. Mogul HR, Weinstein BI, Mogul DB et al. Syndrome W: a new model of hyperinsulinemia, hypertension and midlife weight gain in healthy women with normal glucose tolerance. Heart Dis 2002; 4:78-85.
2. Mogul HR, Peterson SJ, Weinstein BI, et al. Metformin and carbohydrate-modified diet: a novel obesity treatment protocol: preliminary findings from a case series of nondiabetic women with midlife weight gain and hyperinsulinemia. Heart Dis 2001; 3:285-92 cited in Reuters and Medscape, Diabetes and Endocrinology (Nov, 2001).
3. Mogul HR, Peterson SJ, Weinstein BI, et al. Long-term (2-4 year) weight reduction with metformin plus carbohydrate-modified diet in euglycemic, hyperinsulinemic, midlife women (Syndrome W) Heart Dis 2003; 5:384-92.
4. Ludwig DS, Majzoub JA, Al-Zahrani A, et al. High glycemic index foods, overeating, and obesity. Pediatrics 1999; 103:E26.
5. Foster-Schubert KE, Overduin J, Prudom CE, et al. Acyl and total ghrelin are suppressed strongly by ingested proteins, weakly by lipids, and biphasically by carbohydrates. J Clin Endocrinol Metab 2008; 93:1971-79.
6. Lomenich JP, Melguizo MS, Mitchell, et al. Effects of meals high in carbohydrate, protein and fat on ghrelin and Peptide YY secretion in prepubertal children. J Clin Endocrinol Metab 2009; 94:(Nov).

Sources of Research Support: EMPOWIR (ClinicalTrials.gov:NCT00618071) is an unsolicited, investigator-initiated study supported by Glaxo Smith Kline with additional support from a Clinical and Translational Science Award (CTSA) from the National Institutes of Health (UL11RR025740) awarded to Einstein of Yeshiva University.

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Nothing to Disclose: HRM, LAK, BW-C, GDC, MF, RG, SJ, MS, KT

P3-425

High Protein vs High Carbohydrate Diet in Weight Loss, Cardiovascular Risks and Oxidative Stress.

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The prevalence of obesity is on the rise in the US. The detrimental effects of obesity on cardiovascular and metabolic parameters and increased incidence of mortality, as well as, the excess cost to the national health budget are astounding. Therefore, the ability to provide a healthy weight reduction plan is essential to the health and economics of the population in the United States as well as around the world.

A palatable diet providing satiety as well as all essential nutrients may go a long way in treating over-weight individuals. Protein diets have been known to provide greater satiety and reduced energy intake than carbohydrate diets, but definitive long-term studies are sparse.

In this study we tested the hypothesis that a moderately high protein and low carbohydrate (HP) diet causes an equal or greater weight loss and less glucose and lipid excursions resulting in less oxidative stress, markers of inflammation, cardiovascular risk factors other metabolic parameters than the HC diet (American Diabetes Association diet) in obese subjects.

To date 11 female subjects ages 20 to 50 years have been placed on either a HP diet (5 subjects) or HC diet (6 subjects) for the 6 month diet study and monitored at weekly intervals. All subjects had a reduced caloric intake determined by their diet histories and based on their individual Resting Energy Expenditures (REE). All foods were supplied to the subjects for diet compliance.

The mean baseline weight was 245 ± 46 lbs and blood pressure (BP) 129/83. Both diet groups lost weight with the mean weight loss over the 6 months of diet of 20.7 lbs and had a decrease in blood pressure to a mean of 119/74. The mean fasting glucose of the subjects was 94.7 mg/dl before starting the diets and 78 and 84.5 mg/dl after the HP and HC diets, respectively. The glucose area under the curve decreased by 12.1 and 7.8% in the HP and HC groups, respectively. Markers of oxidative stress measured by dichlorofluorescein (DCF) was a mean value of 3.2 uM at the start of the diets and decreased to 2.4 and 2.9 uM in the HP and HC diets, respectively. Lipid peroxidation determined by malondialdehyde (MDA) declined from an initial mean value of 1.1 uM to 0.7 and 0.9 uM at the end of the HP and HC diets, respectively.

This study demonstrates that both the HP and HC diets resulted in weight loss and improved BP, decreased glucose, and markers of oxidative stress with the HP diet having the greatest effects on the parameters studied.

Sources of Research Support: American Diabetes Association.

Nothing to Disclose: FBS, AEK

P3-426

Sustained Weight Loss and Improved Metabolic Profile with Metformin and Carbohydrate Modified Diet in Non-Diabetic Men.

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Introduction

Metformin has known weight reduction benefits in morbidly obese adults, women with PCOS and obese adolescents.(1-3) We have previously reported long term effects of metformin and carbohydrate modified, hypocaloric diet in obese women with Syndrome W - normoglycemic, hyperinsulinemic women with midlife weight gain, waist gain, and white coat hypertension.(4-5) Previous success in treating Syndrome W women leads to investigation of a similar syndrome in men. Comparable studies have not been conducted in men.

Methods

We conducted a retrospective analysis of consecutive hyperinsulinemic men attending a faculty practice based Weight Reduction program from 2000-2009. All 34 patients were treated with metformin 2000 mg/day plus carbohydrate modified diet for 3-55 months. Patients returned at 3, 6, 12 months and then 6 month intervals. Weights and cardiometabolic parameters were assessed with paired t-tests and multivariate analyses (SPSS).

Results

Patients were followed for a mean of 23.9 months, had mean age of 54 years and 2.17 factors for metabolic syndrome. Significant reductions were observed in BMI, waist circumference, HOMA-IR, LDL cholesterol, triglycerides and fasting insulin. HDL cholesterol significantly increased. Mean weight decrease from first to last visit was 22.9 lbs(se 3.9).

Parameters Before and After Metformin Treatment

| Parameters | Baseline | After Metformin Treatment | P Value |
|--------------------------|----------|---------------------------|---------|
| | Mean | Mean | |
| Weight (kg) | 255.6 | 232.7 | 0.001 |
| BMI (kg/m ²) | 36.6 | 33.2 | 0.001 |
| Waist (in) | 46.6 | 42.7 | 0.001 |
| SBP (mmHg) | 132.5 | 127.6 | 0.161 |
| DBP (mmHg) | 81.6 | 77.9 | 0.062 |
| Fasting glucose (mg/dl) | 92.4 | 95 | 0.527 |
| HOMA-IR u | 3.5 | 2.3 | 0.012 |
| Total Chol (mg/dl) | 192.3 | 176.9 | 0.065 |
| HDL(mg/dl) | 42.5 | 45.6 | 0.05 |
| LDL(mg/dl) | 120.2 | 98.2 | 0.004 |
| Triglycerides(mg/dl) | 174.4 | 101.7 | 0.003 |
| Fasting insulin(uIU/ml) | 19.4 | 9.5 | 0.032 |

Conclusion

Our data suggests that the dual regimen of metformin accompanied by a carbohydrate modified diet produces long term weight loss and improves cardio- metabolic risk profiles in obese men. Metformin may be a valuable tool in treating male obesity.

(1)Harborne LR et al., J Clin Endocrinol Metab 2005;90:4593

(2)Srinivasan S et al., J Clin Endocrinol Metab 2006;91:2074

(3)Glueck CJ et al., Metabolism 2001; 50:856

(4)Mogul HR et al., Heart Dis 2003;5:384

(5)Mogul HR et al., Heart Dis 2002;4:78

Nothing to Disclose: MGDS, SJP, MF, HRM

P3-427

Lindaxa Effectiveness in Obesity Treatment Associated with IGT and Arterial Hypertension.

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This research was designed to estimate effectiveness and safety of Lindaxa in treatment of obesity associated with IGT and arterial hypertension. Thus, 20 women (mean age 37.9 years-old) were treated with complex therapy including traditional therapy (diet, physical exercises, dhydration, metabolic and symptomatic treatment) including Lindaxa with dosage of 10-15 mg per day once daily during 6 months. Physical exercises were determined individually according to BMI and metabolic disorders.

Clinical evaluation included brain MRI, Echocardiography, US imaging of abdominal cavity, lipids and carbohydrates metabolism (cholesterol, LDL, HDL, triglycerides, β -lipoproteids, fasting glucose concentration). Besides, BMI, waist circumference, OGTT were evaluated in women with obesity. Control tests were conducted before treatment and after 3, 6 months intervals with evaluation of BMI, waist circumference, lipids profile, glucose levels and OGTT.

Women with arterial hypertension (8 women with mean blood pressure of 145/90 mm Hg) treated with Lindaxa (sibutramine) we recommended hypertension and heart rate monitoring every 2 weeks during first 3 months of therapy and monthly during following 3 months of treatment. Results showed systolic blood pressure (SBP) increase by 2.0 mm Hg whereas diastolic blood pressure (DBP) elevated by 1.6 mm Hg and heart rate increased by 2-5 beats per minute during the first month of therapy, however, with body mass decrease with the Lindaxa therapy blood pressure reliably decreased during the first month of therapy. Thus, after 6 months of treatment SBP decreased by 3.4 mm Hg, while DBP reduced by 2.4 mm Hg.

Comparative evaluation of metabolic data in obese patients treated with Lindaxa after 6 months showed OGTT change from 5.2-11.1-5.6- mmol/L to 4.8-5.8-4.7 mmol/L, decrease in cholesterol levels from 5.2 to 4.6, while β -lipoproteids and LDL decreased from 0.62 and 3.1 to 0.54 and 2.4 respectively; triglycerides reduced from 2.03 to 1.35 while HDL increased from 1.69 to 2.2. The more patient lost weight the more evident improvement in above mentioned tests.

After 6 months of Lindaxa therapy weight loss achieved significant reliable reduction with mean 11.0 kg, where mean body weight reduced from 84.5 kg to 73.5 kg and BMI decreased to 27.8 kg/m². 2 patients who showed no change in body weight developed resistance to the treatment, however, IGT had positive dynamics.

Treatment with Lindaxa improves lipids profile, reduces body mass, decreases elevated blood pressure both systolic and diastolic, and glucose tolerance. Moreover, Lindaxa shows positive effect on lipids metabolism which is important factor in treatment and reducing cardiovascular risk in patients with obesity.

Sources of Research Support: Pharm Company, Zentiva, Chesh Republic.

Nothing to Disclose: SII, ZYK, YMU, GDN, SMS

P3-428

Oxidative Stress and Metabolic Syndrome: Effects of Natural Dietary Antioxidants in Obese Patients with Insulin Resistance.

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Oxidative stress (OS) could play an important role in metabolic syndrome (MS)-related manifestations contributing to insulin resistance (IR). The reciprocal influences between OS and IR are not clear. We investigated the effects of dietary antioxidants on IR, studying 29 obese (16 males and 13 females, 18-66 ys, BMI 36.0±1.0 Kg/m²), with IR evaluated by HOMA index, comparing 4 treatments: hypocaloric diet alone (group A) or plus metformin 1000 mg/daily (group B), diet enriched with natural antioxidants alone (group C) or plus metformin (group D). A personalized program, with mean caloric intake of 1500 Kcal, 25% proteins, low glycemic index CHO and a calculated antioxidant intake of 800-1000 mg/daily, derived by fruit and vegetables, was administered to group C and D. An OGTT and evaluation of total, LDL and HDL-cholesterol, triglycerides, uric acid and albumin were performed before and after a 3-months treatment. Total antioxidant capacity was determined by H₂O₂-metmyoglobin system, which interacting with the chromogen ABTS generates a radical with a latency time (LAG), proportional to antioxidant content. Despite a similar BMI decrease, we found a significant decrease of HOMA only in group C and D and of insulin peak in group B and D. Insulin AUC showed the highest decrease in group D (25.60±8.96%). No differences were observed in glucose AUC and lipid metabolism and LAG values.

MEAN±SEM VALUES OF METABOLIC PARAMETERS AND LAG

| | HOMA-IR | Insulin AUC (mcU/ml/120') | Insulin peak (mcU/ml) | LAG (sec) |
|-------------------|------------|---------------------------|-----------------------|------------|
| GROUP A (n=6) pre | 3.91±0.46 | 12683.25±3493.88 | 168.37±54.50 | 65.00±9.75 |
| GROUP A post | 3.95±0.4 | 9805.25±2770.78 | 161.77±44.59 | 71.00±8.43 |
| GROUP B (n=9) pre | 4.46±0.70 | 12414.67±2177.37 | 181.61±24.64 | 58.33±3.12 |
| GROUP B post | 3.62±0.66 | 12097.17±1790.76 | 157.62±30.59* | 61.11±6.33 |
| GROUP C (n=6) pre | 4.20±0.34 | 11613.90±1310.10 | 156.46±16.38 | 60.00±2.58 |
| GROUP C post | 3.13±0.65* | 10436.40±1866.88 | 124.64±18.59 | 61.00±4.58 |
| GROUP D (n=8) pre | 5.71±1.63 | 17686.29±3347.53 | 246.44±35.00 | 56.25±5.07 |
| GROUP D post | 3.06±0.67* | 11362.88±705.78 | 141.73±12.57* | 60.63±5.55 |

p<0.05 vs pretreatment values

These data suggest that dietary antioxidants ameliorate insulin-sensitivity in obese subjects with IR enhancing the effect with insulin-sensitizing drugs, although the molecular mechanisms still remain to be elucidated.

Nothing to Disclose: AM, GEM, VDD, EL, SR, RF, MM, AM, AS, AP, EM

P3-429

Eating Behavior as Assessed by the Three-Factor Eating Questionnaire and Weight Loss Success in Obese Women with and without the Polycystic Ovary Syndrome.

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BACKGROUND: Low cognitive restraint of eating, combined with high emotional disinhibition of eating control and inability to cope with the sensation of hunger as measured by the Three-Factor eating Questionnaire (TFEQ) have been associated with use and liking of sweet and high-fat items among dieters. We evaluated whether scores on these 3 eating behavior dimensions as measured by the TFEQ predict successful weight loss in obese women with and without PCOS participating in a weight reduction study.

METHODS: Obese (BMI > 30 kg/m²) women with PCOS (n=13) and without PCOS (n=12) participated in a weight loss study. Anthropometric measurements were obtained at baseline and weekly for 8 weeks. The 51-item TFEQ was completed by 20 participants at baseline. All participants received a 1-hr dietary education session provided by a registered dietician at baseline, followed by weekly weight check and follow-up dietary education for 8 weeks. All participants kept a daily food log. Self assessment and dietician assessment of dietary adherence were recorded weekly. Weight loss success was defined as a minimum mean weight loss of 3.6kg (8 pounds) over the 8 week period. **RESULTS:** Scores on TFEQ cognitive restraint domain ranged from 5-19 (higher score = more cognitive restraint); scores on emotional disinhibition of eating control domain ranged from 0-16 (higher score = increased disinhibition); and scores on susceptibility to hunger domain ranged from 0-11 (higher score = inability to cope with hunger sensation). Individuals were divided into tertiles based on their scores on each domain, resulting in high, medium, and low categories for each domain. Low cognitive restraint was significantly associated with unsuccessful weight loss attempt (p=0.0334). However, there were no relationships between disinhibition and susceptibility to hunger scores and weight loss success. When PCOS and normal women were evaluated separately, the same trend was evident in PCOS women, although not statistically significant (p=NS). No consistent trend was evident in women without PCOS. **CONCLUSIONS:** High scorers in the cognitive restraint domain may be especially responsive to dietary information which was used as the diet education method in this study (and in most traditional clinical setting). The lack of a consistent trend in women without PCOS may be due to the limited number of participants studied.

Sources of Research Support: National Institutes of Health Grants K23HD049454 (to K.I.C.) K24HD40237 (to J.E.N.), and Clinical Research Center Grant M01-RR00065, NCCR.

Nothing to Disclose: KIC, SK, SNS, JEN

P3-430

A Social Cohesion Model for Successful Weight Management in Young Women.

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Obesity is increasing in epic proportions, particularly among Mexican-Americans (MA), the fastest growing ethnic group in the United States. MA women are at particularly high risk for obesity and metabolic syndrome and have the highest prevalence of metabolic syndrome among non-Hispanic Whites, African Americans, and MA women. Although the prevalence of metabolic syndrome and cardiovascular disease increases with age, in adults, the greatest increase in weight and waist circumference occurs in the 20-29 year old age range. Thus, young, overweight adults need to be targeted for weight loss and prevention of weight gain. However, recruitment and retention of young adults, in particular those who are ethnic minorities, in weight loss and weight maintenance studies is poor relative to older age groups, suggesting that effective weight loss strategies in young adults and ethnic minorities may be different from those that work in older Caucasian adults. Ecologic models suggest that numerous, context specific, cultural, social and environmental factors may influence health behaviors leading to obesity. Thus, we employed a social cohesion strategy to recruit and retain MA women between the ages of 20-29 years into a weight reduction, nutrition and aerobic class. The class included weight loss principles using food choices, portion sizes and meal preparation, methods to improve self perceptions and self-efficacy and methods to increase physical activity into day-to-day activities. The women responded well to social cohesion strategies that included small, semi-competitive teams, with each team choosing a leader who took the responsibility of making weekly calls and reminders to their team about the week's goals, such as increasing their daily walking to 60 minutes per day, eating healthy food choices, increasing water consumption to 60 ounces per day, as well as other goals that were outlined by course faculty. Nominal rewards were given to the team that achieved the greatest changes to both the behavioral and physical parameter goals on a weekly basis. After 3 months, nearly all subjects had decreased weight (mean 3.5 lbs or 2% of their starting body weight) and no one voluntarily dropped the program. IPAQ and NCI questionnaires demonstrated improvements in physical activity and food choices. These data suggest that a gender and age-specific social cohesion strategy is critical for successful recruitment, retention, and compliance in a weight management program.

Nothing to Disclose: ETV, KLW, WAH

P3-431

Improved Weight Loss with Sleeve Gastrectomy Compared to Gastric Banding at a New York Bariatric Center of Excellence.

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INTRODUCTION

Sleeve gastrectomy (SG) has been proposed as an attractive primary bariatric procedure because of its potential for greater weight loss and favorable effect on co-morbidities. Laparoscopic adjustable gastric banding (LAGB) is widely performed despite certain concerns related to its long term efficacy¹. Few systematic studies have compared weight loss and glycemic control in these procedures. The aim of our study is to compare body weight changes over a twelve month period in patients undergoing these restrictive procedures.

Methods

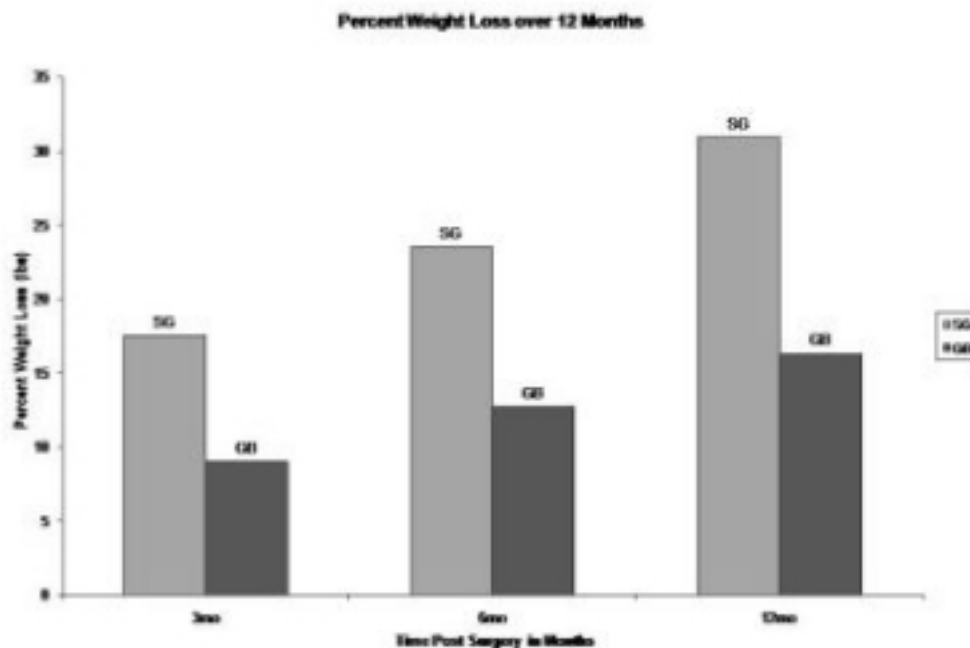
We conducted a retrospective analysis of 143 consecutive adults undergoing LAGB (n= 101) or LSG (n= 42) from Jan -Dec, 2008 at The Westchester Medical Center Bariatric Center of Excellence. Weights were assessed with ANOVA and multivariate analysis (SPSS).

RESULTS [brr]Mean weight change and percent weight change were significantly better at 3, 6, and 12 months with SG vs. LAGB, respectively 60.7((se)3.7) vs 23.3(1.1) 80.2(5.8) vs. 32.4(1.7) and 102.9(10.3) vs 40.9(2.6) lbs; all P=.000. Mean age was 46 and there were 97 females and 48 males. Weight loss was independent of age and sex.

Wt loss over 12 month period:SG vs.LAGB

| Characteristics | SG(n=42) | | LAGB(n=101) | |
|--------------------------|----------|-----------|-------------|-----------|
| | Mean | Std.Error | Mean | Std.Error |
| Wt loss 3 months(lbs) | 60.70 | 3.70 | 23.54 | 1.14 |
| Wt loss 6 months(lbs) | 80.27 | 5.87 | 32.39 | 1.75 |
| Wt loss12 months(lbs) | 102.93 | 10.38 | 40.91 | 2.61 |
| % Wt change at 3 months | 17.52 | 0.75 | 9.02 | 0.41 |
| % Wt change at 6 months | 23.47 | 1.30 | 12.71 | 0.66 |
| % Wt change at 12 months | 30.87 | 2.05 | 16.33 | 1.07 |

SG-Sleeve Gastrectomy;LAGB-Laparoscopic Adjustable Gastric Banding;Wt-Weight



Conclusion:

This data suggests that sleeve gastrectomy may be a preferable alternative procedure to laparoscopic adjustable gastric banding. Prospective randomized controlled studies are needed to determine the long term efficacy of the procedure on weight loss and other comorbidities.

(1)Tice JA et al., Am J Med. 2008 Oct;121(10):885-93

Nothing to Disclose: CK, MDS, AK, HM

P3-432

Laparoscopic Gastric Banding Surgery (LAGB) for Patients with Type 2 Diabetes and Morbid Obesity: Improving BMI and Psychological Status at 1 Year Post-Surgery.

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Background: Surgical treatment is increasingly recognised as the most effective means of achieving weight loss and improving glucose tolerance in morbidly obese patients with Type 2 diabetes (T2DM). The long-term psychological status of morbidly obese individuals (both with and without T2DM) undergoing LAGB remains unclear despite its increasing use.

Methods: Twenty-five participants were recruited (21 female, 4 male; 16 with T2DM, 9 without; age range: 30-58 years; BMI 50.7 ± 5.9 kg/m²; HbA1c $8.9 \pm 2.0\%$). All were considered suitable for surgery after comprehensive assessment. Participants completed psychological scales measuring general anxiety and depression (HADS); quality of life (WHOQoL-Bref); and social anxiety (DAS-24) pre- (T1), six (T2) and twelve (T3) months post-LAGB.

Results: Repeated measures ANOVA resulted in significant improvements across the three time points being recorded for BMI & HbA1c (both $p < .001$), the psychological and physical subscales of the quality of life measure (both $p < .001$), the anxiety and depression subscales of the HADS (both $p < .001$) and for levels of social anxiety (DAS-24, $p < .001$). While both BMI and HbA1c significantly improved at all time points, a different pattern was observed for the psychological variables. Significant improvements were observed between T1 and T2 and between T1 and T3 on the WHOQoL subscales, the HADS subscales and the DAS-24. However, no significant gains were specifically recorded for these measures between T2 and T3, although an improving trend was noted.

Conclusions: These results suggest that LAGB improves BMI, HbA1c and psychological status. Twelve months post-LAGB there is evidence that psychological health is improving in parallel with physiological health.

Nothing to Disclose: SJ, MM, KTL, ABJ

P3-433

Impact of Abdominoplasty on Post-Bariatric Surgery Weight Loss.

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Objective: Body contouring surgery is often performed after bariatric surgery in order to remove residual tissue. In addition, it has been shown to improve quality of life in such patients. However, few studies have evaluated the impact of these procedures on subsequent weight loss. The aim of this study is to compare weight loss in women with or without abdominoplasty performed following a gastrojejunal bypass (GB).

Materials and Methods: We included women in whom GB was successfully performed between January 1997 and October 2009. These women were divided in two groups according to whether abdominoplasty was carried out following GB. Student T test was calculated for comparison of means. We estimated the percentage of women in each group that reached a body mass index (BMI) ≤ 30 kg/m² with Kaplan-Meier analysis.

Results: The cohort consisted of 150 women with no abdominoplasty and 50 in whom abdominoplasty was done after GB. The average follow-up period was 4.9 ± 2.9 years. The decrease in the body mass index (BMI) in the abdominoplasty group was significantly higher than in the non-abdominoplasty group (18.64 ± 6.50 kg vs. 15.47 ± 5.70 kg, respectively $P < 0.001$). Similarly, the percentage of excess of body weight lost was also higher in the prior group ($61.04 \pm 14.39\%$ vs. $54.53 \pm 16.96\%$, respectively $P = 0.015$). In addition, a higher percentage of the women with abdominoplasty reached a BMI ≤ 30 kg/m² (34.0% vs. 28.6% , $P = 0.009$) during follow-up [figure 1].

Conclusions: In this cohort of post-GB women abdominoplasty resulted in a greater reduction of BMI and excess body weight over time. The finding of persistent weight loss suggests that abdominoplasty not only aids in the removal of redundant tissue, but also has a long-term effect in the maintenance of weight loss. This is probably a multifactorial effect and may include improvement in factors such as quality of life and body perception.

Nothing to Disclose: EA-V, PA-V, RR-M, DC-R, RM, EG-G

P3-434

Increased GDF-15 Concentrations in Morbidly Obese Subjects Increase Further Following Gastric Bypass-Induced Weight Loss.

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Growth-differentiation factor 15 (GDF-15) also known as macrophage inhibitory cytokine-1 is implicated in inflammation, cancer, and cancer-induced cachexia, and was recently established as a prognostic marker in cardiovascular disease. GDF-15 is secreted by macrophages and adipocytes, and its systemic concentrations are increased in obesity and type 2 diabetes.

Here we evaluated plasma GDF-15 concentrations in a cohort of 117 morbidly obese patients (37.3 +/-12 years, 89 females, 28 males) as compared to 30 age- and sex-matched healthy lean subjects, as well as in 28 patients before and one year following Roux-en-Y gastric bypass (RYGB) procedure. Using a 75g (2h) glucose tolerance test, obese patients were subgrouped in 68 patients with normal glucose tolerance (NGT), 37 patients with impaired glucose tolerance (IGT) and 12 patients with newly diagnosed diabetes. Circulating GDF-15 concentrations were increased not only in the DM group, but also in the NGT and IGT groups when compared to lean healthy subjects (all $P < 0.001$). Obese patients with DM presented higher GDF-15 than obese patients with NGT. Plasma GDF-15 levels significantly correlated with age, waste, systolic blood pressure, creatinine, glucose, insulin, C-peptide and HbA1c. Age, creatinine and insulin were independent predictors of GDF-15 concentrations in morbidly obese subjects. RYGB led to a significant reduction in weight, CRP, leptin, insulin and HOMA-IR, but further increased GDF-15 levels ($P > 0.001$). One year after RYGB, GDF-15 correlated only with age and free T4 levels. The RYGB-induced increase in GDF-15 was positively associated to the decrease in HOMA-IR ($P = 0.017$).

In summary, we present here that age, insulin and creatinine are independent predictors of increased GDF-15 levels in obese patients. The reduction in weight and insulin resistance following bariatric surgery is associated with a further increase in GDF-15. The prognostic value of increased circulating GDF-15 in obesity and after weight loss remains to be evaluated in further studies.

Nothing to Disclose: GV, MR, CA, MR, MC, BL, GP, MK, AL

P3-435

Effect of Bariatric Surgery on Bone Mass and Other Parameters in Men: Bariatrics in Louisville VA (BAIL VA).

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Bariatric surgery has been shown to be the most effective means of achieving substantial and sustained weight loss, and it decreases morbidity associated with obesity. In post-menopausal women, gastric bypass surgery has been shown to adversely affect bone density. But few studies have addressed bone health in men who underwent similar surgery. In this study, we conducted a retrospective review on men who underwent gastric bypass surgery at the Louisville Veterans Affairs medical center.

Electronic medical records of 136 patients who underwent gastric bypass surgery in Louisville VA Hospital between year 2000 and 2009 were reviewed (men=71, women=65). Twenty-seven male patients who had both lumbar spine and left hip bone density measurement by Hologic densitometer postoperatively were reviewed for the following parameters: weight change, intact parathyroid hormone (PTH), serum calcium, 25-OH vitamin D (Vit D25) levels and post-operative bone mineral density (BMD) measurement.

The mean age of subjects was 58±13 years, pre-operative weight was 340±137 lbs and postoperative weight was 211±49 lbs. The mean weight loss was 110±40 lbs over 2.5 year follow-up period. Mean lumbar spine bone density was 1.161±0.2 gm/cm² and the left hip bone density was 0.906±0.1 gm/cm². The T-score for the lumbar spine density ranges between 4.5 and -2.5, while T-score for the left hip ranges between 1.5 and -2.5. One patient with known hypogonadism was excluded. Thirty-seven percent of the men had low BMD. Vitamin D25 level was less than 30 ng/ml in 87 % of the patients. The mean PTH level was 48±16 pg/ml and the serum calcium was in the normal reference range. There was no correlation between the initial body weight, current body weight or postoperative weight loss to the current BMD (p=0.261). Vit D25 level did not correlate with current body weight or BMD in these subjects.

In our study, we found a high prevalence of low BMD and low Vit D 25 levels in men after bariatric surgery. However, there is no direct correlation between the extent of weight loss and low BMD or low VitD25 level. These negative findings may be related to our low sample size. Future prospective studies are needed to evaluate factors that may contribute to low BMD in men undergoing bariatric surgery in order to develop strategies to prevent bone loss in these subjects.

Nothing to Disclose: CCC, SLM, CW, SSK

P3-436

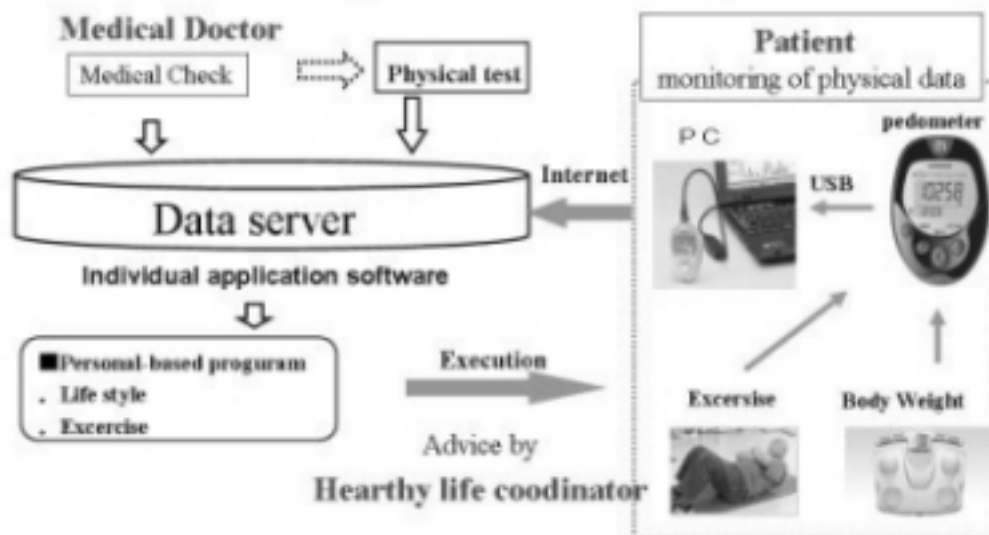
Interactive Life Style Intervention Program by Monitoring the Internet-Based Acquisition of Daily Physical Data with Highly Functional Pedometer and Weighing Machine in Japanese Male Patients with Metabolic Syndrome.

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[OBJECTIVE] Although the life style intervention is known to be critical for the treatment of metabolic syndrome (MeS), it is difficult to understand the precise patient's daily lifestyle, to make a person-based program for life style intervention, and to monitor the patient's life style. In "Chiba healthy life coordination program (CHLCP)", we trained public health nurse and dietitian for 3 weeks with special program for "the healthy life coordinator". In this program, they make each patient-based program for life style intervention using Personal Computer (PC), monitor the patient's daily acquisition of physical data with highly functional pedometer and weighing machine (whose data are also possible for patient to see through the personal web page), and advice the life style by e-mail. We tested the effectiveness of this program at medical clinic.

Fig.1; System of Intervention Program of Internet-based Acquisition of Patient's Daily Physical Data and Life Style



[METHOD] 35 male patients with MeS (50.2 ±7.3 years old), who did not immediately need drug therapy, were enrolled for 6 months. Biochemical examinations and CT scan were performed before and after the study.

[RESULTS] Body weight and body mass index significantly decreased and area of visceral fat also was significantly reduced from 106 to 89.96cm² after 6 months (P <0.05). HDL-cholesterol significantly increased and triglyceride, insulin and HOMA-IR significantly decreased.

[CONCLUSION] The interactive life style intervention program of CHLCP was very effective to improve the metabolic abnormality in MeS. For the effective life style intervention, person-based intervention program, monitoring and interactive intervention are important, and the application of internet-based technology may help us to establish them.

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

P3-437

Efficacy and Tolerability of Testosterone in Hypogonadal Men with Abdominal Obesity: A Prospective, Non-Controlled, Mono-Center Observational Study.

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¹Private Practice for Internal Med and Diabetes Munich, Germany and ²Bayer Vital GmbH Leverkusen, Germany.

Objectives:

The relationship between visceral obesity and hypogonadism is well established. Acting as an active endocrine organ, visceral adipose tissue promotes hypogonadism while hypogonadism acts to facilitate visceral obesity, thus establishing a vicious cycle. The aim of this study was to assess efficacy and safety of testosterone (T) replacement in hypogonadal patients with abdominal obesity.

Design and methods:

29 men (42-58 years) with symptomatic hypogonadism and abdominal obesity (waist circumference ≥ 102 cm) followed a supervised combined strength and endurance exercise program, and a Mediterranean style diet. 28 patients received intramuscular testosterone undecanoate (1000 mg), 1 patient testosterone gel (50 mg T/day), respectively.

Results:

From the first visit to the last follow up visit, the following changes were recorded: Mean waist circumference decreased from 106.0 ± 2.2 cm by 5.4 ± 2.3 cm (-5.1%), mean weight from 100.6 ± 5.9 kg by 4.7 ± 2.7 kg (-4.6 %) and mean BMI from 31.2 ± 1.7 kg/m² by 1.5 ± 0.8 kg/m² (-4.6 %). Mean heart rate decreased from 75.1 ± 4.5 bpm by 1.7 ± 3.8 bpm (-2.1%), mean systolic blood pressure from 135.2 ± 9.9 mm Hg by 5.3 ± 5.2 mm Hg (-3.8%), and mean diastolic blood pressure from 85.3 ± 2.9 mm Hg by 3.4 ± 3.2 mm Hg (-3.9%). Mean HDL-cholesterol increased from 48.7 ± 8.0 mg/dl by 3.3 ± 2.8 mg/dl (7.5%). LDL-cholesterol decreased from 153.6 ± 19.0 mg/dl by 5.7 ± 10.4 (-3.2%) and triglyceride from 226.8 ± 46.0 mg/dl by 37.1 ± 31.5 mg/dl (-14.6%). At the end of the observational period efficacy of testosterone treatment was judged by the physician as "very good" or "good" in 93.1%. Out of all 29 patients, 96.6% rated efficacy as "very good" or "good", with these effects being observed in the majority of patients at Visit 2 or Visit 3, respectively. Prostate Specific Antigen (PSA), hematocrit and hemoglobin were always within the normal range. No AEs or SAEs were reported.

Conclusion:

As previously reported for obese men with new-onset type 2 diabetes, supervised lifestyle modification along with testosterone replacement have shown promise when treating this group of relatively young men with visceral obesity and features of the metabolic syndrome.

Heufelder AE, Saad F, Bunck MC, Gooren L, J Androl 2009; 30(6): 726-733

Sources of Research Support: Partially funded by Bayer Vital GmbH, Leverkusen, Germany.

Disclosures: AEH: Speaker, Bayer Schering Pharma, Berlin, Germany. MH: Employee, Bayer Vital GmbH, Germany.

P3-438

Orlistat: A Review of Liver-Related Medwatch Adverse Reports.

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Background:

Orlistat is a medication available to treat obesity. It prevents the absorption of dietary fat by inhibiting gastrointestinal lipases. Reports have been submitted to the FDA associating orlistat with serious hepatic damage, including a case of death and a few cases of liver failure requiring liver transplantation. The purpose of this study was to review the orlistat MedWatch adverse reports so clinicians can make a more informed decision when prescribing orlistat.

Methods:

26,277 reports on adverse effects of orlistat were submitted to the FDA between Jan 1, 1989 and Oct 23, 2009. The majority of the side effects reported have been gastrointestinal symptoms including abdominal distension, cramps/pain, nausea, vomiting, flatulence, and steatorrhea. We requested the individual safety reports of hepatic injury cases that were determined by the FDA to be directly linked to orlistat; the 33 reports we received were reviewed in this study.

Results:

Of the 33 reports, 29 were females, 3 males, and 1 gender not recorded. The mean age was 44 years and mean BMI was 30.1 kg/m². In 31 reports, orlistat was listed as the primary suspect and in 2 it was listed as the secondary suspect with atorvastatin as the primary. The total daily dose ranged from 120 mg to 360 mg with 46% using 120 mg TID. The average duration of therapy was 76 days. Of the 57% of the reports recording the AST, ALT, and total bilirubin values, 89.5% had elevated AST, 94.4% had elevated ALT, and 83.3% had elevated total bilirubin. 88% of the patients were hospitalized with abnormal LFTs and/or symptoms such as icterus, malaise, pruritis, emesis, dark urine, and/or clay colored stool. Of these, 1 patient died, 4 required liver transplantation, and 19 recovered with supportive care and discontinuation of orlistat; the final outcome was unknown in 5 patients. Of note, none of the 33 patients had a pre-existing hepatic condition listed.

Conclusion:

Clinicians should be aware that serious liver-related adverse events possibly secondary to orlistat use have been reported to the FDA. Healthcare providers need to be cognizant of this when prescribing and monitoring patients on orlistat. If a patient develops signs or symptoms consistent with early liver impairment, consider discontinuing orlistat or closer monitoring.

Nothing to Disclose: RB, DP, AS

P3-439

Dynamics of Plasma Proteome of Genetically Leptin-Deficient Patients during Leptin Replacement Therapy.

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Despite the existence of a large body of publications on the pathways involved in leptin action, the mechanisms of leptin resistance, anti-lipotoxicity, and cross-talk between leptin-insulin are not fully understood.

To investigate the dynamics of plasma proteome of three genetically leptin-deficient adults, we compared protein abundance levels at four stages of treatment with recombinant methionyl human leptin: at the leptin-naïve state, after 18 months and 6 years of continuous treatment, and 7 weeks after temporary interruption of treatment. We used proteomics analysis with iTRAQ (Isobaric Tagging for Relative and Absolute Protein Quantification) approach. A two-dimensional HPLC-ESI/MS scheme for bottom-up proteomics analysis was employed. Protein identification was done by the X!Tandem (GPM) search engine. Pathway and network analyses were performed using proteins identified with E-value $\leq 10^{-3}$. Heat maps and hierarchical clustering of protein abundance time courses were generated.

We identified with extremely high significance (median logE < -24.7) and quantitated 556 proteins (31% extracellular, 69% intracellular). Treatment led to up-regulation of pathways and processes related to cell adhesion, cytoskeleton remodelling, cell cycle, blood coagulation, glycolysis and gluconeogenesis, in all patients. When off-leptin, up-regulation of inflammation, lipoprotein and lipid metabolism pathways was observed only for the oldest and most obese patient. In the insulin resistance network, adiponectin and sex hormone-binding globulin were overabundant for all patients in all stages of treatment.

In conclusion, leptin treatment/withdrawal is associated with the up-regulation of specific pathways. Those results are clinically relevant, and should be taken into account when treating patients with leptin.

Sources of Research Support: National Institutes of Health Grants RR-16996, HG-002500, DK-58851, RR-017611, and K24-RR-016996 to J. Licinio and DK-063240, RR-017365, MH-062777, RR-000865, and K24-RR-017365 to M. -L. Wong and awards from the National Alliance for Research on Schizophrenia and Depression to M. -L. Wong.

Nothing to Disclose: GP-F, VA, M-LW, RCD, OVK, JAW, JL

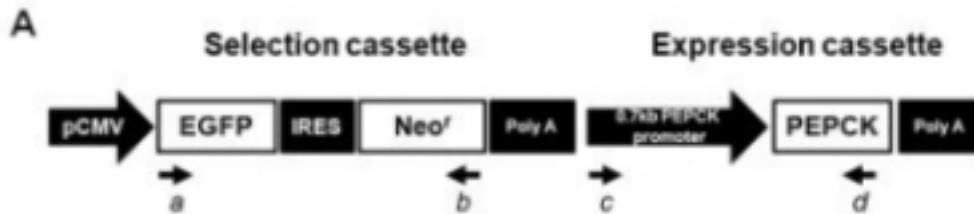
P3-440

Generation of Transgenic Dogs Overexpressing *Phosphoenolpyruvate Carboxykinase* for Type II Diabetes Mellitus Model Via Somatic Cell Nuclear Transfer.

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¹SooAm Biotech Res Foundation Yongin-si, Republic of Korea and ²Chungbuk Natl Univ Cheongju-si, Republic of Korea.

Development of canine disease models is valuable for understanding human disease and therapeutic strategies. Using somatic cell nuclear transfer to produce transgenic offspring for modeling human disorders and for developing therapeutic strategies may have a valuable impact on medicine. Type II diabetes mellitus (T2DM), non-insulin-dependent diabetes mellitus's disease (NIDDM), is one of the most common yet complex metabolic disorders and is characterized by hyperglycemia and metabolic alterations. A primary cell line was established from fetal fibroblasts and transfected with PEPCK containing the enhanced green fluorescent protein gene.



A total of 66 cloned embryos derived from fetal fibroblast transfected with PEPCK transgene was transferred to 4 recipients, 2 of which became pregnant and gave birth to 2 transgenic-cloned offsprings.

Characteristics and status of cloned puppies.

| Offspring identification | Karyoplast passage no. | Gestation length | Birth weight (g) | Delivery method | GFP expression |
|--------------------------|------------------------|------------------|------------------|------------------|----------------|
| DFN59 | 5 | 44 | N/A | Natural | - |
| DFN60 | 5 | 65 | 380 | Cesarean section | - |
| DFN64 | 5 | 63 | 340 | Cesarean section | - |
| DMP108 | 6 | 62 | 380 | Cesarean section | + |
| DMP110 | 6 | 63 | 360 | Cesarean section | + |

All of the live puppies expressed green fluorescent nails, toes and white hairs.



PCR and real-time PCR analysis determined that the cloned offspring contained and expressed the PEPCK transgene. Microsatellite analysis confirmed that the clones were genetically identical to the donor cell. The SCNT-T2DM canine model would offer us basic information necessary to understand underlying biology of type II DM and to develop its therapeutics. We here report our progress in developing a somatic cell nuclear transfer (SCNT) derived transgenic model of T2DM in dogs that express PEPCK for understanding type II DM.

Nothing to Disclose: YWJ, GSL, JJK, SWP, KHK, MK, CJY, TKJ, EMJ, YKK, SHH, TS, EBJ, WSH

P3-441

Regulation of Hepatic Gene Expression and Metabolism by FoxO Proteins.

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FoxO transcription factors play an important role in mediating effects of insulin, and FoxO1 is thought to promote gluconeogenic gene expression and glucose production in the liver. Based on studies in transgenic mice, we previously reported that FoxO proteins also may be important in regulating hepatic glucose utilization, lipogenesis and amino acid catabolism (JBC 281:10105, 2006). Three major mammalian FoxO proteins (FoxO1, FoxO3 and FoxO4) have been identified and recent studies in knockout mice (KO) indicate that these proteins can serve overlapping functions. To better understand their function in the liver, we created two liver-specific FoxO KO mouse models, FoxO1 KO and FoxO1/3/4 KO, using the Cre lox system. Initial studies revealed that 18 hr fasted glucose levels were not altered in FoxO1 or FoxO1/3/4 KO mice compared to "floxed" littermate controls. Glucose tolerance tended to be improved in female FoxO1 mice and was significantly improved in both male and female FoxO1/3/4 KO. This result indicates that hepatic FoxO proteins may not be essential for short-term adaptation to fasting, but that multiple FoxO proteins contribute to the dynamic regulation of glucose metabolism. Serum triglyceride levels were not altered in FoxO1 KO mice but were increased in FoxO1/3/4 KO mice, supporting the concept that endogenous FoxO proteins contribute to the regulation of lipid metabolism in liver. Real-time PCR studies indicated that effects on the expression of IGF binding protein-1 (a known FoxO target gene), and gluconeogenic (PEPCK, G6Pase), glycolytic (glucokinase) and lipogenic genes (SREBP-1c) are most apparent when all 3 FoxOs are knocked out compared to FoxO1 KO alone. Gene profiling studies with Illumina arrays identified 519 genes that are differentially expressed in fasting FoxO1/3/4 KO mice compared to "floxed" controls, including genes associated with diabetes (74), lipid metabolism (55), protein synthesis (54) or degradation (26) and amino acid metabolism (13). Together, these results indicate that multiple FoxO proteins (and not just FoxO1) contribute to the regulation of glucose, lipid, and protein metabolism in the liver.

Nothing to Disclose: ISO-S, JBW, GF, SL, NJ, TU

P3-442

Improved Energy Expenditure, Glucose Utilization and Insulin Sensitivity in VAMP8 Null Mice.

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OBJECTIVE: Previous studies have demonstrated that the VAMP8 protein plays a complex role in the control of granule secretion, transport vesicle trafficking, phagocytosis and endocytosis. The present study was aimed to investigate the role of VAMP8 in mediating GLUT4 trafficking and therefore insulin action in mice.

RESEARCH METHODS: Physiological parameters were measured using Oxymax indirect calorimetry system in 12 weeks old VAMP8 null mice. Dynamic analysis of glucose homeostasis was assessed using euglycemic-hyperinsulinemic (EU) clamp coupled with trace radioactively labeled 2-deoxyglucose. Insulin stimulated GLUT4 protein expressions on muscle cell surface were examined by immunofluorescence microscopy.

RESULTS: VAMP8 null mice display reduced adiposity with increased energy expenditure despite normal food intake and reduced spontaneous locomotor activity.

Figure 1 VAMP8 Null Mice Display Reduced Adipocyte Cell Size

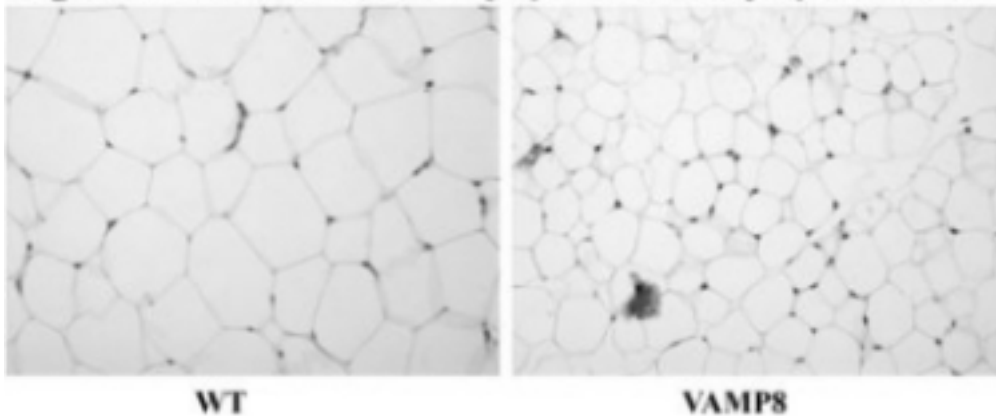
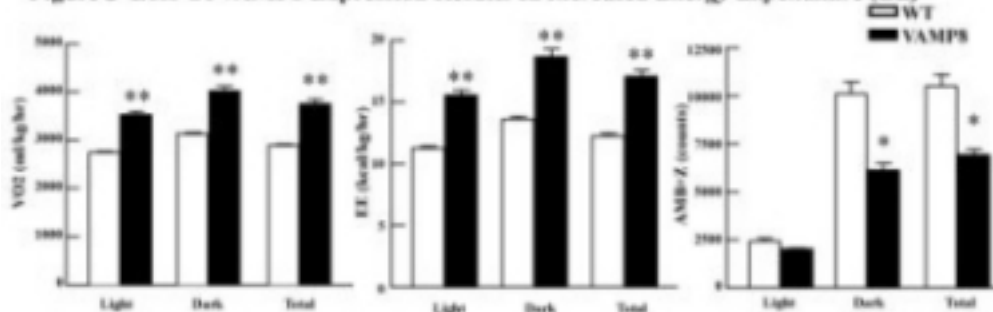


Figure 2 Loss Of VAMP8 Expression Results In Increased Energy Expenditure (EE)



In parallel, the VAMP8 null mice also had fasting hypoglycemia (84.14 ± 11.32 vs 115.11 ± 4.73), improved glucose tolerance with increased insulin sensitivity due to both increased basal and insulin-stimulated glucose uptake in skeletal muscle (0.19 ± 0.04 vs 0.09 ± 0.01 mmol/kg/min during basal, 0.6 ± 0.04 vs 0.31 ± 0.06 mmol/kg/min during clamp in red-gastrocnemius, $p < 0.05$). Consistent with a role for VAMP8 in the endocytosis of the insulin responsive glucose transporter GLUT4, sarcolemma GLUT4 protein levels were increased in both the basal and insulin-stimulated states without any significant change in the total amount of GLUT4 protein, or related facilitative glucose transporters present in skeletal muscle, GLUT1, GLUT3 and GLUT11.

CONCLUSIONS: These data demonstrate that in the absence of VAMP8, the relative subcellular distribution of GLUT4 is altered resulting in increased sarcolemma levels that can account for increased glucose clearance and insulin sensitivity.

Nothing to Disclose: HZ, C-CW, WH, JEP

P3-443

FXR Positively Regulates Hepatic SIRT1 Levels Via MicroRNA-34a Inhibition.

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The nuclear bile acid receptor FXR controls lipid and glucose metabolism by regulating numerous metabolic target genes. The NAD⁺-dependent SIRT1 deacetylase regulates cellular metabolism, stress response, and possibly aging in response to nutritional and hormonal fluctuations. Although both SIRT1 and FXR are critical in liver metabolic regulation, it remains unknown whether the regulation of these two proteins is linked. Here we report that bile acid-activated FXR positively regulates hepatic SIRT1 levels through the inhibition of microRNA-34a (miR-34a). FXR indirectly inhibits expression of the miR-34a gene by induction of SHP, an orphan nuclear receptor and transcriptional corepressor. SHP, then, interacts with and inhibits transactivation of p53, a key activator of the miR-34a gene by inhibiting association of p53 with the miR-34a promoter. MiR-34a decreased SIRT1 protein levels by binding to the 3' untranslated region of SIRT1 mRNA and adenoviral-mediated overexpression of miR-34a substantially decreased SIRT1 protein levels in mouse liver. Remarkably, miR-34a levels were elevated and SIRT1 protein levels were decreased in FXR null mice and in diet-induced obese mice. Activation of FXR by daily treatment with GW4064, a synthetic FXR agonist, for 1 week decreased miR-34a levels and increased SIRT1 levels in obese mice, indicating an intriguing link between FXR activation, decreased miR-34a levels, and increased SIRT1 levels. Our study shows an unexpected role of the FXR/SHP pathway in controlling hepatic SIRT1 levels via miR-34a inhibition and manipulation of this regulatory network may be useful for treating diseases of aging, such as diabetes, obesity, and cancer.

Sources of Research Support: NIH DK062777; ADA Basic Science Award.

Nothing to Disclose: JL, AP, AS, GS, JM, LW, VE, JKK

P3-444

FGF21-Deficient Mice Have Atypical Responses to High Fat and Ketogenic Diets.

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¹Beth Israel Deaconess Med Ctr, Harvard Med Sch Boston, MA.

FGF21 is a secreted hepatic protein that plays an important role in energy homeostasis, and appears to play a role in regulating hepatic fatty acid oxidation and ketogenesis. It is markedly upregulated in fasted mice, as well as in mice fed a ketogenic diet. It also participates in glucose metabolism and has been shown to improve glucose uptake in diabetic rodent models. However, studies of FGF21-deficient mice have produced inconsistent results. To further understand the phenotype associated with FGF21 deficiency, mice lacking FGF21 were fed different diets: either chow, a high-fat high-sucrose diet utilized to induce obesity (HF), or a ketogenic diet (KD) for 8 weeks. FGF21-deficient mice fed either chow or KD gained more weight (40.2 +/- 1.6 vs. 38.1 +/- 1.13 gm on chow; 38.4 +/- 2.0 vs. 33.5 +/- 1.4 gm on KD) and showed significantly impaired glucose metabolism as assessed by glucose and insulin tolerance tests when compared to their wild-type littermates. FGF21-deficient mice on KD had significantly elevated fasting glucose (104.9 +/- 6.4 vs. 63 +/- 4.3 mg/dl, $p < 0.001$) and insulin levels (0.45 +/- 0.08 vs. 0.23 +/- 0.06 ng/ml, $p < 0.05$). In addition, FGF21-deficient mice fed a chow or ketogenic diet had significantly higher triglyceride and non-esterified fatty acid (NEFA) levels compared to their WT littermates. In contrast, when mice were fed a high-fat high-sucrose diet, both WT and FGF21-deficient mice gained the same amount of weight and there was no difference in glucose homeostasis or in lipid levels. Interestingly, obese wild type animals showed signs of FGF21 resistance as assessed by an impaired ability of exogenous FGF21 to induce expected changes in the phosphorylation of target genes and in gene expression of immediate early genes c-Fos and EGR1 in the livers of treated mice. This study provides further evidence that FGF21 plays an important role in glucose and fatty acid metabolism, particularly in the adaptation to ketogenic feeding.

Nothing to Disclose: PCC, FMF, EM-F

P3-445

Neuronostatin Regulates Pancreatic Function.

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Neuronostatin, a recently discovered peptide, is derived from the somatostatin preprohormone. As expected, neuronostatin is produced in the same tissues as somatostatin, including the delta cells of the endocrine pancreas. Intraperitoneal injection of neuronostatin into mice led to c-Jun expression in pancreatic islet cells (1). We therefore hypothesized that neuronostatin may regulate islet function. To test this hypothesis, we isolated islets from rats and after stabilization culture they were treated with either vehicle alone, or vehicle containing 10, 100 or 1000 nM neuronostatin or 100 nM somatostatin as a positive control. Islets were then exposed to either 3 mM or 20 mM D-glucose for 30 minutes, and hormone content of the supernatant was measured by radioimmunoassay. Glucose stimulated insulin secretion was significantly inhibited by neuronostatin at all concentrations and by the positive control, somatostatin. Neuronostatin also significantly enhanced low glucose-stimulated glucagon secretion, suggesting a direct effect on the alpha cell and not the beta cell. In addition neuronostatin increased glucagon expression in isolated rat islets and clonally-derived alpha cells (α TC1-9 cells). To determine if neuronostatin would affect glucose disposal in vivo, adult, male rats were fitted with jugular and carotid catheters to perform an intra arterial glucose tolerance test. Rats received via the carotid catheter a bolus (0.5 ml) of saline alone or saline containing 30 micrograms/kg neuronostatin, followed by a 35 min continuous intra-arterial infusion of saline alone (0.05 ml/min) or saline containing neuronostatin at a dose of 1 microgram per minute. Five minutes later, a blood sample was removed and a bolus of glucose (1g/kg) was injected intra-arterially. Additional blood samples were removed 1, 10 and 30 minutes thereafter. Treatment with neuronostatin not only led to an increased peak in blood glucose levels at 1 minute following the administration of the glucose bolus, but also significantly delayed the clearance of glucose from the bloodstream. As predicted by those blood glucose levels and the in vitro studies described above, neuronostatin treatment also significantly attenuated the insulin response to glucose challenge. These results indicate that the somatostatin gene encodes two hormones, neuronostatin and somatostatin, which can act within the islet to regulate glucose homeostasis.

(1) Samson WK et al., J Biol Chem 2008; 283:31949

Sources of Research Support: NIH Grant HL066023-06 to WK Samson.

Nothing to Disclose: GLCY, ASS, WKS

P3-446

Effects of a Potent and Selective Non-Retinoid Retinol Binding Protein 4 (RBP4) Modulator on Glucose Homeostasis.

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¹Exelixis, Inc San Diego, CA ; ²EMBL, Grenoble Outstation Grenoble, France ; ³Exelixis, Inc South San Francisco, CA and ⁴ Ctr for Molecular Design, Univ of Virginia Hlth Syst Charlottesville, CA.

Retinol-binding protein 4 (RBP4) is a serum retinol transporter protein that is elevated in insulin resistant mouse models and in obese or diabetic humans. In mice, increasing serum RBP4 concentrations through transgenic over-expression or injection of recombinant RBP4 protein causes insulin resistance although the mechanisms by which this occurs are not completely understood. Mice dosed with fenretinide, a synthetic retinoid that increases renal clearance of RBP4 by disrupting the interaction with its serum binding partner transthyretin (TTR), are protected from diet-induced insulin resistance. In our efforts to validate RBP4 as a potential target in type 2 diabetes, we identified a potent, non-retinoid RBP4 ligand, EXEL-9767, which selectively binds to RBP4 and disrupts the interaction between RBP4 and TTR. Binding of EXEL-9767 to RBP4 was confirmed by solving the co-crystal structure of RBP4/EXEL-9767. In vivo, EXEL-9767 reduces circulating RBP4 levels in a dose-responsive manner, with an 80-90% maximal response. The activity of EXEL-9767 on glucose homeostasis was further explored using mouse and rat models of insulin resistance and hyperglycemia. When mice were treated with EXEL-9767 before they developed insulin resistance induced by high fat diet feeding, EXEL-9767 attenuated the increase of insulin/glucose levels and improved measures of glucose homeostasis with the most significant effect seen at 10-12 weeks of treatment. However, in animal models with pre-existing insulin resistance (mice pre-fed with high fat diet for 8 weeks or Zucker fa/fa rats), EXEL-9767 did not improve any measured metabolic endpoint. These results suggest that lowering circulating RBP4 levels by small molecule disruption of the RBP4-TTR complex is insufficient to improve metabolic parameters in insulin resistant animals. Although RBP4 may not be an ideal target for the treatment of diabetes, additional structural studies of RBP4 revealed an unexpected ligand bound in the retinol binding site that allows us to suggest novel hypotheses of its role in vivo.

Disclosures: HH: Researcher, Exelixis, Inc. PF: Researcher, Exelixis, Inc. BF: Researcher, Exelixis, Inc. MN: Researcher, Exelixis, Inc. JQ: Researcher, Exelixis, Inc. IM: Researcher, Exelixis, Inc. TS: Researcher, Exelixis, Inc.

Nothing to Disclose: PW, IS

P3-447

Differential Effect of FoxO1 on Basal and Insulin-Regulated Gluconeogenic Enzyme Gene Expression.

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¹Johns Hopkins Univ Sch of Med Baltimore, MD.

A major cause of hyperglycemia in Type 2 diabetes is inappropriate hepatic gluconeogenesis. Key gluconeogenic genes have promoter elements that bind a myriad of transcriptional cofactors including FoxO1. FoxO1 is inactivated by insulin-mediated phosphorylation. To better understand the role of FoxO1 in hepatic gluconeogenesis, we infected H4IIEC rat hepatoma cells with an adenovirus containing a FoxO1 specific shRNA and then performed quantitative PCR of key gluconeogenic genes including FBP1, G6P, PEPCK, and PGC1- α . After treatment with 10nM insulin for 6 hours, there was a variable, blunted insulin effect in cells subjected to FoxO1 knockdown. Insulin suppression of G6P mRNA levels was completely abolished after knockdown of FoxO1. After 6 hours of 10nM insulin treatment, G6P message levels were 120% of the values measured at baseline in FoxO1 knockdown cells; in cells treated with a scrambled control adenovirus, G6P message decreased by 44%. In contrast, other mRNA levels were not as severely affected. In FoxO1 knockdown cells, PEPCK message was reduced by only 28% compared to a 48% reduction in control cells. The reduction in FBP1 and PGC1- α mRNA levels (82% and 94% in FoxO1 knockdown vs. 89% and 80% respectively in scrambled controls) was not significantly different between FoxO1 knockdown and control cells. These findings demonstrate that FoxO1 plays a variable role in insulin regulation of gluconeogenic genes. Some genes, like FBP1 and PGC1- α , decrease normally after exposure to insulin after FoxO1 knockdown. The effect of insulin on other genes, like PEPCK, is moderately affected by FoxO1 knockdown, while G6P seems to be completely dependent on FoxO1 for insulin regulation. Surprisingly, at baseline, we noted elevated mRNA levels in cells treated with the FoxO1 specific shRNA adenovirus. At baseline, mRNA levels were increased by 1.9 fold for FBP1, 1.1 fold for G6P, 1.9 fold for PEPCK, and 2.3 fold for PGC1- α . The genes with the highest basal induction after FoxO1 knockdown were noted to have the least impairment in insulin regulation. This finding suggests that FoxO1 has a more complex role in hepatic gluconeogenesis than previously considered. Our results might explain the absence of a major phenotype in the hepatic FoxO1 knockout mouse, as there may be a variable effect of FoxO1 on gluconeogenic enzyme gene regulation by insulin as well as an indirect inhibitory function of FoxO1 on basal gene expression.

Nothing to Disclose: CSD, AS, LH, FEW

P3-448

The Effects of GH, IGF-1 or Combined GH/IGF-1 Therapies on Type 2 Diabetes and Nonalcoholic Fatty Liver Disease (NAFLD) in Mice.

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Our laboratory previously demonstrated that growth hormone (GH) treatment in a mouse model of type 2 diabetic mice can reverse obesity, hyperglycemia, glucose intolerance and NAFLD (1). In this study, we compared the use of GH to that of insulin like growth factor 1 (IGF-1) alone or in combination with GH in the same mouse model. Thirty-nine mice were placed on a high fat (HF) diet for 19 weeks. Starting at week 16, the HF fed mice were divided into 4 groups and treated twice daily for 3 weeks with: saline (n=10) as a HF control, 5ug rbGH (n=10), 2.5ug rhIGF-1 (n=10), or both 5ug rbGH and 2.5ug rhIGF-1 (n=9). Ten mice remained on standard chow and served as LF controls. Body composition analyses revealed that the two groups treated with GH (GH and combined GH/IGF-1 treatment groups) had a significant reduction in fat mass, while the IGF-1 treated group had a fat mass similar to HF controls. Lean mass was significantly increased in all 3 treatment groups (GH, IGF-1 and combined GH/IGF-1) with the combination treatment showing the greatest increase. At the end of the 3 weeks of treatment, glucose tolerance was completely normalized in the GH and combined GH/IGF-1 treatment groups as compared to LF mice, while the IGF-1 treatment group showed no significant improvement. However, glucose measurements taken hourly for 8 hours following a morning treatment revealed that the 2 IGF-1 treated groups (IGF-1 and combined GH/IGF-1) had transient (less than 3 hours) decreases in glucose. Surprisingly, all 3 treatments groups improved NAFLD as the liver triglyceride content was decreased to that found in healthy lean controls. Additionally, a variety of circulating hormone and lipid levels as well as dissected tissue weights and histological preparations of liver sections were analyzed and will be presented. In this study, both GH and IGF-1 significantly increased lean mass but only GH treatment decreased fat mass. Since only the GH treated groups had improved glucose metabolism, it appears that decreasing fat was more beneficial than increasing lean mass related to glucose metabolism. Furthermore, we confirmed our previous findings that GH therapy improves NAFLD (1). Most importantly, we have demonstrated that IGF-1 treatment alone can improve NAFLD by decreasing triglyceride content of the liver. This unexpected finding has provided the framework for future studies on the molecule mechanism(s) of GH versus IGF-1 treatment associated with NAFLD.

(1) List et al., *Diabetologia* 2009; 52(8):1647-55

Sources of Research Support: In part by the State of Ohio's Eminent Scholar Program that includes a gift from Milton and Lawrence Goll, by grants from the NIDDK (DK075436) and NIA (AG031736 and AG019899), by a grant from the AMVETS organization, by a grants from the Diabetes Research Initiative and the VP for Research at Ohio University. The bGH used in this study was a gift from the Monsanto Company. The IGF-1 used in this study was a gift from Tercica.

Nothing to Disclose: EOL, DEB, BB, JDB, JW-P, EL, RM, JJK

P3-449

Elevated Hepatic Hexose-6-Phosphate Dehydrogenase and Glucose-6-Phosphate Transporter Expression May Contribute to the Development of Diabetic Syndrome in Diabetic Mice.

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¹Charles Drew Univ of Med and Sci Los Angeles, CA and ²Hamamatsu Univ Sch of Med Hamamatsu, Japan.

Prereceptor activation of glucocorticoids via the NADPH-dependent 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) has been identified as an important risk factor in metabolic syndrome. Hexose-6-phosphate dehydrogenase (H6PDH) mediates intracellular NADPH availability to mouse hepatic 11 β -HSD1 and requires glucose-6-phosphate transporter (G6PT) to maintain its activity. To assess whether the tissue-specific alterations of H6PDH and G6PT expression could contribute to local glucocorticoid action and insulin resistance in type 2 diabetes and obesity, we analyzed the expression of 11 β -HSD1-H6PDH -G6PT system in vivo in liver and adipose tissue and their regulation by endogenous hormones in type 2 diabetic animals. We observed a positive correlation between expression of both H6PDH and 11 β -HSD1 in liver and insulin sensitivity and expression of PEPCK mRNA in diabetic mice and their controls. Increased expression of H6PDH was paralleled by up-regulation of hepatic G6PT expression and responded to elevated circulating levels of corticosterone and insulin. In contrast, pharmacological inhibition of H6PDH and G6PT expression decreased NADPH production accompanied by reduction of 11 β -HSD1 in the liver and subcutaneous fat of diabetic mice but not in epididymal fat, and attenuated the phenotype of type 2 diabetes. These findings suggest that elevated H6PDH and G6PT expression within the liver may contribute to 11 β -HSD1 up-regulating local glucocorticoid action and the development of type 2 diabetes.

Nothing to Disclose: YL, YW, YN, WW, CV, TCF

P3-450

Human C-Peptide Exerts Anti-Inflammatory Activity in TNF- α -Stimulated U-937 Monocytes under High Glucose Conditions.

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Type I diabetes (T1D) is associated with increased risk of both micro- and macro-vascular complications, which are reduced by intensive diabetes therapy targeted at blood glucose control. Hyperglycemia contributes to the endothelial dysfunction present in T1D which leads to pathologic inflammatory processes, such as secretion of inflammatory cytokines (*i.e.* TNF- α), which lead to the development of vascular disease including atherosclerosis. Atherosclerosis is an inflammatory process and monocytes are involved in every phase of atherogenesis. Data suggest C-peptide has an anti-inflammatory and potentially anti-atherogenic effect on endothelial cells. In addition, C-peptide reduces cytokine secretion in LPS-activated monocytes under high-glucose conditions. In this study, we investigated whether C-peptide exerts anti-inflammatory effects in monocytes in an endogenous model of inflammation, by studying C-peptide's effects on cytokine secretion in TNF- α -activated monocytes.

The human monocytic cell line U-937 was plated in a 24-well plate and incubated overnight with C-peptide. Subsequently, the monocytes were exposed for 4 hours to glucose and TNF- α in the presence or absence of varying concentrations of C-peptide or a control. The control was C-peptide with a randomized sequence (scrambled C-peptide). Secretion of the inflammatory cytokines IL-6, MCP-1, IL-8, MIP-1 α , and MIP-1 β in the culture supernatants was measured by Luminex.

Incubation with C-peptide followed by TNF- α -activation led to significantly reduced secretion of the majority of inflammatory cytokines in the supernatants compared to TNF- α alone (*p* values <0.01-<0.05). Addition of the control, scrambled C-peptide, to TNF- α -stimulated U937 cells did not significantly affect cytokine secretion. We conclude that under hyperglycemic conditions, C-peptide decreases TNF- α -activated monocytic cytokine secretion *in vitro*. Thus, C-peptide has been demonstrated to reduce cytokine secretion in a monocytic cell line in 2 different models of inflammation, LPS- and TNF- α -activated models. This suggests an anti-inflammatory, and thus potential anti-atherogenic, effect of C-peptide in conditions associated with hyperglycemia.

Nothing to Disclose: JLH, MT, PL

P3-451

Low-Dose Serum Amyloid A Exposure Decreases Phosphorylated Akt in Liver in Mice.

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Serum amyloid A (SAA) is a proinflammatory adipokine, which was shown recently to correlate with obesity and insulin resistance (IR) in human and cell lines. However, it is not clear whether SAA can induce IR *in vivo*. Moreover, the molecular mechanisms that link SAA and IR are also poorly understood *in vivo*.

In the present study, we tested the effect of low dose infusion of recombinant human SAA (rhSAA) on insulin resistance and the signaling pathway concerning insulin resistance in male C57BL/6 mice. Osmotic pumps were used to keep a stable and persistent injection of rhSAA. 1 μ g per hour rhSAA was delivered in rhSAA treatment group for 7 days. Control group was treated with 0.9% normal saline under the same conditions. On 7th day, intraperitoneal glucose tolerance test (IPGTT) was performed. Blood was collected to test the concentrations of human SAA and mice SAA. Additionally, phosphorylated JNK (p-JNK), total JNK, phosphorylated ERK (p-ERK), total ERK, phosphorylated Akt (p-Akt) and total Akt in liver and skeletal muscle were assessed with Western Blot.

Our results show that the concentration of human SAA in the blood (43.5 \pm 16ng/ml) was increased significantly after treatment with rhSAA. There were no differences between the concentration of mice SAA in the blood in rhSAA treatment group (141.2 \pm 31.9 μ g/ml) and control group (141.4 \pm 34.7 μ g/ml). After low-dose SAA treatment, there were no obvious changes in body weight and IPGTT. However, there was a significant decrease in the expression ratio of p-Akt to total Akt after SAA treatment in the liver (p<0.05) but not in skeletal muscle. Since the phosphorylation of Akt plays very important role in insulin signaling, these results indicate that the insulin signaling pathway was affected with low-dose SAA treatment even though there is no change in IPGTT compared with control group. However, unlike other models, we found no significant changes in the ratio of p-JNK to total JNK or p-ERK to ERK in liver and skeletal muscle. Therefore, our results suggest that phosphorylation of Akt in liver is the most sensitive factor in insulin signaling after an increase in SAA and there is a reduction in phosphorylated Akt before development of insulin resistance in mice treated with low dose SAA.

Sources of Research Support: Chinese Natural Science Fund #30671004; Jiangsu Natural Science Fund #BK2006006; Nanjing Targeted Science and Technology Development Fund #ZKX06014.

Nothing to Disclose: RM, DLZ, YPW

P3-452

Loss of Ceacam1 Leads to the Development of Non-Alcoholic Steatohepatitis.

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Null mutation of Ceacam1 impairs hepatic insulin clearance, leading to hyperinsulinemia, insulin resistance, elevation in hepatic and serum triglyceride levels, and visceral obesity. L-SACC1 mice with liver-specific dominant-negative inactivation of Ceacam1 exhibit the same phenotype; in addition, they develop non-alcoholic steatohepatitis (NASH) when fed a high fat diet. To discern whether this phenotype reflects a physiological function of CEACAM1 rather than the effect of the dominant-negative transgene, we investigated whether *Cc1*^{-/-} null mutant mice develop NASH-like phenotype on a prolonged high fat diet. 3 month-old *Cc1*^{-/-} mice on a C57BL/6 genetic background were fed a high fat diet for 3 months and their NASH phenotype was examined. While high fat feeding elevated hepatic triglyceride content in both wild type and *Cc1*^{-/-} mice, it exacerbated macrosteatosis, fibrogenic changes and inflammatory responses more intensely in the null mouse. Fat feeding induced apoptosis only in *Cc1*^{-/-} mice. This demonstrates that CEACAM1-dependent insulin clearance pathways are linked with both insulin resistance and the pathogenesis of NASH.

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Nothing to Disclose: SG, PRP, MK, MAF, REB, SKE, MFM, SMN

P3-453

Changes in Hepatic Gene Expression Following GH or IGF-1 Treatments in Mice with Diet-Induced Nonalcoholic Fatty Liver Disease.

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Nonalcoholic fatty liver disease (NAFLD) is closely linked to obesity and type 2 diabetes and is the most commonly diagnosed liver pathology in the United States. Defined as fatty depositions in the liver with alcohol consumption below 20 grams per day, NAFLD is an independent risk factor for cardiovascular disease and may progress to nonalcoholic steatohepatitis (NASH), cirrhosis, or malignancy. The pathogenesis of this disease is still not fully understood, and pharmacological interventions have been unsuccessful. C57BL/6J mice have been shown to develop obesity, diabetes, and NAFLD when fed a high-fat (HF) diet. Our laboratory found that growth hormone (GH) treatment can reverse liver triglyceride content in this mouse model of NAFLD to the levels of healthy standard chow-fed control mice (1). We have recently found that treatment with insulin-like growth factor-1 (IGF-1) also decreases liver triglyceride content (List, unpublished results, details of this study have been submitted in a separate abstract at this meeting (2)). This was unexpected since IGF-1 is thought to have minimal activity on liver due to low to non-existent IGF-1R levels in hepatocytes, and also because IGF-1 has not been shown to decrease fat mass.

To better understand the genes that are up or down regulated during the reversal of fatty liver with GH or IGF-1 treatments, Agilent 4 x 44K Whole Mouse Genome MicroArrays were used to study gene expression changes in the livers of HF fed C57BL/6J mice treated with twice daily injections of either GH (5 ug/g body weight) or IGF-1 (2.5 ug/g body weight) for 3 weeks versus HF and low-fat (LF) controls treated with saline. Differentially expressed genes were identified using the RankProd software in R Bioconductor. HF fed versus LF fed mouse livers had ~1000 genes significantly altered with ~500 down regulated and ~500 up regulated, respectively. GH treated versus untreated livers from HF fed mice had ~450 altered genes with ~200 down regulated and ~250 up regulated, respectively. IGF-1 treated versus untreated livers from HF fed mice had ~270 altered genes with ~180 down regulated and ~90 up regulated, respectively. Gene annotation analysis revealed changes in lipid metabolic processes, including genes regulated by PPAR and insulin. These results provide insight into the molecular mechanisms that mediate the reversal of NAFLD due to GH or IGF-1 treatments.

(1) List et al., 2009, *Diabetologia*, 52 (8), 1647-1655.

(2) List et al., (abstract submitted concurrently with this abstract to Endo 2010, San Diego, CA)

Sources of Research Support: In part by the State of Ohio's Eminent Scholar Program that includes a gift from Milton and Lawrence Goll, by grants from the NIDDK (DK075436) and NIA (AG031736 and AG019899), by a grant from the AMVETS organization, by a grants from the Diabetes Research Initiative and the VP for Research at Ohio University. The bGH used in this study was a gift from the Monsanto Company. The IGF-1 used in this study was a gift from Tercica.

Nothing to Disclose: JDB, EOL, DEB, KS, JW-P, AZ, AK, MG, CD, JJK

P3-454

Evaluation of Fatty Acid Profiles in Type 2 Diabetes.

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Clinical data show that dyslipidemia is a critical factor in the development of diabetic retinopathy. The DCCT/EDIC cohort study revealed a strong association between severity of retinopathy and the size of LDL, VLDL and HDL particles as well as concentration of serum lipoproteins in patients with type 1 diabetes (1). Higher levels of serum lipids are associated with an increased risk of development of hard exudates in the macula, and ensuing visual loss (2). Studies have demonstrated that lipid-lowering dietary and drug therapy may lead to regression of retinal hard exudates, and that a diet high in polyunsaturated fatty acids (PUFA) may protect against retinopathy. There are no detailed studies of blood and tissue fatty acid profiles in patients with type 2 diabetes (DM). We analyzed samples from 11 DM patients (8 women, 3 men) without retinopathy, with duration of diabetes 7.27 ± 4.43 years and 10 controls without diabetes (9 women, 1 man). We measured fasting LDL, HDL, total cholesterol, triglycerides, HbA1c, and fatty acid levels (20:5n3, 18:3n3, 18:3n6, 22:6n3, 16:1n7, 16:0, 20:4n6, 22:5n3, 18:2n6, 20:3n6, 18:0 and 18:1n9). Mean ages for DM patients and controls were 53.45 ± 11.28 years and 42.0 ± 11.64 years respectively ($p < 0.05$). Total saponified fatty acids from erythrocytes of controls and DM patients were analyzed by HPLC. Mean glucose, HbA1c and chol/HDL in DM patients were higher than in controls (143.9 ± 47.7 mg/dl, $8.04 \pm 1.29\%$, 3.66 ± 1.11 mg/dL for DM patients and 85.4 ± 10.8 mg/dL, $5.65 \pm 0.4\%$ and 2.91 ± 0.38 mg/dL for the controls; ($p < 0.05$ for all, age-adjusted). Average HDL, 22:5n3, and 20:3n6 in DM patients were lower than in controls (46.27 ± 8.81 mg/dL, 10.70 ± 3.62 mg/dL, and 4.63 ± 2.44 mg/dL for DM patients and 57.7 ± 10.67 mg/dL, 11.50 ± 0.34 mg/dL, and 5.84 ± 1.29 mg/dL for controls; ($p < 0.05$ for all, age-adjusted). There was no difference between DM patients and controls in LDL, total cholesterol, triglycerides, and other fatty acid levels. We observed an inverse relationship between statin use and levels of 16:0. Since most of our DM patients were on statins, this is likely why no significant difference in 16:0 levels was found between DM patients and controls. As expected, lower levels of PUFA were observed in DM (22:5n3, 20:3n6). The role of these differences in the development of retinopathy in patients with type 2 diabetes remains to be determined.

(1) Lyons, T.J., et al., Diabetic retinopathy and serum lipoprotein subclasses in the DCCT/EDIC cohort. Invest Ophthalmol Vis Sci, 2004.45(3):910-8.

(2) Rice, T. Early Treatment Diabetic Retinopathy Study. Trans Pa Acad Ophthalmol Otolaryngol. 1982 Spring;35(1):24-30.

Nothing to Disclose: SRM, VVG, MT, KM, YX, JVB

P3-455

Glucocorticoid Related Gene Expressions in the Liver of Calcium- or Magnesium-Deficient Rats.

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Objective: Altered peripheral glucocorticoid metabolism may be important in the pathogenesis of metabolic syndrome. The effects of glucocorticoids on glucose metabolism, such as an impaired peripheral glucose uptake or hepatic insulin resistance, are well characterized. There is strong evidence that glucocorticoid action underlies metabolic disease, largely from rodent obesity models. Furthermore, many epidemiologic studies have reported the link between calcium- or magnesium-deficiency and metabolic syndrome. We have examined whether calcium- or magnesium-deficiency in rats induces the changes in glucocorticoid metabolism.

Methods: Female Wistar rats were weaned onto very low calcium diet (0.008% calcium), magnesium deficient (0.003% magnesium) or control diets (0.9% calcium, 0.082% magnesium). Quantitative real-time PCR was used to assess messenger RNA for 11 β hydroxysteroid dehydrogenase-1 (11 β -HSD1), 11 β hydroxysteroid dehydrogenase-2 (11 β -HSD2), phosphoenolpyruvate carboxykinase (PEPCK), peroxisome proliferators activated receptor α (PPAR α), and glucocorticoid receptor (GR) in liver taken from calcium- or magnesium-deficient rats. Concentrations of adiponectin and insulin were determined in fasting plasma using rat-specific enzyme-linked immunosorbent assay.

Results: After 2 weeks, no difference in blood pressure was observed among the groups. Magnesium deficient rats showed increased heart rate and lesser body weight gain. Calcium deficient rats showed lower adiponectin. In magnesium deficient rats, insulin levels were increased, while no difference in serum glucose level was observed. The expression of 11 β -HSD1 mRNA in the liver was increased in calcium deficient rats, but not in magnesium deficient rats. The expression of hepatic PEPCK was down regulated in magnesium- or calcium-deficient rats. GR, PPAR α and 11 β -HSD2 expressions showed a similar tendency without significant intergroup differences.

Conclusions: We observed a lower expression of PEPCK, which involves first committed step in hepatic gluconeogenesis, in the liver of calcium- or magnesium-deficient rats suggesting a possible compensatory mechanism to diminish the glycogenesis. Low magnesium diet may trigger hyperinsulinemia in consequence of insulin resistance. Low calcium diet alters glucocorticoid metabolism, predicting hepatic up-regulation of 11 β -HSD1, which may be a key mechanism inducing the metabolic complications of calcium deficiency.

Nothing to Disclose: JT, AI, YO, KK

P3-456

Hepatic Stellate Cells Express AKT1 and AKT3 but Not AKT2.

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Hepatic stellate cells (HSC) are liver perisinusoidal cells that store vitamin A and triglycerides and play a role in maintaining vascular tone. They are regarded as the principal cell type responsible for the development and progression of liver fibrosis. Although Akt is known to play major role in the proliferation of HSC, the relative expression and the contribution of the three Akt isoforms during HSC activation and collagen production remained to be determined. In this communication we used human HSC obtained from morbidly obese patients undergoing bariatric surgery. HSC were isolated and cultured as previously described. Using quantitative RT-PCR and Western analysis with isoform specific antibodies we determined the expression of the three isoforms during culture activation and also after treatment of the cells with acetaldehyde. We show that akt1 and akt3 but not Akt 2 are expressed in stellate cells . We further show that Akt1 is substantially more abundant than Akt3. When HSC were activated in culture the levels of akt1 increased during the first 10 days of differentiation. During incubation with acetaldehyde, a known fibrogenic agent for HSC, Akt1 levels remained constant, while akt3 levels doubled. In summary, our findings demonstrate that Akt1 and Akt3 are expressed in HSC and that the expression of Akt3 is up-regulated during culture activation and after incubation with acetaldehyde.

Sources of Research Support: NIH: 5R01AA009231-17.

Nothing to Disclose: FK, MR

P3-457

Increased Renal Gluconeogenesis in a Rat Model of Intrauterine Growth Restriction.

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We have shown that low-sodium diet given to dams during the last week of gestation consistently results in giving birth to intrauterine growth restricted (IUGR) offsprings. This model is characterized by a significant decrease in maternal circulating volume, a reduced uterine arcuate artery diameters and decreased placental weights implying a reduction in placental perfusion. In addition, IUGR fetuses showed a significant increase in brain and heart masses relative to body weight whereas kidney-to-body ratio remained unvaried suggesting a redistribution of a preferential blood flow to the brain and heart. DNA microarray analyses of fetus kidney showed an overexpression of gluconeogenic enzymes in the IUGR group compared to control. Although the liver is the main organ in maintaining plasma glucose levels, the kidney can contribute to more than one third of the whole body glucose production. In this view, we hypothesize that the kidney in the IUGR fetus will show a significant increase of the gluconeogenesis providing the glucose production required compensating the diminished placental perfusion. The specific aim of this study was to measure the expression of gluconeogenic enzymes in the liver and the kidney of the IUGR fetus. During the last week of gestation, female Sprague-Dawley rats received a normal diet or a low-sodium diet (0.03% sodium). On Day 22 (term=23 days), animals were sacrificed, kidneys and livers were quickly removed and snap-frozen. Protein expression of the gluconeogenic enzymes was measured by western blot. Aldolase B, fructose-1,6- biphosphatase (FBPase) and glucose-6-phosphatase (G6Pase) were significantly increased in the IUGR fetal kidneys compared to controls, whereas PEPCK-C was unchanged. Most of the protein expression was affected by the sex of the rats. Indeed, the expression of FBPase and G6Pase was more marked in IUGR male kidneys while aldolase B showed an overexpression in IUGR female kidneys. On the other hand, gluconeogenic enzymes did not show any significant change in the IUGR liver. Since protein expression of the studied enzymes is increased in the fetal IUGR kidney, an elevated renal gluconeogenesis is more certain to be present. The kidney will then provide a support to the hepatic glucose homeostasis for the IUGR fetuses, in order to insure the basal metabolic rate, thus the fetus survival. The sex of the fetus has an impact on the ezymes expression.

Nothing to Disclose: EK, MB

P3-458

Mitochondrial Mutations in Tumors Arising in a Transgenic IGF-1 Mouse Model of Mammary Tumorigenesis.

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Warburg originally observed that malignant cells have elevated levels of aerobic glycolysis and proposed that defects in mitochondrial oxidation drive cancer cells to utilize the alternative lactate-producing pathway. Recently, there has been renewed interest in Warburg and it has been suggested that the shift from oxidative to glycolytic pathways is a fundamental property of cancer cells and represents a critical step in transformation. Several studies have identified mutations in mitochondrial DNA (mtDNA) in human neoplasms, including breast cancer, implicating mtDNA damage in carcinogenesis. As there are few reports of mitochondrial mutations in animal models of cancer, we evaluated mtDNA in mammary tumors arising in BK5.IGF-1 transgenic (Tg) mice. Previous studies with this model revealed that 31% of the females develop spontaneous mammary tumors by 1 year of age. Following DMBA treatment, 74% of the BK5.IGF-1 mice develop mammary adenocarcinomas compared to 29% of the wild types. Using massively redundant, high-throughput sequencing, we analyzed the mitochondrial genome of 13 Tg DMBA-induced tumors, 1 Tg vehicle tumor, 5 WT DMBA-induced tumors and 3 control mammary glands, including samples from virgin and pregnant WT and Tg mice. Nine of 13 (69%) Tg DMBA-induced tumors had a total of 23 different mitochondrial mutations with some tumors having more than one mutation. Among the 23 mutations, 14 were deletions of one or more adenosine residues in various poly-A repeats in the mt genome, while 9 were point mutations. The vehicle-treated Tg tumor had 6 different mitochondrial gene mutations. Of the 5 WT DMBA-induced tumors analyzed, 3 (60%) had mt mutations, with one tumor having more than one mutation. Three of these mutations in WT DMBA-induced tumors were point mutations and one was a deletion of two adenosine residues. Mutations in the mouse mammary tumors were predominantly found in the Cox 1, ND 2, ND 4 and ND 6 genes. None of the control mammary glands had detectable mitochondrial mutations. Our results demonstrate the presence of mitochondrial mutations in mammary tumors arising in a metabolically related animal model of mammary cancer. The profile of mtDNA mutations in tumors in our model is comparable with reports of mutations found in human tumors, suggesting that animal models like this one are useful for studying the role of altered glucose metabolism and mitochondrial mutations in the pathogenesis of mammary cancer.

Sources of Research Support: R01 CA104114 from NCI/NIH and BC07436 from the DOD Breast Cancer Research Program (to RFY).

Nothing to Disclose: CJC, TB, JT, GJ, IL, RF-Y

P3-459

Treating Diabetes in Mice with an Implantable Bioartificial Pancreas.

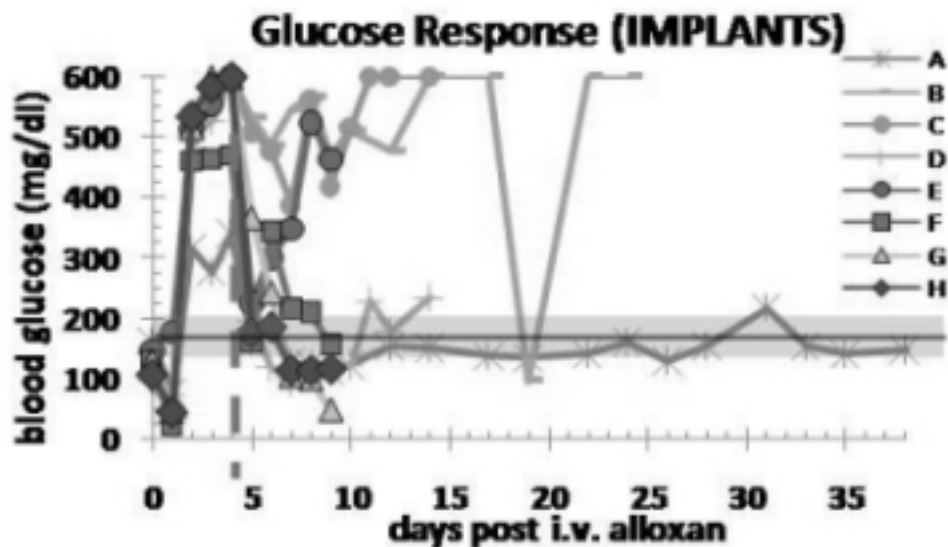
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Introduction: Mouse models of diabetes offer fertile ground to study β -cell regeneration, effects of hyperglycemia on organ systems, and cures. Rendering an animal diabetic is easy: maintaining it in hyper- or normoglycemic states for extended periods is difficult, but necessary for our experimental goals. Our therapeutic approach to this end is the implantable bioartificial pancreas; a construct comprised of insulin-secreting cells entrapped in bioinert materials. When implanted, cells in the construct sense glucose and secrete insulin appropriately.

Methods: Female C3H/HeN mice (n=8) were rendered diabetic by one i.v. injection of alloxan (62.5 mg/kg). Once diabetic (3 consecutive days fasting blood sugar >300 mg/dl), constructs containing β TC-tet insulinoma cells were crafted through a proprietary process, implanted i.p., and fasting blood sugars were followed. Animals with sustained blood glucose >600, or >15% mass loss were euthanized (B,C), and the construct, eyes, liver, pancreas, heart & kidneys removed for histology.

Results: Figure 1 illustrates this ongoing study.



Alloxan induces hypoglycemia 24 h post administration (7 of 8 mice) due to β -cell death and insulin dumping. Thereafter, hyperglycemia occurs, until corrected by construct implantation (on day 4). To date, all animals show reduced fasting blood sugars immediately post-implantation. Five animals exhibit recovery to near normal levels (A,D,F,G,H), three others maintained a hyperglycemic state for an extended time (B,C,E). On day 19 post alloxan, animal B exhibited a drop indicative of insulin dumping due to construct cell death. After euthanasia, minimal immune response to the implant (e.g., fibrotic overgrowth) was noted.

Conclusions: These preliminary studies indicate our immune-acceptable implantable constructs regulate glucose levels of diabetic mice for extended periods critical for diabetes research studies. The promise for clinical applications is enticing. Work focuses on improving construct efficacy, determining if β -cell regeneration is occurring in the damaged pancreata, and observing effects of extended hyperglycemia (in those deemed 'failures') on target organs.

Sources of Research Support: NIH Grant RO1 DK47858 awarded to NES.

Nothing to Disclose: NES, MMC, KSA, JAR, MJB

P3-460

Age-Related Metabolic Dysfunction in Mice Is Attenuated by Treatment with a Form of Soluble Activin Receptor Type IIB.

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Control over metabolic homeostasis declines with advancing age and is associated with an increase in body adiposity and a loss of muscle mass. Low muscle mass, high adiposity, and poor glycemic control are all markers which independently predict functional decline and increasing dependency in the elderly. Therefore, changes to body composition which increase lean mass, decrease adiposity, and improve metabolic function might ameliorate the loss of independence in the elderly. Several ligands of the activin receptor type IIB (ActRIIB), including myostatin, have been implicated in the regulation of muscle mass and may also modulate adipose tissue mass. Previous studies in normal mice have demonstrated that administration of a non-signaling, decoy ActRIIB promotes a gain of muscle mass and a loss of fat mass. To determine whether treatment with a soluble ActRIIB could offset the aging-induced changes in body composition and metabolism, we examined the effects of RAP-435, a fusion protein comprised of an optimized extracellular domain of ActRIIB linked to a murine Fc, in aged mice. In this study, 76-week old male C57BL/6 mice were treated with either vehicle (VEH) or 10mg/kg RAP-435 (RAP) for 8 weeks. Changes in body composition were measured longitudinally across the study by NMR scanning. Over the course of the study, the RAP cohort gained a significant amount of muscle mass (+19.6%), while the VEH cohort lost lean tissue mass relative to baseline (-2.74%; $p < 0.0001$). Concurrently, the RAP cohort lost significantly more fat mass (-44.3%) compared with the VEH cohort (-18.2%; $p < 0.0001$). Additionally, after 8 weeks of treatment, glycemic control (as measured by Hemoglobin A1C testing) was significantly improved in the RAP cohort (4.06% RAP versus 4.38% VEH; $p < 0.005$). Finally, serum insulin levels were also reduced in the RAP cohort relative to the VEH cohort (1530 pg/mL VEH versus 902 pg/mL RAP; $p < 0.05$). Taken together, these data support the hypothesis that a soluble ActRIIB would be efficacious in attenuating the metabolic decline and changes in body composition which are associated with normal aging.

Nothing to Disclose: LH, JLL, RSP, MS, JS

P3-461

Repair of Defective Glucagon Counterregulation (GCR) in Insulin Deficiency: An *In Silico* Study.

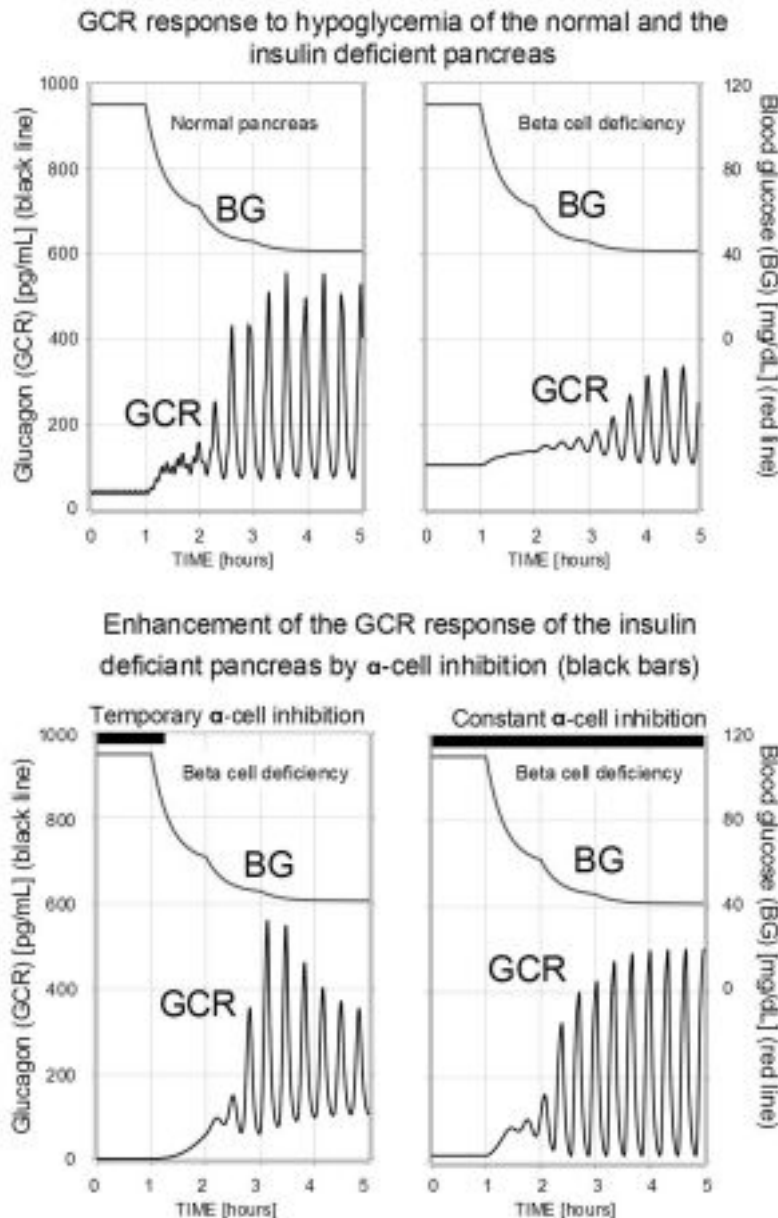
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Background. GCR is a key protection against hypoglycemia compromised in insulinopenic type 1 diabetes. Our recent work (1-3) suggests that suppression of hyperglucagonemia, particularly reducing basal glucagon may repair defective GCR (4). This *in silico* study tests different infusion strategies using α -cell inhibitors (ACI) to test their ability to repair defective GCR.

Methods. We use a differential equation-based mathematical model of GCR, which assumes that α -cells are suppressed by blood glucose (BG), β -cells, and auto-feedback. This model replicates the GCR control mechanism and its impairment in diabetes (3). We used the construct to simulate a response to hypoglycemia and design ACI infusion strategies to repair GCR assuming different ability of these signals to suppress basal and pulsatile glucagon.

Results. Figure 1 (top left) shows the normal GCR response to hypoglycemia and the defective GCR in the insulin-deficient pancreas (top right). If a signal suppresses basal rather than pulsatile glucagon, repair of GCR is possible by a constant ACI (bottom right). If a signal suppresses both basal and pulsatile glucagon, GCR repair is possible either by a low-rate constant infusion (not shown) or by switching off a higher rate infusion (temporary ACI) when BG reaches the range of 70-80 mg/dL (bottom left). Early (before the onset of BG decline) or late switch-off were less effective. Repair of GCR is possible only with signals that can inhibit basal glucagon.



Conclusion. We tested whether α -cell inhibitors can repair defective GCR. Signals which suppress basal rather than

pulsatile glucagon were the most effective. GCR repair is also possible by signals which suppress both basal and pulsatile glucagon, but they may need to be switched off during hypoglycemia. The results emphasize the need to study experimentally different α -cell inhibitors to determine the extent to which they suppress basal and pulsatile glucagon. Such studies would allow future simulations to design GCR repair strategies to be used in artificial pancreas design for protection against hypoglycemia.

- (1) Farhy LS, Du Z, Zeng, Veldhuis PP, Johnson ML, Brayman KL, McCall AL. Amplification of pulsatile glucagon secretion by switch-off of α -cell suppressing signals in Streptozotocin (STZ)-treated rats. *Am J Physiol Endocrinol Metab*, 2008; 295: E575 - E585.
- (2) Farhy LS, McCall AL. System-Level Control to Optimize Glucagon Counterregulation by Switch-Off of α -Cell Suppressing Signals in β -Cell Deficiency. *Journal of Diabetes Science and Technology*, 2009;3(1):21-33
- (3) Farhy LS, McCall AL. Pancreatic network control of glucagon secretion and counterregulation. *Methods in Enzymology*, Dec, 2009, Vol. 467:547-81 (in press).
- (4) Farhy LS, McCall AL. Inhibition of Basal Glucagon Secretion May Repair Defective Glucagon Counterregulation (GCR) in β -Cell Deficiency. American Diabetes Association 68th Scientific Sessions, San Francisco, CA, 2009

Sources of Research Support: NIH grants DK072095; AG032555; RR019991.

Nothing to Disclose: LSF, ALM

P3-462

Nebivolol Attenuates Ultrastructural Glomerular Hypertrophy and Restores Podocytopenia, Proximal Tubule Mitochondria Fragmentation, and Basal Polarity in the Zucker Obese Rat.

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Background: Obesity and insulin resistance are major determinants of kidney disease and proteinuria, largely as a result of maladaptive tissue remodeling at the level of the glomerular filtration barrier and the proximal tubule cell. Nebivolol, a beta-1 antagonist capable of increasing bioavailable endothelial derived nitric oxide, has been shown to improve insulin sensitivity and albuminuria. Thereby, we sought to investigate whether nebivolol improves proteinuria through restoration of abnormal ultrastructural glomerular and proximal tubule remodeling in young Zucker obese (ZO) (fa/fa) rats which demonstrate insulin resistance, hypertension and albuminuria between the ages of six and nine weeks of age.

Methods: Six week old male Zucker obese (ZO) and age-matched Zucker lean (ZL) rats were treated with nebivolol (10 mg•kg⁻¹•day⁻¹) for 21 days. At nine weeks all animals were sacrificed and ultrathin sections were obtained from the left kidney for ultrastructural analysis utilizing transmission electron microscopic (TEM).

Results: ZO rats displayed increases in proteinuria concurrent with glomerular remodeling which consisted of hypertrophy with dilated capillaries, widened Bowman's space, and podocytopenia. Podocytes contained increased perinuclear mitochondria (Mt) and increased protein and lipid inclusions. Concurrently, the proximal tubule cell(s) (PTC) demonstrated mitochondrial fragmentation with spherical changes and loss of elongation. There was a loss of basilar invaginating canalicular plasma membrane infoldings and overall a loss of basal polarity in the PTCs. Additionally, there was evidence of an early tubulointerstitial inflammatory reaction with intracapillary and interstitial mononuclear cell accumulation. These outcomes were largely abrogated with nebivolol treatment.

Conclusions: The ZO model demonstrated concurrent abnormal ultrastructural glomerular and proximal tubule remodeling at nine weeks of age, which was attenuated and/or restored with three weeks of nebivolol treatment.

Sources of Research Support: NIH and VA Merit to JRS and CDA-2 for AWC and Forest Research Institute.

Nothing to Disclose: MRH, JH, DE, YY, ATW-C, JRS

P3-463

Higher Complement C4 Protein Levels Are Positively Correlated with β -Cell Preservation in New Onset Type 1 Diabetes Mellitus.

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Background: Type 1 diabetes mellitus has been extensively characterized as a T-cell mediated autoimmune disease. Complement proteins function in both adaptive and innate immune defense and patients with complement C4 deficiencies are prone to developing autoimmune diseases. However, the role of complement in the development of type 1 diabetes has not been thoroughly and accurately investigated.

Objective: The aim of this study is to explore the relationship between complement C3 and C4 protein concentrations and C-peptide levels in new onset type 1 diabetes patients.

Methods: We studied 20 patients of European ancestry, ages 4 to 16 years (10.85 ± 3.12 , mean \pm SD) with recently diagnosed type 1 diabetes mellitus at Nationwide Children's Hospital in Columbus, Ohio. The diagnosis of type 1 diabetes was confirmed by the presence of positive diabetes associated antibodies. Complement C3 and C4 protein concentrations were elucidated by radial immunodiffusion using EDTA-plasma. At 1 month post type 1 diabetes diagnosis, both fasting C-peptide and stimulated C-peptide levels were measured during a standardized mixed meal tolerance test.

Results: Type 1 diabetes patients with higher complement C4 protein concentrations at diagnosis were more likely to have higher levels of fasting C-peptide ($p=0.003$; $F=12.23$, $R^2=0.40$) and stimulated C-peptide ($p=0.05$; $F=4.36$, $R^2=0.20$) at 1 month post diagnosis. There was no apparent relationship between complement C3 protein concentration and fasting C-peptide ($p=0.44$; $F=0.63$; $R^2=0.03$) or stimulated C-peptide levels ($p=0.71$; $F=0.14$; $R^2=0.01$).

Conclusion: Our findings show that complement C4 protein levels are positively correlated with C-peptide levels in recently diagnosed type 1 diabetes patients indicating that greater β -cell function are preserved in patients with higher C4 protein levels. These results suggest that high complement C4 protein levels may be a protective modifier in type 1 diabetes mellitus.

Nothing to Disclose: SEK, RPH, YLW, CYY

P3-464

Nebivolol Attenuates Renal Renin-Angiotensin System-Dependent Oxidative Stress in Zucker Obese Rat.

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Rationale: Insulin resistance is associated with activation of the renin-angiotensin system (RAS), increases in NADPH oxidase-dependent generation of reactive oxygen species (ROS) and tissue damage. In this context, nebivolol, a β 1-adrenergic receptor antagonist has been reported to improve insulin sensitivity and reduce NADPH oxidase-dependent ROS. Thereby, we hypothesized that nebivolol would attenuate RAS-mediated oxidative stress and tissue damage in the kidney of the insulin resistant, hypertensive Zucker Obese rat.

Methods: Six week old male Zucker obese (ZO) and age-matched Zucker lean (ZL) rats were treated with nebivolol (10 mg•kg⁻¹•day⁻¹) for 21 days followed by measurement of markers of renal cortical RAS activation (Ang II and AT₁R expression levels), renal oxidative stress (NADPH oxidase subunit protein expression, 3-nitrotyrosine labeled proteins), podocyte (nephrin and desmin) and proximal tubule cell-specific proteins (megalin and LAMP-2), and generalized metabolic abnormalities (proteinuria).

Results: Zucker obese rats displayed significant increases in proteinuria in relation to 1) increases in immunologic intensities of Ang II and the AT₁R indicative of RAS activation, 2) increases in NADPH oxidase subunits (Nox2, Rac1, and p47^{phox}) along with 3-nitrotyrosine indicative of oxidative stress, and decreases in nephrin, desmin, megalin and LAMP-2, indicative of podocyte and proximal tubule cell dysfunction. These outcomes improved with with nebivolol treatment.

Conclusions: In conclusion, these data suggest that nebivolol treatment in the insulin resistant, hypertensive ZO rat improves proteinuria through improvements in RAS-dependent generation of ROS and in glomerular podocyte and proximal tubule integrity.

Sources of Research Support: NIH and VA Merit to JRS and CDA-2 for AWC and Forest Research Institute.

Nothing to Disclose: JH, AW-C, MRH, RDT, RIS, MSJ, JMR, NR, JRS

P3-465

Effects of Intermittent Hypoxia on Blood Pressure, Serum Plasminogen Activator Inhibitor 1 and Glucose Metabolism in Male Sprague Dawley Rats.

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Sleep apnea is the most common sleep disorder in the United States affecting up to 20% of the population and has been linked to hypertension, insulin resistance, inflammation, endothelial dysfunction and atherosclerosis. Interestingly, these conditions are also associated with high levels of serum Plasminogen Activator Inhibitor 1 (PAI-1) and with adverse cardiovascular and metabolic outcomes. Many aspects of Sleep apnea can be simulated in vivo by exposing rats to intermittent hypoxia (IH). Using this animal model, we hypothesized that rats exposed to IH would develop hypertension, increased PAI-1 and impaired glucose tolerance.

Adult male Sprague Dawley rats underwent surgery to implant femoral artery catheters. All experiments were started at least one week after surgery. Rats were exposed for 7 hrs during sleep to either IH (20 short exposures to 5% oxygen each hour) or air cycles (21% oxygen) for 2 weeks. Fasting glucose, insulin, and plasma PAI-1 were measured weekly. Intraperitoneal glucose tolerance tests (IPGTT) were performed at baseline and repeated weekly.

After 14 days, IH rats had elevated systolic blood pressure (Air= 100 mm Hg +/-3, IH=110 mm Hg +/-2, p<0.05, n=10), higher plasma PAI-1 levels compared to air rats (Air = 1.4 ng/mL +/- 0.1, IH = 2.3 ng/mL +/- 0.3, p = 0.0153, n = 6) and lower average body weight (Air=338.5 grams, n=4; IH= 284.66 grams, n=12). Despite the fact that there were no significant differences in fasting blood glucose or intraperitoneal glucose tolerance test IPGTT responses, there was a trend to higher fasting insulin and lower glucose to insulin ratio at wk 1 and 2 compared to baseline suggestive of increased insulin resistance after IH exposure (0.0047 at week 0, n=5; 0.01 at week 1, n=4, p=0.13; 0.007 at week 2, n=3, p=0.21).

These data demonstrate that short term intermittent hypoxia, in male Sprague Dawley rats, short term IH to simulate adult obstructive sleep apnea and leads to mild hypertension, elevated plasma levels of PAI-1 and a trend towards a hyperinsulinemic state. This rat model of experimentally induced sleep apnea independent of obesity may represent an innovative tool to further evaluate the relationship between intermittent hypoxia sleep apnea, metabolic dysfunction and cardiovascular disease.

Nothing to Disclose: LEA, XD, KLS, BRW, KMC, NLK

P3-466

Impact of Glucocorticoids upon Lipogenesis and β -Oxidation in Skeletal Muscle.

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Glucocorticoid excess is characterized by increased adiposity, skeletal myopathy and insulin resistance. Although there is a strong inverse correlation between intramuscular triglyceride (IMTG) levels and insulin sensitivity, the impact of glucocorticoids upon the processes that regulate skeletal muscle lipid metabolism has not been explored in detail. Mouse C2C12 skeletal myocytes were grown to confluence and differentiated into myotubes in chemically defined media. Expression of key components of the lipogenic and β -oxidation pathways were examined by RT-PCR. Functional impact of glucocorticoids upon *de novo* lipogenesis (ACC activity) was assessed by measuring 1-[¹⁴C]acetate incorporation into intramuscular lipids and β -oxidation was assessed by measuring the accumulation of [³H]water following 9,10-[³H]palmitate treatment. Experiments were performed using synthetic glucocorticoid dexamethasone (DEX) in the presence and absence of insulin.

C2C12 myotubes treated with DEX has reduced expression of the key lipogenic genes: FAS (0.63-fold, $p < 0.01$), ACC1 (0.79-fold, $p < 0.05$) and GPAT (0.69-fold, $p < 0.05$). The effects of DEX was reversed when cells were coincubated with the glucocorticoid receptor antagonist RU38486. Endorsing these findings, *de novo* lipogenesis was decreased by DEX in a dose dependent manner (2.9 ± 0.04 vs. 1.9 ± 0.03 [5nM] vs. 1.3 ± 0.02 dpm $\times 10^4$ [500nM], $p < 0.05$). DEX conversely increased the rate of β -oxidation (25.8 ± 2.1 vs. 27.7 ± 2.9 [5nM] vs. 28.9 ± 3.1 dpm $\times 10^4$ [500nM], $p < 0.05$). Importantly, an increase PDK4 expression was observed with DEX (11-fold, $p < 0.001$) and reversed by RU38486, suggesting metabolic switching from glucose to fatty acids as fuel.

In the presence of insulin (5nM), low dose DEX was without effect upon lipid accumulation (3.1 ± 0.05 vs.

3.2 ± 0.06 dpm $\times 10^4$ [5nM], $p = ns$), but decreased β -oxidation (26.5 ± 2.8 vs. 25.7 ± 4.2 [5nM] vs. 22.0 ± 2.9 dpm $\times 10^4$ [500nM], $p < 0.05$). Moreover, insulin inhibited DEX induced PDK4 expression (7.77-fold [Ctrl vs. 500nM DEX] vs. 3.35-fold [Ctrl+insulin vs. 500nM DEX+insulin], $p < 0.05$), suggesting that insulin reduces the ability of DEX to stimulate lipid metabolism.

These data highlight the impact of glucocorticoids to decrease lipid accumulation and increase β -oxidation in the absence of insulin. By contrast, in the presence of insulin, they act together to promote IMTG accumulation, potentially contributing to reduced insulin sensitivity.

Nothing to Disclose: SAM, LLG, IJB, PMS, JWT

P3-467

The Comparison of Peripheral Nerve Damage According to the Glucose Control Period in the Experimental Diabetes.

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Besides just tight glucose control, early intensive therapy has been reported to be more important for the prevention of diabetic micro-and macro-complication. However, it was not known exactly about the quantitative difference according to the timing delay in the glucose control and whether early period control is really better than late control in the diabetic peripheral neuropathy. Therefore, in this study we investigated the effect of timing difference in glucose control on the peripheral nerves in the course of diabetes. The five groups (6-8 number in each group) comprised : Normal glucose rats (Normal), rats with hyperglycemia (designated: DM), rats with glucose control for entire 28-week period (designated: INS (W0-28)), rats with glucose control for early 14-week period followed by hyperglycemia for late 14 weeks (designated: INS (W0-14)), and rats with hyperglycemia for early 14 weeks followed by glucose control for late 14-week period (designated: INS (W15-28)). In the results, the current perception threshold (CPT) was more reduced in INS (W0-28) and INS (W15-28) group compared with INS (W0-14) or DM group ($P<0.05$). Mean myelinated axon area was larger significantly in INS (W0-28) and INS (W15-28) group (63.5 ± 2.32 and 60.1 ± 2.14 μm) than INS (W0-14) or DM group (55.5 ± 2.81 or 51.5 ± 2.64 μm) ($P<0.05$) and intraepidermal nerve fibers (IENF) density was less reduced significantly INS (W0-28) and INS (W15-28) group (6.9 ± 0.46 and 6.8 ± 0.11) than INS (W0-14) or DM group ($5.9.5\pm 0.32$ or 5.3 ± 0.39) ($P<0.05$). More increased trend of nerve fiber quantity was also observed in INS (W0-28) group than INS (W15-28) group although there was no significant difference. Our results indicate that continuous glucose control is necessarily important to alleviate the peripheral nerve damage and moreover poor glycemic control during the later period prone to aggravate the neuropathy is more harmful than inappropriate early period management. Therefore, besides earlier management, the importance of continuous glucose control including later period of diabetes should also be emphasized in the diabetic peripheral neuropathy.

Nothing to Disclose: HYJ, TSP, HSB, EDJ, JHP, KAL

P3-468

Metabolic Consequences of High-Fat Cafeteria Diets in Rats: Comparison of Ad Libitum and Pair Feeding Settings.

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“Western-” or “Cafeteria-style” diets (Caf) are high in fat and carbohydrates (CHO) and characterized by a high caloric density. Prolonged consumption leads to weight gain, fat accumulation and associated metabolic complications like insulin resistance. Therefore, these diets are frequently considered to be “unhealthy”. We aimed to dissect whether the dietary macronutrient composition or the high caloric density of these diets causes the detrimental effects. Wistar rats were fed either standard chow (CH; n=15) or a Caf diet [% of metabolizable energy (ME), fat/protein/CHO: CH 18.8/19.8/68.8, ME: 16.1MJ/kg; Caf 65.6/19.7/20.5, ME:20.3MJ/kg]. Caf was offered in 2 different feeding regimes: One group was pair-fed isoenergetic amounts to CH (CafPF; n=8), the other group had ad libitum access to the Caf diet (CafAL; n=5). After 3 weeks on the respective diets, i.p. glucose tolerance tests (i.p.GTTs) were performed. After 4 weeks, rats were sacrificed, truncal blood was collected and fat pads were excised and weighed. Compared to CH, mean daily food intake was 1.3g higher in CafAL, which translates to a 29.7% higher daily energy intake. CafAL rats showed the highest bodyweight gain and CafPF the lowest (CH: +14.5±4.3%, CafPF: +9.1±2.1%, CafAL: +27.3±5.7%, CH vs. CafPF/CafAL: p<0.01; CafPF vs. CafAL: p<0.01). Lean body mass was not significantly increased with CafAL, but significantly lower in CafPF. Compared to CH, fat pads were heavier with CafAL (inguinal +72.2%; perirenal +110.3%; epididymal +67.4%; p<0.001), but lighter in CafPF (vs. CH: inguinal -33.3% (p<0.01); perirenal -21.2% (p<0.05); epididymal -10% (n.s.)). After 4 weeks, fasting blood glucose was not different between the groups. However, insulin concentrations were significantly higher in CafAL (CH: 0.69±0.31ng/mL, CafPF: 0.4±0.08ng/mL, CafAL: 0.89±0.32ng/mL, CafPF vs. CafAL, p<0.05). Initial analysis of i.p.GTTs revealed no significant differences in area under the curve for glucose (insulin pending). In summary, ad libitum consumption of a high-fat Caf diet, increased fat accumulation and led to higher fasting insulin levels. In contrast, isoenergetic pair-feeding of the Caf diet neither affected fat pad weights, nor fasting insulin levels. Thus, Caf diets per se seem not to be “unhealthier” than standard low-fat diets. We conclude, that the overconsumption of calories, related to the high energetic density of Caf diets, rather than the macronutrient composition causes detrimental effects in rats.

Nothing to Disclose: DM, MB, BJMS, MB

P3-469

Low-Carbohydrate/High Fat Diets: Effects on Glucose and Insulin Metabolism in Rats.

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Low-carb/high fat (LC-HF) diets have been suggested to lead to weight loss in adults and to exert beneficial effects on glucose and insulin metabolism. To explore this hypothesis in an animal model, we pair-fed male Wistar rats ($n > 8$ /group) isoenergetic amounts of 2 different LC-HF diets or standard rodent chow (CH) diet (% of metabolizable energy, fat/protein/CHO: LC-HF-1 (66/33/1), LC-HF-2 (94.5/4.2/1.3) and CH (9/33/58)). After 3 weeks of feeding oral and intraperitoneal glucose tolerance tests (oGTTs, i.p.-GTTs) were performed ($n = 4$ /group). Rats were sacrificed after 4 weeks, blood samples stored for further analyses and fat pads extracted and weighed. Blood glucose was measured by glucose-oxidase method and plasma insulin was measured by immunoassay (Linco, USA). Bodyweight was significantly ($p < 0.05$) lower with LC-HF-2 when compared to CH (CH: 405 ± 21 g, LC-HF-1: 405 ± 10 g, LC-HF-2: 375 ± 17 g). Absolute epididymal and perirenal fat pad weights were higher with both LC-HF diets; as were serum leptin concentrations ($p < 0.05$ vs. CH). Whereas fasting glucose levels were significantly reduced in LC-HF-2, fasting insulin levels were significantly higher with LC-HF-1, but lower with LC-HF-2 (CH: 0.3 ± 0.16 ng/mL; LCHF-1: 0.46 ± 0.13 ng/mL; LC-HF-2: 0.15 ± 0.07 ng/mL; $p < 0.05$). The same was true for serum C-peptide levels (CH: 657 ± 66 pM; LC-HF-1: 1007 ± 95 pM; LC-HF-2: 491 ± 79 pM; $p < 0.05$), whereas glucagon concentrations were not significantly different. During oGTTs, area under the curve (AUC) for glucose was significantly higher in LC-HF-2 group. AUC for plasma insulin was not different between CH and LC-HF-2, but about 2-fold higher with LC-HF-1. Surprisingly, preliminary data from i.p.GTTs indicate that only the protein matched LC-HF-1 diet leads to higher glucose concentrations, potentially suggesting that LC-HF diets might affect incretin hormones. In summary, both LC-HF diets led to accumulation of visceral fat mass. However, while rats fed the protein matched LC-HF-1 diet showed insulin resistance, rats fed the LC-HF-2 diet low in protein appear to be very insulin sensitive despite of high fat mass. We conclude that effects of LC-HF diets on glucose and insulin metabolism in rats are dependent on the relative abundance of dietary macronutrients - especially protein - but not on the diet induced unfavourable changes in body composition per se. In contrast to what might have been expected, the extreme LC-HF-2 diet seems to protect against development of insulin resistance.

Nothing to Disclose: MB, DM, BJMS, MB

P3-470

Hyperglycemic Response to Oxidative Stress in SHROB Rats Can Be Attenuated by an Antioxidant from Garlic.

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Oxidative stress is an underlying process causing cellular damage and disruption of homeostasis, leading to aging and possibly diabetes. Many claims have been made for therapeutic actions of antioxidants based on in vitro quenching of free radicals, but clinical trials of antioxidant supplements have not confirmed benefits. We sought to determine the in vivo antioxidant activity of a representative dietary antioxidant from garlic in an animal model of human disease. The spontaneously hypertensive obese (SHROB) rat is a model of prediabetes and metabolic syndrome characterized by normal fasting and high postprandial glucose, severe insulin resistance, hyperinsulinemia, hypertension and dyslipidemia. We hypothesized that oxidative stress can induce hyperglycemia in SHROB rats, and that selected antioxidants may block this effect. SHROB rats (n = 23) were subjected to increased oxidative stress via a combination of pro-oxidant agents: hydroquinone, a source of superoxide radicals, and L-buthionine sulphoximine, a glutathione depleting agent (both 50 mg/kg ip) following a 4h fast. In prior research, this pro-oxidant cocktail after 5d of daily injections of SHROB induced sustained fasting hyperglycemia (234 ± 29 versus 93 ± 6 mg/dL in vehicle treated control SHROB). A subset of SHROB (n = 10) were also injected with the antioxidant diallyl sulfide (50 mg/kg ip), an active component of garlic oil. Tail blood was obtained under local anesthesia and glucose was measured by glucometer (One-Touch Ultra). Plasma insulin was assayed by using rat insulin ELISA kits (ALPCO, Salem, NH). Pro-oxidant injections of SHROB rats increased glucose levels relative to baseline or to lean SHR controls at 30, 60, 120 and 180 min after injection, with a peak response at 30 min (net increase of 55 ± 8 mg/dL). Pro-oxidant injections had no significant effect on insulin levels. Diallyl sulfide attenuated the hyperglycemic response (31 ± 10 mg/dL at 30 min) without affecting insulin levels. We conclude that oxidative stress evokes hyperglycemia by impairing insulin action. Diallyl sulfide attenuated the hyperglycemic response without affecting insulin, implying that this antioxidant may counter insulin resistance. Diallyl sulfide is an effective antioxidant and may prevent the induction of hyperglycemia by oxidative stress.

Sources of Research Support: Endocrine Society Summer Research Fellowship (to D. Hou) and Ophthalmology Education Worldwide (to R.J. Koletsky and P. Ernsberger).

Nothing to Disclose: DH, BC, LS, RJK, PE

P3-471

Expression of Myostatin and ActRIIB in Diabetics Rats Subjected to Exercise.

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Aims: The objective of this study was to determine the influence of exercise in the expression of myostatin (MSTN) and ActRIIB receptor in fat and muscle in rats with diabetes mellitus (DM) induced by streptozotocin. **Methods:** Adult male Wistar rats were housed under controlled conditions (20-22^o C, 10-14h light-dark cycle) and were allowed free access to standard rodent chow. The DM was induced from 60mg/Kg to the streptozotocin. The blood glucose was determined in tail blood, using portable glucometer and soon after the diabetics animals received insulin injection in alterned days. After 2 weeks, the group CG and DG were randomly divided into 4 groups assigned to a swimming training group (CGE and DGE) or a sedentary group (CGS and DGS). CGE and DGE swam individually in water tanks (50x30cm) at 34^o C, for 45 minutes at 0900h and 1700h, 5 day week⁻¹, for 4 weeks. After this period, rats were decapitated. White gastrocnemius muscle and fat pads were dissected, weighted, immediately cooled in liquid nitrogen and stored at -70^o C for subsequent analysis. MSTN mRNA was quantified by real time RT-PCR. The animals were maintained according to the local University Committee guidelines for the care and use of laboratory animals. **Results:** The blood glucose of the animals of groups EC and ED was significantly lower than the blood glucose of the groups SC and SD respectively. In group SD, the expression of MST increased significantly in muscle and in subcutaneous fat and decreased significantly in the BAT, compared to the group SC. In animals of the group EC there were significant reduction in the expression of MST and ActRIIB in BAT when comparing to the group SC. In the group ED, occurred significantly decrease of MST in subcutaneous fat and significantly increase in mesenteric fat. The expression of ActRIIB in group SD was significantly higher in muscle, BAT and mesenteric fat, compared to group SC. The expression of ActRIIB was significantly lower in muscle and BAT of group ED compared to group SD. In conclusion, the results show that the expression of MST and ActRIIB changes in muscle and fat tissues in diabetic animals subjected to exercise.

Sources of Research Support: Fapesp and CNPQ.

Nothing to Disclose: DB, PGB, HSSdA, KON, AMdOL

P3-472

Antioxidant Effects of Indian Medicinal Plants on Blood and Seminal Plasma Enzymes of High Glucose Fed Rats.

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Oxidative stress (OS) due to hyperglycemia has been suggested in the blood and semen of diabetic male (1). Brahmi (*Bacopa monniera*) and Triphala (an Indian ayurvedic medicine, composed of equal parts of three plants: *Terminalia chebula*, *T. belerica*, and *Emblica officinalis*) have been shown to have significant antioxidant property (2-4). Our aim was to evaluate the effects of Triphala (Tr), Brahmi (Br) as well as of their combination on activities of the catalase and glutathione peroxidase (GPx) (antioxidant enzymes) in red blood cells (RBC) and on sorbitol dehydrogenase (SDH) in semen of high glucose fed rats. The rats were divided into 8 groups containing 6 rats in each group.

| Group | Details of feeding |
|-------|--------------------|
| 1 | Lab diet (C) |
| 2 | Glu+c |
| 3 | Br+c |
| 4 | Tr+c |
| 5 | Tri+Br+c |
| 6 | Br+gl +c |
| 7 | Tr+ glu + c |
| 8 | Tr+ Br + glu+c |

C= control, Glu=Glucose

All rats were given lab feed and water ad libitum. Rats in glucose diet group were given high glucose diet consisting of 50 grams glucose added to each 100 grams of feed. The rats in Brahmi (or Triphala or Brahmi and Triphala groups) were orally administered Brahmi (and/or Triphala) powder (3.6 mg/ 100g body weight /day) dissolved in 0.9% normal saline during every morning for 30 days. After 30 days feeding the weights of the rats were taken and deprived of food overnight. The next day the rats were sacrificed. Enzymes activities were measured in RBC and seminal plasma. Our results showed significant decreased activity of catalase in high glucose fed rats compared to rats on lab feeds; significant increase in activity of antioxidant enzymes (catalase and GPx) and decrease in activity of SDH in semen after Triphala and Brahmi administration.

Comparison of enzyme activities between groups

| Group | Catalase | GPx | SDH |
|-------|-----------|-----------|-----------|
| 1&2 | P<0.001 ↓ | NS | NS |
| 1&3 | P<0.001 ↑ | P<0.001 ↑ | P<0.001 ↓ |
| 1&4 | P<0.001 ↑ | P<0.01 ↑ | P<0.001 ↓ |
| 1&5 | P<0.001 ↑ | P<0.001 ↑ | P<0.001 ↓ |
| 1&6 | P<0.001 ↑ | P<0.001 ↑ | P<0.02 ↓ |
| 1&7 | P<0.001 ↑ | NS | P<0.001 ↓ |
| 1&8 | P<0.001 ↑ | P<0.001 ↑ | NS |

Triphala- Brahmi combination had higher effect than Triphala or Brahmi alone. Increase in activity of catalase and glutathione peroxidase while decrease in activity of SDH after Triphala and Brahmi administration suggests beneficial role of these herbs on hyperglycemia induced OS.

- (1) Ceriello A., *Diabetes Care* 2003, 26(5):1589-1596.
- (2) Naik GH et al., *Phytother Res* 2005, 19(7):582-586.
- (3) Russo A et al., *Phytother Res* 2003, 17(8):870-875.
- (4) Sandhya T et al., *Mutat Res* 2006, 609(1):17-25.

Nothing to Disclose: NRD, AV, SS, PD, JR, AA

P3-473

Effect of Antioxidant Diet on Expression of Renal NOS Isoforms and Splicing in Male Obese Zucker Rats.

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Previous investigations of diabetic nephropathy (DN) have shown a major role of decreased renal nitric oxide (NO) production and increased oxidative stress in kidney damage. The aim of this study was to observe the expression of three NO synthase (NOS) isoforms and test the effect of an antioxidant-fortified (AO) diet during the progression of DN in obesity-related type 2 diabetes mellitus (T2bDM).

Male obese Zucker rats (fa/fa), as a model of T2bDM, were studied at 6, 13 and 20 weeks of age, on a regular (REG) or AO diet. Glomerular filtration rate (GFR) was measured and NOS isoforms (n-neuronal, e-endothelial and i-inducible) analyzed with Western blots. There was a significant difference in GFR on the regular diet between 6 and 13 weeks and between 13 and 20 weeks with the 13 week values being lower than either 6 or 20 weeks. The AO diet prevented the decrease in GFR at 13 weeks. NOS protein levels were higher in several groups given the AO diet; eNOS and nNOS were higher than on the REG diet at 6 and 20 weeks and iNOS was higher only at 6 weeks. With age, levels of eNOS and nNOS increased and became significantly higher at 20 weeks on both diets. Levels of iNOS went up with age only in the group on AO diet, but not on REG diet. This is a possible explanation of the protective effect on GFR at 13 week in males on AO diet. Multiple monomeric splice forms, up to 7 for eNOS, 6 for iNOS and 9 for nNOS were detected; 3-5 dimeric forms were also detected for each isoform. A greater number of monomer splice variants of eNOS and nNOS were observed in the AO diet groups compared to REG diet groups at 6 and 20 weeks. This did not result in a difference in total amount of dimers. In the case of iNOS, a decreased prevalence of dimers was observed at 20 weeks in animals on AO diet with no change in monomeric splice forms. Conclusion: The AO diet corresponds to an increase in eNOS and nNOS early on (6 weeks) and a decrease in presumably active dimeric iNOS at the later stage (20 weeks). The early differences in enzyme levels may have contributed to the relative preservation of GFR at 13 weeks.

Sources of Research Support: NIH R15 DK073066 to SRI and FVN.

Nothing to Disclose: YS, SSI, FVN

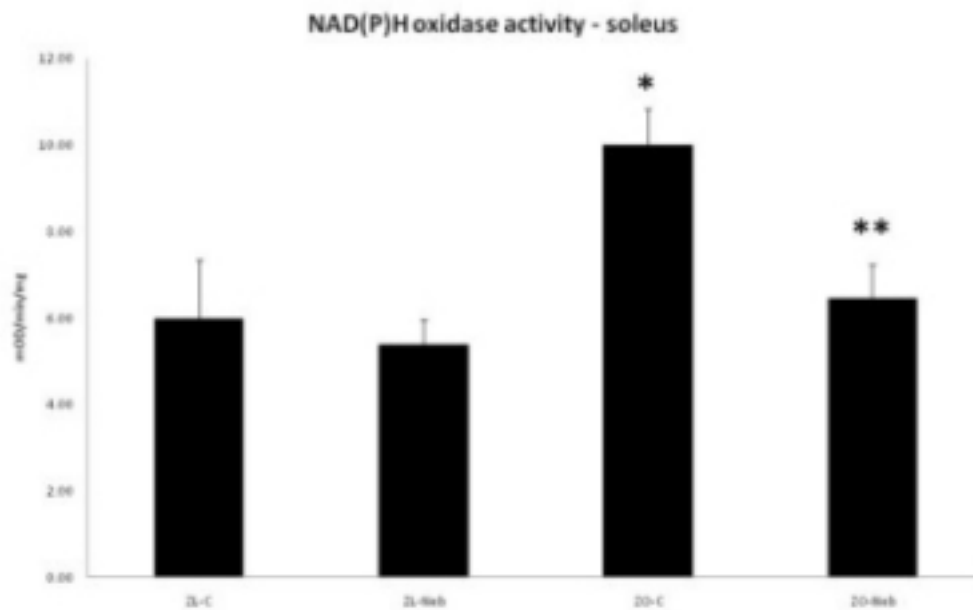
P3-474

Nebivolol Reduces Oxidative Stress and Improves Insulin Resistance in Skeletal Muscle in the Zucker Obese Rat.

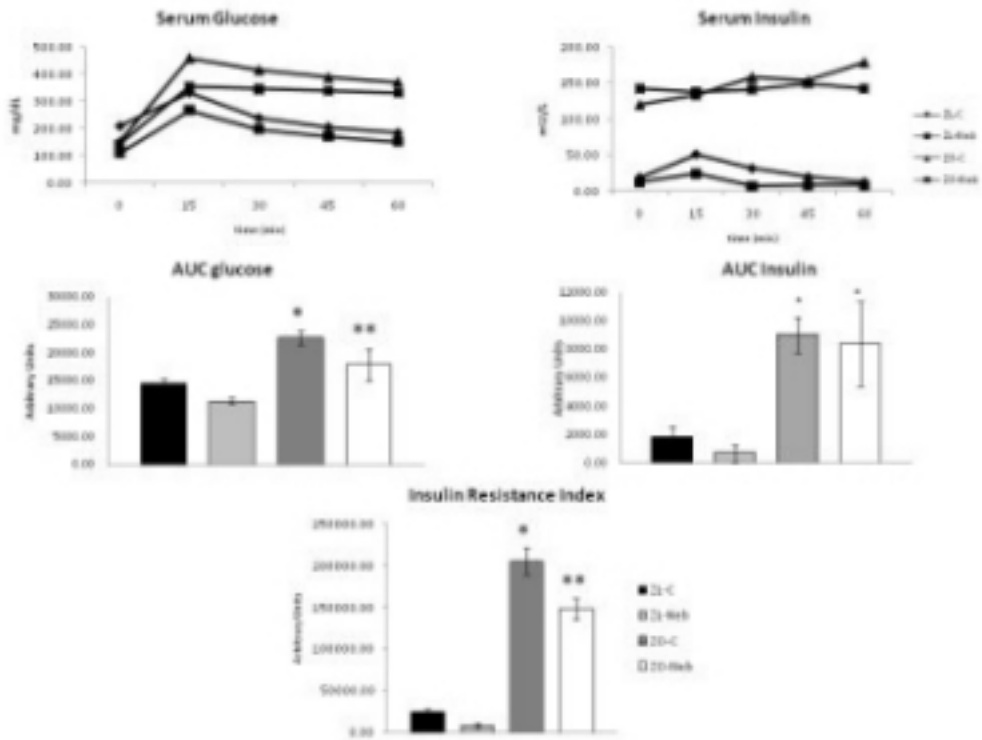
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Insulin resistance is linked to obesity, hypertension and increased oxidative stress. Use of β -blockers in hypertension is controversial due to influences on weight, lipids, and glucose metabolism. Nebivolol is a selective β_1 blocker with antioxidant and vasodilatory activities that has a neutral impact on insulin sensitivity. We studied the role of nebivolol on NADPH oxidase activity, whole-body insulin sensitivity and glucose transport in skeletal muscle in the Zucker Obese rat (ZO), which exhibits obesity, insulin resistance, and hypertension. Six week old male Zucker Obese rats and age-matched Zucker Lean (ZL), were treated with nebivolol 10mg/kg/day for 21 days or placebo. Systolic blood pressure (SBP) was measured by the tail-cuff method, and an Intravenous Glucose Tolerance Test (IVGTT) was performed at the end of treatment. In soleus samples, we assessed NADPH oxidase activity by spectrophotometry and insulin-stimulated 2-deoxyglucose uptake. Results are presented as means \pm SEM. SBP was higher in ZO compared to ZL (162 ± 12.24 vs 147 ± 7.25 mm Hg, $p > 0.05$), and was reduced by nebivolol (126 ± 4.6 mm Hg, $p < 0.05$). NADPH oxidase activity was increased in ZO relative to ZL, and was significantly reduced by nebivolol.



The Area Under the Curve (AUC) for glucose and the insulin Resistance Index (IRI) ($AUC_{\text{Glucose}} \times AUC_{\text{Insulin}}$) were increased in ZO compared to ZL, and were significantly reduced by nebivolol.



Incremental insulin-stimulated glucose uptake was reduced in ZO relative to ZL controls (0.05 ± 0.003 vs 0.13 ± 0.06 mmol/mg/20min, $p > 0.05$), and was non-significantly increased by nebivolol (0.067 ± 0.02). In conclusion, our data demonstrate that nebivolol modulates oxidative stress and improves systemic insulin resistance (with a trend towards improved glucose uptake in skeletal muscle), in a rodent model of the Metabolic Syndrome.

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Nothing to Disclose: CMMA, GLG, JRS

P3-475

Comprehensive Upstream Regulatory Exploration Reveals That cMyc Is a Key Regulator of Proliferation in Both Commonly Employed Rat Insulinoma Cell Lines.

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Human β -cell lines do not exist. Accordingly, rodent, e.g., rat (INS1, RIN) and mouse β -cell cell lines are widely substituted as model systems for human β -cells. Surprisingly, the fundamental mechanisms driving proliferation in these 4 rodent cell lines are not known. Prior work suggested that 7 cell cycle regulators are increased: 3 "early G1/S molecules" (cyclin D3, cdks 4,6), together with 4 "late G1/S molecules" (cyclins A,E, cdks 1,2). Forced overexpression of these 7 molecules in normal rat β -cells reproduces the accelerated replication phenotype of rodent insulinoma cells. Collectively, these observations suggest that the 7 molecules are responsible for driving rodent insulinoma replication. However, the mechanisms responsible for the upregulation of these 7 molecules are unknown.

We surveyed possible upstream regulatory pathways for the 7 G1/S molecules in INS1 and RIN, and compared them to normal rat islets. The 4 late G1/S molecules (cyclins A/E, cdks 1,2) displayed marked increases both in their respective mRNAs as well as proteins, suggesting that they are activated transcriptionally, likely as a result of activation by events upstream in the pRb pathway, such as E2F activation.

In marked contrast, the 3 early G1/S molecules (cyclin D3, cdks 4,6) showed no increases in mRNA, but in each case appeared to result from increases in protein synthesis or stability, suggesting that upstream signaling pathways serve to augment these molecules at the protein level. Accordingly, we surveyed multiple potential upstream regulatory pathways in insulinoma vs. normal rat islets. No evidence of activation of MAPK, PI3K, PKB/Akt, CREB, PKC, Jun, JunK, STAT3, SMAD, cAbl, cSrc, FoxO1 or β -catenin pathways was identified. In marked contrast, striking (6-fold) upregulation of cMyc was reproducibly observed in INS1 and RIN, both at the protein and mRNA levels. cMyc gene amplification was absent. Importantly, pharmacologic inhibition of cMyc transcription using the cMyc-MAD inhibitor, 10058-F4 (1RH), reduced proliferation (³H-thymidine) rates in INS1 and RIN cells near to those observed in rat islets, indicating that cMyc is required for driving pathological INS1 and RIN proliferation.

Thus, cMyc is central in driving pathological proliferation in rat INS1 and RIN insulinoma lines. Elucidating the mechanisms responsible for cMyc overexpression in rodent insulinomas may reveal clues as to how best to develop continuously growing human β -cell lines.

Nothing to Disclose: EK, KT, GH, JK, FS, IC-C, AG-O, DS, AFS

P3-476

Examining the Role of CTGF in Regulating β -Cell Replication.

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CTGF (connective tissue growth factor) is a secreted protein that modulates TGF- β , BMP, and Wnt signaling. In the pancreas, CTGF is expressed in embryonic β cells, ductal epithelium, and pancreatic vasculature but is silenced in β cells, after birth. In other cell types, CTGF plays a role in cell migration and cell proliferation, as well as vasculogenesis. We previously showed that CTGF is required for proper allocation of endocrine progenitor cells to the different hormone-producing lineages and also for embryonic β cell proliferation. CTGF null mutant mouse embryos have increased a cell mass and a concomitant reduction in β cell mass compared to wild-type embryos. CTGF is re-expressed in maternal β cells during pregnancy suggesting that it plays a role in β cell proliferation under physiologically stimulatory conditions. We hypothesize that CTGF over-expression in adult mice of various ages will increase proliferation and subsequently β cell mass. To test this hypothesis, we have generated mice in which CTGF over-expression in β cells is doxycycline-dependent. CTGF expression will be induced with doxycycline at various ages to test whether β cell replication is enhanced, and whether CTGF can function in this capacity even in older islets that are more resistant to proliferative stimuli. In addition, CTGF will be induced in the setting of islet transplantation to determine whether this factor would be beneficial for β cell survival and islet revascularization. We anticipate that enhanced CTGF expression will promote sustainability of islet transplants.

Nothing to Disclose: UG, MAG

P3-477

Prolactin and Glucose Act in Concert To Induce beta Cell Replication: Co-Induction of Prolactin Receptor Gene Expression.

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The growth and function of pancreatic beta cells are controlled by nutrients, cytokines, growth factors, and hormones including the lactogens produced by the pituitary gland (prolactin, PRL) and placenta (placental lactogen). PRL enhances glucose-stimulated insulin secretion (GSIS) through induction of beta cell glucose transport, glucokinase, and pyruvate dehydrogenase activity. Here we examined the interactions of PRL and glucose in the control of beta cell replication in isolated rat islets. PRL increased 3H-thymidine incorporation 47.3% and 59.8% respectively in islets incubated in 2.5mM and 5.5 mM glucose (p<0.05). However, PRL stimulated a 2-fold increase in 3H-thymidine incorporation (p<0.001 vs its effects in 2.5 or 5.5 mM glucose) in islets incubated in 17.8 mM glucose; thus, glucose potentiated the effect of PRL on islet replication.

The synergistic effects of glucose and PRL on beta cell replication may be explained in part by glucose induction of beta cell PRL receptor (PRLR) expression: glucose increased by 2-5-fold the mRNA levels of the PRLR and potentiated the effect of PRL. The PRLR is encoded by a gene with distinct V1 and V2 promoters; only the isoform containing the V2 promoter is induced by glucose. PRL increased binding of insulinoma cell nuclear proteins to oligonucleotides containing a consensus STAT5 sequence at -190 bp of the V2 promoter; in contrast, neither glucose nor insulin up-regulated STAT5 in insulinoma cells. These findings suggest that glucose may enhance PRL action in beta cells by induction of PRLRs through a STAT5-independent mechanism.

These findings suggest novel interactions between glucose and lactogens in the control of beta cell growth and function. PRL increases glucose uptake and metabolism, facilitating nutrient action, while glucose facilitates PRL action through induction of PRLR expression and acts in synergy with PRL to promote beta cell replication.

Sources of Research Support: NICHD, ADA, Duke Children's Miracle Network, Pfizer Corporation.

Nothing to Disclose: DF, RA, EH, MF

P3-478

Protein Kinase C (PKC) Zeta (ζ) Is Required for Glucose-Induced Beta Cell Proliferation In Vitro and In Vivo.

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PKC ζ activation is essential for growth factor-induced mitogenesis in INS-1 cells and mouse primary beta cells in vitro. Glucose is a well-known beta cell mitogen that enhances PKC ζ activation in MIN-6 cells. However, whether activation of PKC ζ is required for glucose-mediated beta cell proliferation is unknown. Glucose increased PKC ζ phosphorylation (2-3-fold) in a dose- and time-dependent manner in INS-1 cells. Exposure to 20mM glucose for 24h increased INS-1 cell proliferation 2-fold. Importantly, downregulation (70-80%) of PKC ζ , but not PKC λ , the other atypical PKC family member, eliminated glucose-induced proliferation. Furthermore, expression of kinase-dead PKC ζ (KD-PKC ζ) in INS-1 cells blocked glucose-mediated proliferation. In mouse and human islets, 20-25mM glucose induced atypical PKC phosphorylation, and expression of KD-PKC ζ inhibited glucose-mediated beta cell proliferation. Collectively, these results indicate that activation of PKC ζ is required for glucose-induced beta cell proliferation in vitro. To address whether this is the case in vivo, we generated transgenic mice in which the rat insulin II promoter (RIP) drives expression of KD-PKC ζ in beta cells (RIP-KD-PKC ζ mice). RIP-KD-PKC ζ transgenic mice (TG) at 2-3 months of age displayed normal body weight, blood glucose, plasma insulin, glucose tolerance and insulin tolerance in basal conditions. Pancreatic weight, beta cell proliferation and beta cell mass were also similar in TG and normal (NL) mice. Therefore, PKC ζ activity is not required for glucose and beta cell homeostasis in basal conditions. To determine whether PKC ζ activity is required for glucose-induced beta cell replication, we infused glucose (Glu; 2.5 g/kg/hr) or saline (Sal; matched volume) intravenously for 4 days in 10-12 week male TG and NL littermates. Mice receiving glucose showed significantly ($p < 0.01$) increased blood glucose (mg/dl; 106 ± 5 (NL-Sal), 103 ± 2 (TG-Sal), 141 ± 9 (NL-Glu) and 135 ± 2 (TG-Glu)) and plasma insulin (ng/ml; 0.8 ± 0.1 (NL-Sal), 0.8 ± 0.1 (TG-Sal), 2.4 ± 0.3 (NL-Glu) and 1.3 ± 0.3 (TG-Glu)). Importantly, the significant increase in beta cell proliferation observed in NL mice infused with glucose (5.0-fold, $p = 0.02$) was decreased in hyperglycemic TG mice (1.6-fold, $p = ns$). These results suggest that activation of PKC ζ is required for glucose-mediated compensatory beta cell proliferation in vivo in mice. Inadequate PKC ζ activation is a potential mechanism driving beta cell failure in diabetes.

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Nothing to Disclose: LCA, SV-G, JLP, TCR, DAH, SRV, BZ, LR, TT, CPO, AG-O

P3-479

Systemic Administration of Parathyroid Hormone-Related Peptide (PTHrP) 1-36 Enhances β -Cell Proliferation and Increases β -Cell Mass in Adult Mice.

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Diabetes results from a deficiency of functional β -cells. Therefore, a major focus in the treatment of diabetes is to identify small molecules that enhance endogenous β -cell mass and function. PTHrP is a strong candidate in this regard as: 1) PTHrP and its receptor are expressed in the β -cell, 2) PTHrP enhances rodent and human β -cell function, proliferation, and survival in vitro, as well as in vivo in PTHrP-overexpressing transgenic mice, 3) only 36 amino acids of PTHrP are necessary to manifest its salutary effects, 4) and PTHrP(1-36) is currently in clinical trials for the treatment of osteoporosis, demonstrating its safety.

This study examines whether short-term systemic administration of PTHrP(1-36) can induce beneficial effects on the β -cell in vivo. Normal eight-week old male BalbC mice were injected subcutaneously with 40, 80, or 160 μ g of PTHrP(1-36) peptide/Kg body weight, or vehicle as control, 5 days/week for 4 weeks. There were no changes in body weight, blood glucose, or insulin sensitivity, or incidence of hypercalcemia with all three PTHrP doses. In the second week, PTHrP significantly improved glucose tolerance in a dose-dependent manner, as well as increased plasma insulin to 2.5 ± 0.5 ng/ml at the 160 μ g PTHrP dose vs 1.1 ± 0.1 ng/ml in control mice. By week four, the glucose tolerance in the PTHrP-treated mice was completely normalized; however, plasma insulin was significantly higher in both the 40 and 160 μ g PTHrP-treated mice vs controls.

All three doses of PTHrP significantly increased β -cell proliferation by two-fold, resulting in a 30% increase in β -cell mass at the 80 and 160 μ g doses. β -cell replication was also enhanced in the first week of treatment with 160 μ g of PTHrP. No major changes were observed in islet or β -cell size, β -cell death, or expression of differentiation and cell cycle markers at the mRNA level, after four weeks of PTHrP treatment. However, cyclinD2 protein, an activator of the G1/S phase of the cell cycle, was increased, whereas p16, an inhibitor, was decreased, in islets from PTHrP-overexpressing transgenic mice compared to normal mice.

These results clearly indicate that normal mice treated with PTHrP(1-36) peptide show an initial improvement in β -cell function, and a significant persistent increase in β -cell proliferation, resulting in enhanced β -cell mass. Whether systemic administration of PTHrP(1-36) can improve diabetes outcomes in models of Type 1 and Type 2 diabetes is currently being investigated.

Sources of Research Support: National Institutes of Health (DK078060 and DK072264) to RCV; JDRF Research Awards (1-2008- 46) to RCV.

Disclosures: AFS: Member, Osteotrophin LLC.

Nothing to Disclose: KRW, DKA, SJG, HL, NKG, XYZ, AB, AG-O, RCV

P3-480

Elevated Circulating Free Fatty Acids Increase Cell Cycle Inhibitors p16 and p18 and Block Glucose-Induced beta Cell Replication In Vivo in Mice.

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Elevated circulating free fatty acids (FFA) increase the risk of type 2 diabetes, independent of BMI. We hypothesized that FFA might reduce compensatory beta cell replication in vivo. C57bl/6j male mice (n=11-12/group) were infused intravenously with heparin plus saline (SAL), Liposyn II (LIP), glucose (GLU), or Liposyn II + glucose (L+G) for four days. Mice receiving lipid showed increased plasma FFA (Table 1; p<0.001). Mice receiving glucose showed increased blood glucose (p<0.001) and plasma insulin (p<0.05).

| Table 1 | SAL | LIP | GLU | L+G |
|------------------------|---------|---------|---------|---------|
| FFA (mM) | 1.6±0.2 | 3.7±0.3 | 1.1±0.1 | 4.3±0.2 |
| Blood Glucose (mg/dl) | 104±2 | 83±4 | 138±3 | 139±3 |
| Plasma Insulin (ng/ml) | 1.2±0.1 | 0.7±0.1 | 2.1±0.3 | 2.3±0.6 |
| % BrdU (+) Beta Cells | 1.0±0.2 | 1.3±0.2 | 2.7±0.4 | 1.3±0.3 |

Surprisingly, elevated FFA did not alter the blood glucose and plasma insulin attained by glucose infusion. Elevated glucose and FFA did not increase beta cell apoptosis, as determined by TUNEL staining. However, elevated FFA completely blocked the glucose-induced increase in beta cell replication (Table 1; p<0.01). In hyperglycemic mice, we observed a clear dose-dependent inhibition of beta cell replication with increasing circulating FFA (Table 2).

| Table 2 | GLU No heparin | GLU w/ heparin | L+G w/ heparin |
|-----------------------|----------------|----------------|----------------|
| Blood glucose (mg/dl) | 136±3 | 138±3 | 139±3 |
| FFA (mM) | 0.3±0.1 | 1.1±0.1 | 4.3±0.2 |
| % BrdU(+) beta cells | 4.6±0.9 | 2.7±0.4 | 1.3±0.3 |

As previously shown, immunoblot analysis of islets from GLU mice showed up-regulated cyclin D2 expression. Cyclin D2 was also up-regulated in islets from L+G mice, suggesting that FFA does not block replication by interfering with cyclin D2 induction. Therefore, we hypothesized that FFA might inhibit beta cell proliferation by increasing cell cycle inhibitors. Cip/Kip proteins p21 and p27 were not altered in islets from infused mice. Intriguingly, Ink proteins p16 and p18 were significantly (p<0.05) increased in islets from L+G mice. In summary, no evidence of classical glucolipotoxicity (insulin secretion deficit or beta cell apoptosis) was observed in this model of short term hyperglycemia with increased circulating FFA. However, elevated FFA completely inhibited glucose-induced beta cell replication, possibly through induction of cell cycle inhibitors p16 and p18. This may represent a novel lipotoxicity mechanism that contributes to failure of compensatory beta cell expansion in people at risk for diabetes.

Sources of Research Support: NIH grants DK076562 and DK046204 and a Junior Scholar Award from the Department of Medicine at the University of Pittsburgh to LCA.

Nothing to Disclose: LCA, JLP, DAH, TCR, LR, BZ, CPO, AG-O

P3-481

In Vivo Glucose-Induced Beta Cell Replication Involves the Insulin Signaling Intermediate Insulin Receptor Substrate 2.

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Glucose, the primary physiologic insulin secretagogue, is an important beta cell mitogen. However, the contribution of insulin signaling to glucose-induced beta cell replication in vivo is completely unknown. Insulin Receptor Substrate (IRS) proteins are critical mediators of intracellular response to insulin. IRS2 is activated in INS-1 cells, rat islets, and mouse beta cells in response to glucose in vitro. We hypothesized that glucose-induced beta cell proliferation in vivo might require IRS2. To address this question, we induced hyperglycemia in mice mutant for IRS2 and assessed beta cell proliferation.

8-12 week old male mice, wild type (WT), heterozygous (HT), or knockout (KO) for IRS2 were catheterized and infused with 0.9% saline [Sal: WT (n=18), HT (n=20) or KO (n=15)] or 50% glucose [Glu: WT (n=22) or HT (n=19)] to determine whether haploinsufficiency or loss of IRS2 would impair glucose-induced beta cell replication. Baseline (saline-infused) four-day average circulating blood glucose (mg/dl) was similar in WT and HT mice [111±5 (WT-Sal), 111±4 (HT-Sal)] but was variably elevated in KO mice [range, 124-565, mean 322±43 (KO-Sal)]. Glucose infusion significantly increased blood glucose in WT and HT mice [134±4 (WT-Glu; p<0.01 vs. WT-Sal), 139±5 (HT-Glu; p<0.001 vs. HT-Sal; p=ns for WT-Glu vs. HT-Glu]. Saline-infused baseline plasma insulin (ng/ml) was slightly higher in HT than WT mice [1.2±0.1 (WT-Sal), 1.6±0.2 (HT-Sal, p<0.05)]. Glucose infusion significantly raised plasma insulin in WT and HT mice [2.6±0.4 (WT-Glu, p=0.001 vs. WT-Sal), 3.6±0.5 (HT-Glu, p<0.001 vs. HT-Sal), p=ns for WT-Glu vs. HT-Glu]. Plasma insulin in KO-Sal (1.9±0.3) was significantly higher than WT-Sal (p<0.05) but similar to WT-Glu mice (p=ns). Under these conditions, haploinsufficiency for IRS2 caused no significant deficit in glucose-induced beta cell replication [3.2±0.5% (WT-Sal), 2.5±0.4% (HT-Sal; p=ns vs. WT-Sal), 5.3±0.5% (WT-Glu; p<0.01 vs. WT-Sal), 4.5±0.6% (HT-Glu; p<0.01 vs. HT-Sal), p=ns between WT-Glu and HT-Glu]. In KO mice, despite higher blood glucose in this group, replication rates were significantly lower than WT-Glu mice [2.4±0.6% (KO-Sal); p<0.001 vs. WT-Glu]. When severely hyperglycemic (BG>200) KO mice were excluded, replication was still significantly lower than WT-Glu mice (2.7±0.9%; p<0.05). Taken together, these data provide the first evidence that insulin signaling may be required for in vivo glucose-induced pancreatic beta cell replication.

Sources of Research Support: NIH grant DK076562 and a Junior Scholar Award from the Department of Medicine at the University of Pittsburgh to LCA.

Nothing to Disclose: LCA, JLP, DAH, QJ, LR, BZ, CPO, AG-O

P3-482

Glucose Increases Human Beta Cell Proliferation In Vitro and In Vivo.

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Glucose is a well-documented mitogen for rodent beta cells, both in vitro and in vivo. However, the relevance of glucose as a pro-proliferative signal in human beta cells has been debated. To address this question, we performed a careful characterization of human beta cell replication in vitro, across a wide range of glucose concentrations and exposure duration. Human islets from 14 donors were dispersed to single cells, plated on coverslips, and exposed to 2, 5, 11 or 25mM glucose for 24, 48, 72 or 96 hours. Replication, determined by BrdU/insulin immunofluorescence, was significantly increased at 24h and 48h in high glucose ($0.30\pm 0.06\%$ (2mM) vs. $0.68\pm 0.16\%$ (11mM) at 24h; $0.33\pm 0.09\%$ (2mM) vs. $0.61\pm 0.11\%$ (25mM) at 48h); $p<0.05$. High glucose did not increase human beta cell replication at 72 or 96 hours exposure in vitro. To test whether physiologically elevated circulating blood glucose is mitogenic for human beta cells in vivo, human islets were transplanted into STZ-diabetic NOD-SCID mice and the mice were intravenously infused with glucose. Islets from 13 non-diabetic donors, ages 7-76 years and BMI 19.6-43.8 kg/m² were transplanted, into 2-4 mice per donor. Blood glucose was normalized in most recipients 3 weeks after transplant (412 ± 13 mg/dl at day 0, 145 ± 35 mg/dl at day 21). Transplanted mice were catheterized in the femoral artery and vein, allowed to recover for 3 days, and then infused with either 0.9% saline or 50% glucose for 4 days. Circulating blood glucose was moderately increased in mice infused with glucose (81 ± 13 mg/dl for saline vs. 110 ± 7 mg/dl for glucose, $p=0.05$). Circulating human insulin significantly increased over baseline in glucose-infused mice ($61\pm 11\%$ for saline vs. $203\pm 50\%$ for glucose, $p=0.03$). Immediately after infusion, human islet grafts were recovered, fixed, processed, and analyzed. Quantification of proliferation (immunofluorescence for insulin and BrdU) revealed a significant positive correlation between circulating blood glucose and graft human beta cell replication (ranges, 0.03% - 2.2% BrdU (+) and 49-255 mg/dl blood glucose; $p<0.05$ for correlation). Analysis of TUNEL (+) beta cells showed no relationship between blood glucose and human beta cell apoptosis (range 0.0%-1.1%, $p=ns$). In conclusion, we have determined that glucose acts as a mitogen for human beta cells, both in vitro and in vivo in a transplant setting.

Sources of Research Support: Juvenile Diabetes Research Foundation grant 5-2008-189 to LCA and AG-O.

Nothing to Disclose: HEL, TJC, JLP, DAH, NA, JMDM-G, TR, RJL, LR, BZ, AFS, CPO, AG-O, LCA

P3-483

β Cells Can Proliferate at an Extra-Pancreatic Site in an Insulin Resistance Model.

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Adaptations of the β cell to insulin resistance include increased insulin secretion and an increase in β cell number resulting from increased β cell proliferation. To test the hypothesis that the β cell responds to inductive signals associated with insulin resistance independently of the pancreatic environment, we transplanted 200 mouse islets, isolated from 6 week-old male wild type mice (wt/wt), under the kidney capsule of 6 week-old male insulin-sensitive, wt/wt and insulin-resistant, ob/ob recipients. Ob/ob mice receiving an islet transplant had similar non-fasting hyperglycemia (276 ± 20 vs 338 ± 21 mg/dl, $p > 0.05$, $n = 18-50$ measurements per group) as control ob/ob mice. Proliferation of β cells was analyzed by Ki67 labeling in the pancreas and islet grafts 3 and 10 weeks post-transplantation. Three weeks after transplantation, β cells in the pancreas of the ob/ob recipients had a greater proliferation rate compared to wt/wt recipients (1.92 ± 0.46 vs. 0.51 ± 0.21 , $p = 0.03$, $n = 5-7$ mice). In islet grafts removed 3 weeks after transplantation, there was a greater proliferation rate in wt/wt islets transplanted into ob/ob mice than those transplanted into wt/wt mice (2.75 ± 0.68 vs 0.53 ± 0.22 , $p = 0.02$, $n = 5-7$ mice). Similarly, 10 weeks after transplantation, the proliferation rate was greater in the endogenous islets (1.56 ± 0.10 vs. 0.23 ± 0.13 , $p < 0.01$, $n = 3$ mice) as well as transplanted islets into ob/ob recipients (1.43 ± 0.04 vs. 0.15 ± 0.15 , $p < 0.01$, $n = 3$ mice) compared to wt/wt controls. The β cell proliferation rate was similar in the native islets and islet grafts in both the insulin-sensitive and insulin-resistant models at both time points ($p > 0.05$). These results indicate that systemic factors in ob/ob mice stimulate β cell proliferation, suggesting that β cell proliferation can occur independently of the pancreatic environment.

Nothing to Disclose: IGP, CD, GP, MB, ACP

P3-484

GLP-1 Can Protect Proinflammatory Cytokines Induced beta Cell Apoptosis.

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Background: Diabetes mellitus is a metabolic disease caused by progressive impaired pancreatic beta cell. The etiology of diabetes mellitus is even less clearly understood. Proinflammatory cytokines has been known to one of the cause of diabetes mellitus. However, the exact molecular mechanism by which proinflammatory cytokines induce beta cell death are not clear. Glucagon like peptide-1 (GLP-1) is a new drug for treatment of type 2 diabetes and has effects on stimulation of insulin secretion and beta cell preservation. Also, it may have an antiapoptotic effect on β cells, but detailed mechanisms are not proven. Therefore, we investigated the protective effect of GLP-1 in ER mediated beta cell apoptosis by proinflammatory cytokines.

Methods: For induction of the ER stress, HIT-T15 cells (hamster β cell line) were treated with mixture of cytokines (TNF- α : 50 ng/mL, INF- γ : 50 ng/mL IL-1 β : 10 ng/mL). Apoptosis was evaluated with MTT assay, hoechst 33342 staining and Annexin/PI flow cytometry. Expression of ER stress-related molecules was determined by real-time PCR or western blot. For blocking ER stress, Nitric oxide was measured by griess reagent. Analysis of iNOS protein and iNOS mRNA were real-time PCR and western blot. iNOS protein degradation was evaluation by immunoprecipitation. we pretreated HIT-T15 cells with exendin-4 (Ex-4; GLP-1 receptor agonist) for 1 hour before stress induction.

Results: After induction with ER stress (mixture of cytokines), beta cells were lost by apoptosis. We found that Ex-4 had a protective effect through ER stress related molecules (GRP78, GRP94, CHOP) modulation and nitric oxide and Ex-4 stimulate the degradation of iNOS protein. Also, Ex-4 recovered the expression of insulin2 mRNA in β cells.

Conclusion: These results suggest that GLP-1 may protect beta cells apoptosis through the modulation of iNOS protein degradation.

Nothing to Disclose: DML, BJK, KYP, KWL, JYK, SWK

P3-485

Differentiation of Uterine Stem Cells into beta Pancreatic Cells.

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Introduction:

Pancreatic islet cell transplantation is an effective approach to treat type 1 diabetes but not widely used because of the severe shortage of transplantable donor islets

Human uterine endometrial stem cells are a potential source of multipotent mesenchymal stem cells of for tissue engineering and cell based therapies.

Objectives:

The aim of this study was to generate beta pancreatic cells from uterine mesenchymal stem cells. Endometrial cells from human biopsies were isolated and induced to differentiate into insulin secreting cells by an adjusted 3-step protocol.

Differentiation was evaluated by expression of insulin, glucagon, Hnf6a (Hepatocyte Nuclear Factor 6 alpha), PDX1 (Pancreatic and duodenal Homeobox gene-1), Ngn3 (Neurogenin 3), Pax4 (Paired box gene-4), β -actin and other genes implicated in the posttranslational metabolism of insulin such as prohormone convertases (PC1, PC2) and Carboxypeptidase E (CE).

Materials and Methods:

Endometrial tissue from 12 patients who underwent surgery for benign pathology were processed and plated in plastic flasks. After 2 passages cells were induced to differentiate into insulin secreting cells by an adjusted 3-step protocol. In step 1, the cell monolayer was treated for 24 hours with H-DMEM with 10% FBS and 10^{-6} M retinoic acid then the medium was changed to H-DMEM with 10% FBS for 2 days. In step 2, the cells were seeded on ECM gel and L-DMEM, supplemented with 10% FBS, 10 mM nicotinamide, 20 ng/ml epidermal growth factor, 300nM Indolactam-V and 50 ng/mL Fibroblast Growth Factor 10 (FGF10) for 12 days. In step 3, the medium consisted of L-DMEM with 10% FBS, 10 nM exendin-4 and Activin A 10ng/mL for 7 more days. Cellular differentiation was monitored by observation of islet-like cell clusters and gene expression using RT-PCR.

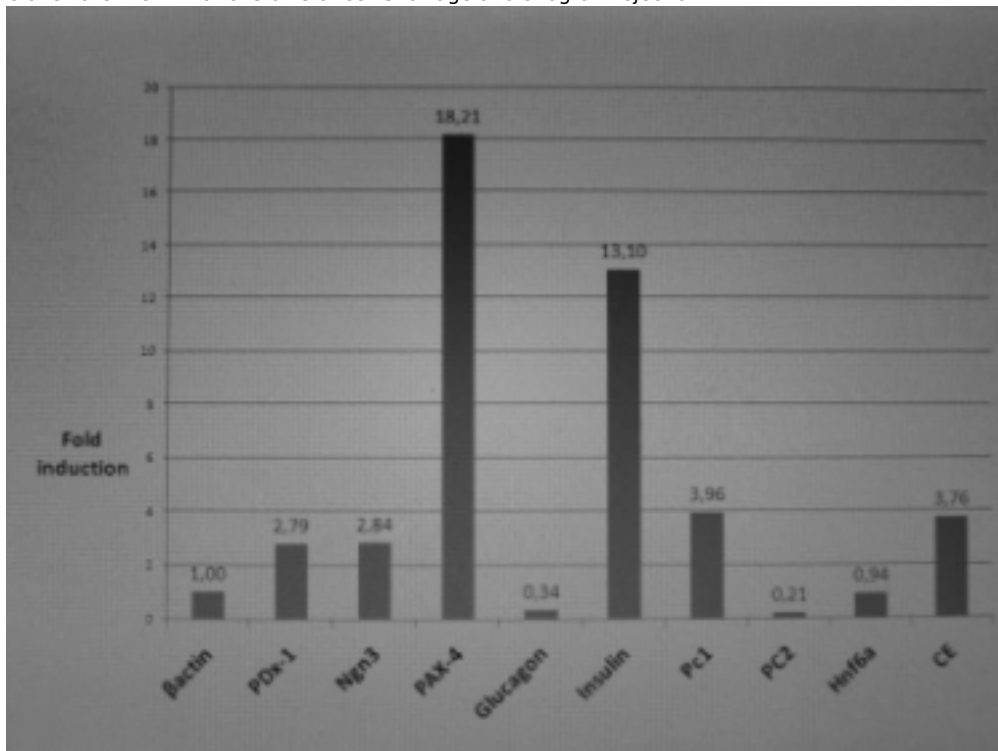
Results:

At Step 1 no changes in morphology were observed. In Step 2 cells formed islet-like cell clusters. After step 3 expression of insulin, PDX-1, Ngn 3 and PAX-4 were identified using qRT-PCR as shown in [figure 1].

Insulin was 13.10 fold more expressed in the treated cells as PAX-4 (18.40 fold).

Conclusions:

Endometrial stem cells can be induced to adopt a β -pancreatic cell endocrine phenotype, including insulin expression. These cells may be useful as a therapeutic tool in the treatment DM. This source of renewable stem cells has potential to alleviate the limitations of islet cell shortage and allograft rejection.



Nothing to Disclose: JS, EM, YF, HST

P3-486

The Thyroid Hormone T3 Improves Function and Survival of Rat Pancreatic Islets during In Vitro Culture.

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Ex vivo islet cell culture in the presence of stimulating factors prior to transplantation is considered a good strategy to contrast the short outcoming of islets transplantation. We previously demonstrated how T3 can increase beta cell function via specific activation of Akt; therefore we hypothesized that thyroid hormone T3 can be considered a promising candidate for the in vitro expansion of islet cell mass. Rat pancreatic islets have been isolated by the collagenase digestion and cultured in the presence or not of the thyroid hormone T3 10⁻⁷ M. Islets viability has been evaluated by the use of two different dyes, one cell-permeable green fluorescent dye and propidium iodide, and by the analysis of core cell damage upcoming. Moreover, islets function has been evaluated by insulin secretion. The ability of beta cells to counteract apoptosis induced by streptozotocin has been analyzed by TUNEL assay. We demonstrated that treatment of primary cultures of rat pancreatic islets with T3 results in augmented beta cell vitality with increase of their functional properties. Contemporary a sensible reduction of the core cell damage has been observed in the T3 treated islets, suggesting the preservation of the beta cells integrity during the culture period. Nonetheless, the insulin secretion is sensibly augmented after T3 stimulation. The strong increment we evidenced in Akt activation suggests the involvement of this pathway in the observed phenomena. In conclusion we indicate T3 as a good factor to improve ex vivo islets cell culture.

Nothing to Disclose: SM, CVF, CM, SR, AS, MT, VT

P3-487

Islet Innervation Is Regulated by VEGF-A Expression in the Developing Pancreas.

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Pancreatic islets contain abundant blood vessels and nerve endings, yet the mechanisms involved in the development of islet vascularization and innervation are incompletely characterized. Vascular endothelial growth factor A (VEGF-A) is an important regulator of islet vascularization and function, as genetic inactivation of pancreatic VEGF-A in mice reduces vascularization of the mature islet. To test the hypothesis that pancreatic VEGF-A expression during development also regulates islet innervation, we used genetic mouse models to either increase or decrease VEGF-A expression in the developing pancreas. To inactivate VEGF-A in the embryonic pancreas, we bred Pdx1-Cre and VEGF-A-loxP mice to generate VEGF^{Δpanc} mice. To induce beta cell-specific overexpression of VEGF-A, we administered doxycycline to pregnant dams of RIP-rtTA; Tet-O-VEGF-A bitransgenic (BiTg) mice starting at embryonic day 5.5 (E5.5). Pancreata were harvested at E16.5, E18.5, postnatal day 1 (P1), P7, and P21. Immunohistochemistry was performed on whole-mount embryonic tissue and postnatal pancreatic cryosections to label PECAM-1+ endothelial cells, class III beta-tubulin+ (TUJ1+) nerve fibers, and insulin+ beta cells or Pdx1+ pancreatic cells. Compared to controls, pancreata from VEGF^{Δpanc} mice had a reduced density of islet-associated PECAM-1+ endothelial cells at each time point, and fewer insulin+ cells. In contrast, pancreata from BiTg mice displayed a dramatic expansion of PECAM-1+ endothelial cells and disrupted endocrine cell clustering, but had no apparent change in the total insulin+ cell number. In control embryos, TUJ1+ nerve fibers were found at the periphery of cell clusters expressing high levels of Pdx1 (Pdx1^{HI}), and a TUJ1+ nerve network connected distinct clusters of Pdx1^{HI} cells. This network was not disrupted in VEGF^{Δpanc} mice at E16.5, E18.5, or P1. However, at P7 and P21, TUJ1+ nerve fibers penetrated the vascularized insulin+ cell clusters in control mice but not in VEGF^{Δpanc} mice. In contrast, pancreata of BiTg mice at P1 and P7 had an increased density of TUJ1+ fibers in hypervascularized pancreatic regions associated with VEGF-A-overexpressing beta cells. These data indicate that developing islets become innervated after becoming vascularized, and that islet innervation is regulated by the level of VEGF-A expression and/or formation of a normal vascular network in the developing endocrine pancreas.

Nothing to Disclose: RBR, QC, MB, ACP

P3-488

Silencing Epigenetic Modifications Regulate Tissue Specific Expression of Pdx1.

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Pdx1 is a homeobox transcription factor that is obligate for beta cell function and development, and is preferentially expressed in pancreatic beta cells, with little to no expression in other tissues. Absence of Pdx1 leads to pancreatic agenesis, and haploinsufficiency of Pdx1 leads to β -cell dysfunction and type 2 diabetes. Epigenetic modifications play a key role in regulation of Pdx1 transcription in islets. In previous studies, we found that robust histone acetylation (H3Ace) and trimethylation of H3K4 at the proximal promoter of Pdx1 is requisite for normal expression in islets. In contrast, H3K9 dimethylation (H3K9me2) and DNA methylation result in a marked decrease in Pdx1 expression. USF-1 is a critical activator of Pdx1 transcription and decreased binding of USF-1 markedly reduces or abolishes Pdx1 transcription. Previously we have shown that loss of USF-1 binding leads to an increase in repressive chromatin modifications such as methylation of H3K9 and H3K27. To determine if silencing of Pdx1 expression in liver and muscle is due to epigenetic modifications at the proximal promoter of Pdx1, which preclude USF1 binding. Liver, muscle, and islets from male Sprague-Dawley rats were harvested at 6-8 weeks of age. Gene expression was measured by quantitative PCR analysis. Histone modifications were evaluated by chromatin immunoprecipitation assay and quantitative PCR analysis. DNA methylation was measured by pyrosequencing analysis. Statistical analysis was done by unpaired t-tests between islet and liver, and between muscle and liver. Pdx1 gene expression was significantly decreased in liver compared to islets (5000 fold decrease, $p=0.001$) as well as muscle compared to islets (32,000 fold decrease, $p=0.001$). Surprisingly, acetylation of H3 at Pdx1 was not significantly different between liver, muscle and islets. H3K9me2 at Pdx1 in liver was 6.6 fold higher compared to the islet ($p=0.028$), whereas H3K9me2 at Pdx1 in muscle was 60 fold greater compared to islets ($p=0.025$). Unexpectedly, binding of the transcription factor USF-1 at the proximal promoter of Pdx1 was not significantly different between the three tissues. Further, the CpG island at the proximal promoter of Pdx1 was not methylated in any tissue. These data demonstrate that silencing of Pdx1 in muscle and liver is not due to DNA methylation or lack of USF-1 binding at the proximal promoter, but rather due to histone modifications including methylation of H3K9.

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

Nothing to Disclose: YH, SEP, RAS

P3-489

Developmental Programming: Gestational Testosterone Excess Compromises Fetal Pancreatic Differentiation.

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Prenatal testosterone (T) excess leads to insulin resistance as early as 5 wks of age in female sheep. It is unclear if pancreatic defects programmed during fetal life contribute towards this altered function. We hypothesized that prenatal T excess alters the fetal ontogeny of pancreatic development and function. Pregnant Suffolk sheep were given T propionate (100 mg, i.m. twice weekly) in cottonseed oil from days 30-90 of gestation (term: ~147 d). Uterine arterial samples were collected from 5 control (C) and 5 T-treated mothers and fetal umbilical arterial samples from their female fetuses (one fetus/mother) on day 90 of gestation for measurement of glucose and insulin. Fetal pancreas was dissected out, weighed, and split into head and tail portions. The tail portion was fixed, paraffin embedded and serially sectioned (5 μ m) for morphometric analyses. To determine effects of prenatal T excess on insulin (β -cells) and glucagon (α -cells) positive cells, 6 sections (100 μ m apart) were stained for insulin (guinea pig anti-porcine insulin) or glucagon (mouse anti-human glucagon) and nuclei counterstained with DAPI. To determine β -cell apoptosis, 2 sections (300 μ m apart) from each pancreas were double-stained for insulin and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick translation end labeling (TUNEL). Images were captured (400X) and total number of positive α -, β - and β -TUNEL cells (<2,000 cells/measure/pancreas) were counted. Findings indicated that gestational T excess did not alter mean glucose (G), insulin (I) and I/G ratio in fetal but increased ($P<0.05$) I/G ratio in maternal samples. The pancreatic/fetal weight ratio was lower in T-treated compared to C fetuses (C: 0.14 \pm 0.01; T: 0.12 \pm 0.00g; $P<0.05$). Percentage of β -cells (C: 17.4 \pm 2.1; T: 17.7 \pm 3.3%) did not differ between treatment groups. In contrast, % of α -cells (C: 18.7 \pm 1.5; T: 13.1 \pm 1.8%; $P<0.05$) was reduced in the T-treated compared to C fetuses. Percentage of apoptotic β -cells (C: 7.8 \pm 0.9; T: 12.1 \pm 1.7; $P=0.09$) did not differ between treatment groups. In summary, gestational T excess impacts fetal pancreatic differentiation, specifically α -cell population. Considering the role of glucagon in controlling hepatic glycogenolysis and gluconeogenesis processes, differences in number of α -cells may underlie the hepatic compromise found in T-treated female fetuses.

(1) 91st Endocrine Society Meeting, Washington, DC, USA. P1-273.

Sources of Research Support: NIH P01 HD44232.

Nothing to Disclose: AV-L, MM, VP

P3-490

Differential TGF β Superfamily Expression and Action in Mouse and Rat Islets.

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Diabetes is a hyperglycemic state that arises when β -cells are no longer able to secrete sufficient insulin to control blood glucose. This might result from increased insulin demand such as from insulin resistance, or from β -cell damage or loss from a variety of factors. Thus, a more complete understanding of regulatory mechanisms governing β -cell mass and function in adults would facilitate development of novel diabetes treatments that increase insulin bioavailability. Recent studies indicate that members of the TGF β superfamily of growth factors have roles in regulating β -cell function and/or mass in adults, although mouse and rat studies do not always agree. To better understand the biological basis for such differences and to begin to dissect the roles of TGF β ligands, we characterized the mRNA expression of 10 TGF β ligands in isolated islets from C57BL/6 mice and Lewis rats by qPCR. For comparison, the islet depleted fraction was also investigated. Activin and FST proteins were confirmed by IHC. In mice, myostatin (MSTN) was highly and exclusively expressed and activins A and B were preferentially expressed in islets. BMP7 and TGF β 2 were highly expressed in both islet enriched and depleted fractions. In contrast, MSTN mRNA was undetectable in rat islets while activin A and BMP7 were preferentially expressed in the islet fraction. Activin B, GDF11, TGF β 1,2, and 3, and BMP2 and 4 were all preferentially expressed in the islet-depleted fraction. In static cultures of mouse islets, none of these ligands altered GSIS at low or high (2.8 or 16.7 mM) glucose but TGF β 1 and follistatin, an activin/myostatin/GDF11 antagonist, both inhibited GSIS. In contrast, cultured rat islets responded to TGF β 1 and MSTN with enhanced GSIS at low glucose (1.6 & 2.6 fold respectively) whereas activin A, TGF β 1 and myostatin enhanced GSIS at high glucose (1.5, 1.3 & 1.4 fold respectively). These results indicate that mouse and rat islets synthesize different subsets of TGF β superfamily ligands which may be dictating their differential responses to these ligands. In addition, ligand production by islets may influence the outcome of static islet culture experiments due to ligand accumulation indicating that perfusion cultures might be required to elucidate their true activity. Synthesis of TGF β ligands by islet cells supports the hypothesis that these ligands have important roles in adults that might be exploited to develop novel therapeutic strategies for diabetes.

Sources of Research Support: NIH grant R01DK075058.

Nothing to Disclose: MLB, LB, FK, NU, ALS

P3-491

Early Pancreatic Islets Defects in IUGR Rodent Models: Gene Expression and Morphological Alterations.

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Obesity and insulin resistance represent a growing global public health concern. Recently, accumulating evidences indicate that the risk of developing chronic diseases in adulthood is highly influenced by deleterious intrauterine environment, a concept commonly called "fetal origin of adult diseases". Our work attempts to elucidate mechanisms linking deleterious events during intrauterine life and metabolic disturbances at adulthood.

Methods: To investigate islets alterations caused by intrauterine growth retardation (IUGR), we used 2 rat models: undernutrition of gestating dams (30% of normal food consumption = CR group, for caloric restriction) or infusion of dexamethasone (100 µg/kg/d during the last week of gestation = DEX group). We combined gene expression studies with an ABI real time PCR and morphologic analysis, using MetaMorph® Imaging System, to determine the total area, the number and the size of Langerhans islets classified as small (<25µm²), medium (between 25 and 300µm²) or large (>300µm²).

Results: At postnatal day 7, we detected gene expression alterations for specific hormones (insulin, glucagon) and islets "markers" such as Pdx1, Glut2, Kir6.2, glucokinase, ngn3 and HNF6. Insulin protein content in the pancreas of CR pups was also decreased (15.06 ± 1.64 µg of insulin per pancreas) when compared to control rats (20.26 ± 1.51 µg). Moreover, the ratio of endocrine to whole pancreas area was significantly reduced in CR (1.18 ± 0.11%) and DEX pups (1.32 ± 0.08%) compared to control animals (1.66 ± 0.10%). This reduction in the endocrine pancreas was related to a concomitant decrease in mean islet size and number. In fact, at postnatal day 7, small islets (315 ± 33 per slide), medium islets (261 ± 31) and large islets (53 ± 4) were reduced in CR pups compared to control pups (respectively 532 ± 63; 453 ± 61 and 78 ± 4 per slide) whereas only large islets were decreased in DEX pups (60 ± 5). At postnatal day 21, gene expression alterations mainly disappeared and only large islets were decreased in CR pups (51 ± 3 per slide) compared to control (79 ± 5), whereas no difference was detected in DEX pups, independently of the size of islets considered.

Conclusion: The early postnatal period is important for islet mass development. Compensatory mechanisms during lactation appear to allow increasing endocrine cells since at weaning, gene expression and the morphology of islets are globally normalized in IUGR rodent models.

Nothing to Disclose: ES, DV, PSH, MLA, VMS

P3-492

Biomarkers of Endothelial Dysfunction and Glycemic Variability in Type 2 Diabetes Mellitus.

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CONTEXT: HgA1c determines the risk of developing microvascular diabetic complications.[1] It is less clear however, how extreme fluctuations in glucose contribute to vascular complications. Glycemic variability(GV) is associated with oxidative stress which in turn has been correlated to endothelial damage; a critical event lending way to the vascular complications seen in many disease states.[2-4] **OBJECTIVE:** The aim of this study is to examine the relationship between short term GV obtained from continuous glucose monitoring(CGM) and biomarkers of endothelial dysfunction; sE-selectin, intercellular adhesion molecule-1(ICAM-1) and vascular cell adhesion molecule-1(VCAM-1). **RESEARCH DESIGN:** 28 adult T2DM subjects undergo CGM for 72 hours. To isolate the effect of GV, subjects with extremes in age, HgA1c, blood pressure, lipoproteins and known inflammatory diseases were excluded. Serology for the determination of endothelial biomarkers was collected on day 3. Measurements of GV[5-6]were quantified from CGM data. Statistical analysis was performed using Spearman rank-order correlations. **RESULTS:** Interim analysis(n=13): Mild to moderate correlations between VCAM-1 and mean percentage of blood sugar 5-minute readings above 200(r0.40;p0.16); simple variability(r0.47;p0.09); insulin(r0.50;p0.13); HOMA(r0.47;p0.16). Mild correlations between ICAM-1 and simple variability(r0.38;p0.19); area under the curve postprandial(r0.40;p0.17). Statistically significant correlations between ICAM-1 and HgA1c(r0.62;p0.02) and urine microalbumin to creatinine ratio(r0.69;p0.05). Moderate to strong correlations between sE-selectin and BMI(r0.53;p0.06); triglycerides(r0.49;p0.08) fasting glucose(r0.54;p0.057). **CONCLUSION:** Interim data analysis from 13 of 28 anticipated subjects suggests a positive correlation between short-term GV and endothelial dysfunction. The statistical significance of this observation including the relative importance of fasting blood sugar on GV may be determined after analysis of the remaining subjects. Consistent with published findings of endothelial dysfunction in nephropathy, a significant relationship between microalbuminuria and ICAM-1 was found. A strong correlation between elevated biomarkers and variables associated with insulin resistance was observed. This association may offer the clinician surrogate markers for the mechanistic basis behind the efficacy of treatments aimed to prevent vascular complications.

(1)DCCT research group. N Eng J of Med 1993; 329(14):977-86

(2)Monnier et al., JAMA 2006; 295:1681-87

(3)Brownlee M. Diabetes 2005; 54(6):1615-25

(4)Virella et al., Diabetes Care 2008; 31(10):2006-12

(5)Service et al., Diabetes 1970; 19(9):644-55

(6)Schlichtkrull et al., Ugeskr Laeger 1964; 126:815-20

Nothing to Disclose: CLB, THD, LES, RS, CEH, PY, EAE, TB, MF

P3-493

Disparate Improvement in Peripheral Tissue Sensitivity and Hepatic Tissue Insulin Sensitivity Following Bariatric Surgery.

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The changes in weight and improvement in glucose homeostasis following different types of bariatric surgery have been attributed to incretins, B-cell sensitivity to incretins and peripheral tissue sensitivity to insulin. The role of the liver has not been studied. We examined the changes in peripheral tissue sensitivity and hepatic tissue sensitivity to insulin following the two most commonly performed bariatric surgery; Roux-En-Y (RYGB) and adjustable gastric banding (AGB). Five RYGB volunteers and four AGB volunteers were studied before, 1 month, 3 month, 6 months and 12 months after surgery. All volunteers underwent a two hour hyperinsulinemic-euglycemic ($480 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) clamp coupled with $3\text{-}^3\text{H}$ -glucose methodology to evaluate hepatic glucose production (rate of glucose appearance, Ra) and peripheral tissue sensitivity (rate of glucose disappearance, Rd). In non-obese healthy adults this dose of insulin completely suppresses Ra during the last hour of the clamp (from a basal rate of 2.43 ± 0.13 to $0.1 \pm 0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Fat mass and fat free mass (FFM) changes were assessed using an iDXA. No significant changes were observed in either the RYGB or AGB group in Ra either at the basal state or during the 90-120 minute period of the clamp. The basal and the suppressed levels were $\sim 2.21 \pm 0.18$ and $0.5 \pm 0.18 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ at each assessment. In the RYGB, there was a significant progressive improvement in peripheral tissue sensitivity to insulin (Rd) starting at three months and reaching a level of $5.7 \pm 0.54 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for the 90-120 minute period. AGB group showed no significant improvement in peripheral tissue sensitivity to insulin over a 12 month period. There was a significant reduction in fat mass in the RYGB group (from 63.5 ± 5.2 to $26.2 \pm 4.2 \text{ kg}$). No significant loss in fat mass was observed in the AGB group. There was an association between Rd and fat mass explaining 31% of the variance ($p < 0.01$). The improvement in glucose homeostasis up to one year following RYGB cannot be attributed to changes in HGP. There is a gradual improvement in the peripheral tissue sensitivity to insulin in these patients that is mostly attributed to loss of fat mass. The lack of improvement in hepatic glucose production may be due to persistent steatohepatitis which is not resolved 12 months after RYGB.

Nothing to Disclose: AR, PG, RS-C, AOL, KES, MDS, THM, MH, BRG, DKA, DE

P3-494

Cd226 Gene Polymorphisms and Susceptibility to Type 1A Diabetes in a Brazilian Cohort.

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Introduction: Cd226 molecule is a 67kDa Type 1 membrane, intracellular adhesion protein involved in co stimulation and activation of effector functions of T-helper Type 1 (Th1) cells. Cd226 appears to contribute to multiples and adaptative responses which include leukocyte migration, activation, expansion, and differentiation of CD8 T cells and CD4 cells, and effector responses of T cells and NK cells. Observations of Cd226 and its receptors expression in thymocytes suggests that this interaction may regulate the generation of autoreactive T cells, further emphasizing the importance of Cd226 gene in the immune response. Influences in CD4 T cells and perhaps T regulatory cell responses remains to be explained. Genome Wide Studies has recently identified a robust association of type 1 diabetes with a nonsynonymous single nucleotide polymorphism (SNP) rs763361 Gly307Ser in exon 7 of Cd226 gene. This amino acid change could alter the molecule phosphorylation sites. **Objective:** identify new genetics variants and polymorphisms in CD226 coding sequences in type 1 diabetes (T1D) patients and determine the influence of them as risk factor to type 1 diabetes in our population. **Patients and methods:** genomic DNA was extracted from 70 T1D patients (25M/45F) aged 15,17 ± 9,819, ketosis prone and treated with insulin. Coding regions (exon 2, 3, 4, 5, 6, and 7) and boundaries of Cd226 gene (ENSG00000150637) were amplified. Direct sequencing of PCR amplified products was performed using ABI 3100 capillary sequencer. Results were compared with 50 health controls and databases. **Results:** frequency of polymorphisms already described and identified in this cohort of T1D patients was similar to health controls, including the SNP rs763361 (T/C) in exon 7, related to diabetes susceptibility, considering both its allelic (P= 0,876) or genotypic (P=0,375) frequency. This result, in disagreement with Caucasian data, is probably related to our heterogeneous ethnical population. Unexpectedly, a huge prevalence of rs 763361 TT genotype was observed in female diabetic patients (50%) in comparison to male (21,7%) (P=0,035) that was not observed in controls (P=0,27). **Conclusion:** in this Brazilian cohort of T1D patients polymorphisms in Cd226 gene seems not to contribute to diabetes risk. We found an increased prevalence of TT genotype of the SNP rs763361 in diabetics female patients, nevertheless an increase in sample will be necessary.

Nothing to Disclose: TCM, VSC, ASS, EMP, RTF, ED, MERS

P3-495

Association of the G276T Polymorphism of the Adiponectin Gene with Ischemic Heart Disease in Patients with Type 2 Diabetes.

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Adiponectin, a hormone expressed by adipocytes, has insulin-enhancing and anti-atherogenic effects. The intronic variant rs1501299 (G276T, G allele) at the adiponectin gene is associated with ischemic heart disease (IHD) in Western populations. We assessed the contribution of this variant with IHD in a cross-sectional study of Brazilians with type 2 diabetes (n=682; 47.7 % males; age 59.5 ± 10.1 years, diabetes duration 11.0y [6.0-18.0], median [IQR], 73.3% whites). IHD was diagnosed by the presence of angina or possible infarct (World Health Organization Cardiovascular Questionnaire), and/or resting (Minnesota code)/stress ECG test abnormalities, and/or perfusion abnormalities upon myocardial perfusion scintigraphy. Subjects with different genotypes at rs1501299 (GG=331, GT=280, TT=71, in Hardy-Weinberg equilibrium; P=0.990) did not differ by sex, age, diabetes duration, hypertension prevalence, smoking habit, family history of IHD, body mass index, waist-hip ratio, A1c, triglycerides, HDL, LDL and total cholesterol levels. However, homozygous subjects for the T allele had a greater prevalence of IHD than subjects with the G allele comparing the 3 genotypes (TT=57.7 vs. GT=35.0 vs. GG=37.2%; P=0.002) and in an autosomal recessive model (TT vs. GT/GG; 57.7 vs. 36.2; P<0.001). In a multiple logistic regression analyzes homozygous subjects for the T allele had a greater risk of IHD (OR=1.83; C.I. 95% 1.04 - 3.23; P=0.037) than subjects with the G allele, while adjusting for sex, age, A1c, smoking habit, HDL cholesterol and creatinine levels using the autosomal recessive model. Different from other Western populations, Brazilian diabetic subjects homozygous for the T allele had a greater prevalence of IHD than subjects with the G allele.

Menzaghi C et al., Diabetes 2007; 56: 1198

Heid IM et al., Diabetes 2006; 55: 375

Sources of Research Support: Brazilian National Research Council (CNPq); Coordination of Higher Education and Graduate Training (CAPES); International Scholarship Program of the Endocrine Society; Hospital de Clínicas de Porto Alegre Research and Events Incentive Funds (FIPE-HCPA); Rio Grande do Sul Research Foundation (FAPERGS).

Nothing to Disclose: FG, GG, DC, EPCCR, CMS, JFZ, SPG, ACX, ALS, JLG, LHC

P3-496

Inverse Association between Sleep Duration and Hemoglobin A1C in Healthy Adults with Parental History of Type 2 Diabetes.

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BACKGROUND: Epidemiological data show an association between reduced sleep duration and diabetes risk in the general population. Whether this relationship applies also to individuals with high pre-existing risk of type-2 diabetes has not yet been examined. The goal of this ongoing study is to explore the potential association between objectively measured habitual sleep duration and hemoglobin A1c levels, a surrogate marker of metabolic risk, in healthy adults with parental history of type 2 diabetes.

METHODS: Participants (10F/1M; mean \pm SD age 25.7 ± 3.5 y; BMI 24.3 ± 3.0 kg/m²) were screened to be free of any medical, psychiatric, or sleep disorders. All of them reported having no more than a modest amount of exercise 0 to 2 times per week. Each study participant was monitored for 14 days under free-living conditions. During this period, habitual sleep duration was measured by wrist actigraphy (Actiwatch, Respironics) and daily physical activity was measured by waist accelerometry (Actical, Respironics). Hemoglobin A1C was measured at the time of screening in the clinical laboratory of our medical center. Multiple linear regression was used to examine the association between individual sleep duration and hemoglobin A1C values when BMI and daily physical activity (minutes of moderate and vigorous activity) were controlled for (SPSS, Version 17.0).

RESULTS: The total sleep time of the study participants during the 2-week monitoring period was 6 h 14 min \pm 54 min/day (range: 296 to 461 min/day) and their moderate and vigorous physical activity averaged 143 min \pm 46 min/day. Mean hemoglobin A1C was $5.4 \pm 0.2\%$. In this small preliminary set of data, habitual sleep duration was inversely associated with hemoglobin A1C ($P=0.04$) independent of BMI and physical activity.

CONCLUSION: So far, the results from this ongoing study suggest that reduced sleep duration may be a predictor of higher hemoglobin A1C levels even in individuals with considerable pre-existing risk of developing type 2 diabetes.

Sources of Research Support: NIH grants R01-HL089637, CTSA-RR 04999, and P60-DK020595.

Nothing to Disclose: AD, JB, LA, MK, JI, PP

P3-497

Utilizing Modern Geographic Information System Methods To Investigate the Relationship between the Los Angeles County Environment and the Metabolic Disease Burden in the Underserved, Predominantly Hispanic LA County Community.

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Background: The diabetes prevalence of Hispanics in the urban setting of Los Angeles County(LAC) is twice that of non-Hispanic white.(1,2) Apart from genetic predisposition, undefined environmental variables are involved, such as poverty, an independent factor. Our aim was to evaluate the utility of Geographic Information Systems(GIS) in characterizing possible environmental variables which may be associated with higher prevalence of diabetes in the underserved, predominantly Hispanic community of LAC. This could lead to cost-effective community-based interventions.

Methods: We used GIS technology to study 50 patients who attended the LAC Medical Center Diabetes and Metabolism Clinic, a specialty clinic serving the predominantly Hispanic community of East Los Angeles. From the GIS generated maps, we looked at the cluster patterns of obesity(BMI), glycemic burden(HBA1C), and dyslipidemia(TG).

Results: This method allowed for greater granular mapping of regional metabolic burden. Figure 1 illustrates spatial distribution of diabetes patients.

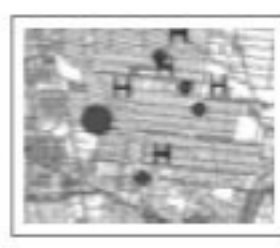
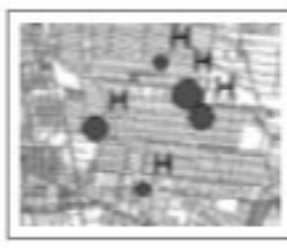
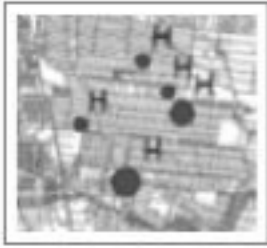


The magnitude of glycemic control is represented by the size of the data points. The cluster pattern of HBA1C, BMI, and Triglycerides were all different. An example a cluster variability is show in figure2.

BMI

HbA1C

TG



Discussion: Our pilot GIS study shows unique spatial distribution for each metabolic variable, which may suggest different environmental influences. Further study of this could lead to better targeted approaches in treating metabolic diseases in this underserved Hispanic community in LAC.

- (1) Stern MP, Mitchell BD. Diabetes in Hispanic Americans. In: Harris MI, Cowie CC, Stern MP, Boyko EJ, Reiber GE, Bennett PH, eds. Diabetes in America, 2nd ed. Washington, DC: U.S. Department of Health and Human Services, National Institutes of Health, 1995; DHHS publication no. (NIH)95-1468.
- (2) CDC. Self-reported prevalence of diabetes among Hispanics---United States, 1994--1997. MMWR 1999;48:8--12.
- (3) U.S. Census Bureau. The Hispanic population. Census 2000 brief. Washington, DC: U.S. Department of Commerce, Economics and Statistics Administration, 2001.
- (4) Hinman SE, Blackburn JK, Curtis A. Int J Health Geogr. 2006 Mar 27;5:13.
- (5) Curtis AJ. Int J Health Geogr. 2008 Aug 22;7:47.
- (6) Stern MP, Mitchell BD. Diabetes in Hispanic Americans. In: Harris MI, Cowie CC, Stern MP, Boyko EJ, Reiber GE, Bennett PH, eds. Diabetes in America, 2nd ed. Washington, DC: U.S. Department of Health and Human Services, National Institutes of Health, 1995; DHHS publication no. (NIH)95-1468.

Nothing to Disclose: WAL, AC

P3-498

A Nutraceutical Preparation Containing Resveratrol and Polyphenols Modulates Oxidative and Inflammatory Response to a High Fat High Carbohydrate Meal: A Randomized Controlled Study.

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We investigated whether a nutraceutical preparation containing resveratrol and polyphenols reduces the oxidative stress induced by a high fat high carbohydrate meal (HFHC). Ten normal subjects were given a 910 kcal HFHC meal either with a nutraceutical preparation (group A) (100mg of resveratrol and 75 mg of total polyphenols) or placebo (group B). Blood samples were collected at baseline and at 1, 3 and 5 hours following meal intake. The intake of the meal in group B induced a significant increase in protein levels of p47^{phox} (NADPH oxidase subunit) by 148±38% over the baseline in the MNC while it did not change following the meal with the supplement (group A). There was a significant increase in plasma concentrations of LPS in group B from 0.23±0.02EU/ml to 0.36±0.03EU/ml (60±16% over the baseline). In contrast, there was a significant decrease in LPS concentrations by 28±7% (from 0.30±0.04EU/ml to 0.22±0.04EU/ml) below the baseline at 1hr in group A. In group A, the DNA binding activity of anti-oxidant transcription factor Nrf-2 was significantly up regulated by 150±39% over the baseline and the expression of its inhibitor, KEAP-1 was depressed by 30% (p<0.05). In group B, there was a significant reduction of Nrf-2 activity and an increase in its inhibitor, Keap-1, by 48±6%. mRNA expression of Nrf2 regulated anti-oxidant target genes, NADH-quinone-oxidoreductase (NQO-1) and glutathione-sulfotransferase-P1(GST-P1), increased significantly by 33±7% and 24±8% above baseline, respectively, in group A (P<0.05). Group B had a 39±13% increase over the baseline in SOCS-3 protein while group A had no change. Thus, the nutraceutical preparation containing resveratrol and polyphenols reduces the magnitude of oxidative stress, endotoxemia and SOCS-3 expression and stimulates specific Nrf-2 related anti-oxidative enzymes, NQO-1 and GST-P1 after an HFHC meal.

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, Sanofi-Aventis, GlaxoSmithKline, Amylin Pharmaceuticals.

Nothing to Disclose: HG, CLS, KK, TL, SA

P3-499

Severe Diabetic Lipemia Associated with Diabetic Ketoacidosis in a Boy with Compound Heterozygous Mutations in the Lipoprotein Lipase Gene.

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Introduction

Elevated triglycerides (TG) in the setting of DKA is well described, termed diabetic lipemia. In an insulin deficient state, carbohydrate metabolism decreases and lipid metabolism increases, resulting in chylomicronemia and elevated VLDL. Lipoprotein lipase (LPL) is the key enzyme that breaks down these lipids. It is insulin dependent, and activity is transiently decreased in DKA. This further potentiates the hypertriglyceridemia. A small number of patients develop severe diabetic lipemia. This subset of patients may be genetically predisposed to extreme hypertriglyceridemia in an insulin deficient state.

We describe a case of severe diabetic lipemia in an 18 month old boy who presented in DKA, and was found to be a compound heterozygote for a known and a novel mutation in the LPL gene.

Case

The patient is an 18 month old boy who presented to the PICU with new onset T1DM in DKA after a 1 week history of polyuria and polydipsia. He was previously healthy, with negative family history for diabetes, and positive family history for unspecified cholesterol disorder in maternal grandparents.

Initial physical exam revealed moderate dehydration. There was no hepatosplenomegaly or eruptive xanthomas, and no lipemia retinalis. His blood was creamy pink in appearance, and TG were found to be elevated to 9935 mg/dl. They peaked at 21,063 mg/dl on hospital day 2. He was placed on a restricted diet with 6 grams of fat per day. During his 12 day admission, his TG trended down to 807 mg/dl. Pancreatic enzymes remained normal throughout his stay. Genetic testing revealed 2 mutations in the LPL gene: G130R in exon 4, and N291S in exon 6.

Discussion

Patients who are homozygous for a loss of function mutation in the LPL gene have familial hypertriglyceridemia with TG >1000 mg/dl. Patients who are heterozygous for mutations in the LPL gene have normal or slightly elevated TG, and cannot be identified by lipid levels alone. However, patients with a heterozygous mutation may develop severe diabetic lipemia in DKA as a result of decreased activity of LPL. This can affect the management of DKA, as the risk of pancreatitis necessitates close observation. Depending on severity, management may include limiting dietary fat while ensuring normal growth and development in children. Further studies are warranted to elucidate the association between heterozygous mutations in the LPL gene and extreme diabetic lipemia, and regarding the impact of genetic testing on its management.

Nothing to Disclose: LAM, RCP, YA-D

P3-500

Positive Association between Overnight Sleep and Habitual Physical Activity in Healthy Adults with Parental History of Type 2 Diabetes.

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BACKGROUND: Short sleep has been associated with increased risk of type 2 diabetes, but the underlying mechanisms of this relationship are poorly understood. Since sedentary living may be an important mediator of metabolic risk, we designed this ongoing study to examine the relationship between overnight sleep duration and habitual physical activity in healthy adults with type 2 diabetic parents.

METHODS: Participants (7F/2M; mean \pm SD age 28.2 ± 4.7 y; BMI 24.5 ± 2.9 kg/m²) were screened to be free of any medical, psychiatric, or sleep disorders and reported having no more than a modest amount of exercise 0 to 2 times per week. After one habituation night of polysomnography at the end of the screening period, participants returned to the laboratory for a repeat measurement of their overnight total sleep time. We then monitored the free-living activity of each participant by waist accelerometry (Actical, Respironics) for 2 weeks. Data were analyzed using multiple linear regression controlling for individual BMI (SPSS, Version 17.0). The inclusion of additional control for gender and race/ethnicity in the regression model did not affect the results of this analysis.

RESULTS: The total sleep time of the study participants during the night of laboratory polysomnography was 7 h 31 min \pm 43 min. Participants averaged $194,662 \pm 99,110$ activity counts per day during the 2-week monitoring period. In this preliminary small set of data, longer total sleep time was a significant predictor of higher daily physical activity ($\beta = 1909$ counts/min of sleep; 95%CI: 933 to 2884; $p = 0.005$).

CONCLUSION: So far, the results from this ongoing study suggest the presence of a positive association between overnight sleep time and habitual daily activity in healthy individuals with a high lifetime risk of developing type 2 diabetes.

Sources of Research Support: NIH grants R01-HL089637, CTSA-RR 04999, and P60-DK020595.

Nothing to Disclose: JB, AD, LA, MK, YB, PP

P3-501

Identification of Cardiac Biomarkers in a Mouse Model of Obesity Induced Type 2 Diabetes.

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Type 2 diabetes (T2D) is a metabolic disorder characterized by insulin resistance (IR) and hyperglycemia. The development of T2D is strongly associated with obesity. In humans, T2D increases the risk for serious complications. Among these, heart disease has been ranked as a leading cause of death in patients with T2D. Thus, it is of interest and importance to discover biomarkers of cardiac dysfunction associated to obesity and T2D. In this study we evaluated the protein profiles in heart tissues of mice made obese and diabetic by high-fat feeding. C57BL/6J male mice were reared on a normal or high-fat diet upon weaning, and their physiological responses (*i.e.* weight, fasting plasma glucose and insulin) were monitored at regular time intervals. Mice presenting significant increases in body weight, fasting blood glucose and insulin were classified as diabetic. Hearts were harvested from diabetic mice (n=5) and non-diabetic controls (n=5) and used for proteomic studies employing 2D-gel electrophoresis followed by mass spectrometry. Over 300 proteins spots per gel were resolved and 15 spots were found to be differentially expressed (8 being increased and 7 decreased) in diabetic hearts as compared to controls. We have identified these proteins as desmin, peroxisomal enoyl coenzyme A hydratase, malate dehydrogenase, nucleoside diphosphate kinase, mitochondrial ATP synthase, troponin T2, α -cardiac actin, isocitrate dehydrogenase 3, adenylate kinase and α -crystallin b. These findings indicate that diabetic hearts show an increase in expression of enzymes related to fatty-acid oxidation and a decrease in proteins involved in ATP production and contractibility. Our results provide new biomarkers which are potential targets for further studies to ultimately detect and perhaps prevent and treat T2D-induced cardiac failure.

Sources of Research Support: In part by the State of Ohio's Eminent Scholar Program that includes a gift from Milton and Lawrence Goll, by NIH grants DK075436-01, AG019899-06, and 1P01AG03173601A and by grants from BioMolecular Innovation and Technology Partnership (BMIT) and the Diabetes Research Initiative (DRI) at Ohio University, and by the AMVETS.

Nothing to Disclose: DC-T, EOL, SO, BK, JJK

P3-502

Associations between Adiposity and Insulin-Mediated Glucose Disposal in African American and European American Females.

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Background:

Obesity, particularly intra-abdominal fat, adversely impacts insulin sensitivity at the whole-body level. However, the extent to which adiposity specifically affects glucose uptake versus endogenous glucose production is less clear. The purpose of this study was to examine associations of total and visceral adiposity with insulin-mediated glucose disposal in healthy females, and to determine whether these associations differ by ethnicity and age.

Methods:

Participants were 147 African American (AA) and European American (EA) females, categorized into one of three groups based on reproductive status: prepubertal, premenopausal, or postmenopausal. Frequently-sampled intravenous glucose tolerance testing was combined with a stable isotope tracer, (6,6-²H₂)glucose, to determine an index of whole-body insulin sensitivity (**total S_i**) and an index of insulin-mediated glucose disposal (**disposal S_i**). Percent body fat (%fat) and intra-abdominal adipose tissue (IAAT) were measured by dual energy X-ray absorptiometry and computed tomography, respectively. Adiposity and S_i indexes were examined by 2-way ANOVA for effects of ethnicity and age group. Correlation analyses and ANCOVA were used to explore relationships between adiposity and each index of S_i.

Results:

%fat was a significant predictor for total S_i (P<0.05) and disposal S_i (P<0.05) after adjustment for ethnicity, age group, and IAAT. Conversely, IAAT independently predicted total S_i (P<0.01) but not disposal S_i. Despite less IAAT (p=0.01), AA had lower total S_i (P<0.001) and disposal S_i (P<0.01). Among EA, IAAT was inversely associated with both indexes of S_i (p<0.001). Among AA, IAAT was not associated with either S_i index. Among adults, %fat and IAAT were inversely related to disposal S_i (P<0.05, adjusted for ethnicity). Among prepubertal girls, the only association observed was between %fat and total S_i, and this relationship was significant only for EA (r=-0.47, P=0.02 for EA, r=-0.38, P=0.06 for AA).

Conclusions:

These results suggest that total fat depresses skeletal muscle uptake of glucose, whereas visceral adiposity may predominantly affect hepatic glucose production. The impact of IAAT on S_i appears stronger for EA than AA, and increases with age.

Sources of Research Support: R01DK58278; R01DK067426; M01-RR-00032; P30-DK56336; P60DK079626.

Nothing to Disclose: ACE, JAA, WMG, CDM, CC, JRF, FO, BAG

P3-503

Polymorphisms in Human AHSG (Fetuin-A) Gene Are Associated with Risk of Nephropathy in Type 2 Diabetes.

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α 2-Heremans-Schmid glycoprotein (AHSG), also called Fetuin-A, is a plasma protein known to act as a transcriptional repressor via inhibition of the insulin receptor tyrosine kinase. It has been linked to lower BMI, inhibition of ectopic vascular calcification and reduced renal and cardiovascular morbidity and mortality in numerous studies. Ten single nucleotide polymorphisms (SNPs) were selected across the AHSG locus using Hapmap and Ensembl Biomart and were genotyped using Sequenom iPLEX in 751 genomic DNA samples taken from subjects with type 2 diabetes (T2DM) from the Salford Diabetes Collection as previously described (Stephens et al 2005). Minor allele homozygosity in five SNPs (in intron 4, exon 6 and exon 7) was associated with reduced prevalence of nephropathy, while in one SNP (in intron 6) it was associated with increased prevalence, when tested using logistic regression correcting for age and gender. In addition, five SNPs were associated with reduced corrected calcium levels. We conclude that polymorphisms of the AHSG gene are associated with altered nephropathy prevalence in T2DM and that this may be due to altered gene function or to coding changes in the protein. The absence of clear association in the promoter region suggests that altered gene expression is not involved.

Stephens RH, McElduff P, Heald AH, New JP, Worthington J, Ollier WE, Gibson JM: *Diabetes* 2005; 54:3547-3553.

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

Nothing to Disclose: RHS, XXP, RJM, PAK, DJO, WERO, JMG

P3-504

Genome-Wide Association and Replication Study of the Metabolic Clearance Rate of Insulin.

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Insulin sensitivity and insulin secretion have been the focus of many genetic studies, while little attention has been paid to the metabolic clearance rate of insulin (MCRI). Better understanding of the physiology of MCRI is needed to determine whether therapeutic measures targeted to this trait might improve type 2 diabetes or other metabolic disorders characterized by abnormal insulin levels (e.g. obesity, metabolic syndrome, fatty liver, polycystic ovary syndrome). Thus, our goal is to discover genetic determinants of MCRI. We conducted a pilot genome-wide association study (GWAS) in 310 subjects from the Mexican-American Hypertension-Insulin Resistance (HTN-IR) cohort, all of whom had undergone euglycemic hyperinsulinemic clamps. MCRI was derived from the steady-state plasma insulin level (SSPI) during the clamps (MCRI = insulin infusion rate/SSPI). The GWAS identified 88 single nucleotide polymorphisms

associated with MCRI at $P < 1 \times 10^{-5}$. Four intergenic SNPs reached genome-wide significance ($P < 5 \times 10^{-8}$). As part of a 7600-SNP Illumina iSelect, the 88 SNPs were subsequently genotyped in the entire HTN-IR cohort (939 subjects), and a subset (17) of the genes identified by these GWAS hits were comprehensively genotyped (SNPs tagging entire gene, plus 50 kb upstream) in the entire cohort. Replication was evaluated by examining the HTN-IR subjects who were not part of the initial GWAS. Eight of the 88 GWAS SNPs replicated association with MCRI. Two of these SNPs ($P < 0.01$) were in the *ADAMTSL3* gene. Seven other SNPs in *ADAMTSL3* were associated with MCRI in the entire cohort ($P = 0.0002-0.05$). *ADAMTSL3* is highly expressed in the liver, kidney, heart, and skeletal muscle, which are key insulin-clearing tissues. A different *ADAMTSL3* SNP has been associated with height in GWAS studies (1); as insulin is a growth factor, an effect of this gene on both MCRI and height is plausible. Another of the GWAS SNP associations that replicated in the remaining HTN-IR cohort is located in the *CMIP* gene. Fifteen additional SNPs in *CMIP* that were included in the iSelect also demonstrated association with MCRI in the entire cohort. *CMIP* interacts with filamin A and inhibits NF- κ B activity, suggesting possible involvement in MCRI via cytoskeletal organization and/or modulation of inflammation. To our knowledge, this is the first GWAS in MCRI, and has begun to shed new light into the genetic control of this poorly-understood trait that may prove critical to metabolic disorders.

(1) Weedon MN et al., Nat Genet 2008;40:75-83

Sources of Research Support: NIH grant R01-DK079888 (to MOG); NIH grant M01-RR000425 (General Clinical Research Center Grant at CSMC); Winnick Clinical Scholars Award (to MOG).

Nothing to Disclose: MOG, KDT, MRJ, JC, TH, YDIC, TAB, LJR, XG, JIR

P3-505

Genetic Variants of TCF7L2 Are Strongly Associated with Increased Risk of Type 2 Diabetes in Lebanese Subjects.

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BACKGROUND. Transcription factor 7-like 2 (TCF7L2) is a key element of the Wnt signaling pathway, and is involved in glucose homeostasis. Genome-wide association studies (GWAS) validated TCF7L2 gene as a significant type 2 diabetes (T2DM) locus in several ethnic populations, and a strong association between T2DM susceptibility and TCF7L2 variants was reported. Marked differences in the risk imparted by each variant was reported, thus pointing to ethnic contribution of specific TCF7L2 variant to overall T2DM susceptibility.

AIM. The aim of this study was to investigate the associations of TCF7L2 gene variants (rs4506565, rs7903146, rs12243326, and rs12255372) in a Lebanese population (376 T2DM patients and 376 ethnically-matched control subjects).

METHODS. Genotyping of the four TCF7L2 single nucleotide polymorphisms (SNPs) was done by allelic discrimination/real-time PCR method. We also identified specific TCF7L2 haplotypes associated altered T2DM risk.

RESULTS. The distribution of the four TCF7L2 variants was in Hardy-Weinberg equilibrium among study participants. The minor allele frequencies of rs4506565 [$P = 2.1 \times 10^{-4}$, OR (95% CI) = 1.48 (1.20 - 1.82)], rs7903146 [$P = 3.0 \times 10^{-5}$, OR (95% CI) = 1.57 (1.27 - 1.93)], rs12243326 [$P = 2.0 \times 10^{-6}$, OR (95% CI) = 1.67 (1.35 - 2.06)], and rs12255372 [$P = 1.8 \times 10^{-7}$, OR (95% CI) = 1.82 (1.48 - 2.24)] were significantly higher in cases. All variants showed increased risk when homozygous than heterozygous, with the strongest risk for rs12255372 [OR (95% CI) = 4.39 (1.82 - 10.62)]. The four TCF7L2 variants were found to be in complete linkage disequilibrium. Four-locus (rs4506565 - rs7903146 - rs12243326 - rs12255372) haplotype analysis identified strong association of the all-mutant haplotype (haplotype 2222) with increased T2DM risk ($P = 1.0 \times 10^{-8}$). Regression analysis confirmed the positive association of haplotype 2222 ($P < 0.001$; aOR = 1.90; 95% CI = 1.47-2.46), and negative association of haplotypes 2221 ($P = 0.023$; aOR = 0.21; 95% CI = 0.05-0.81), 1121 ($P = 0.008$; aOR = 0.11; 95% CI = 0.02-0.57), and 2111 ($P = 0.045$; aOR = 0.27; 95% CI = 0.08-0.97), with T2DM after controlling for a number of covariates.

CONCLUSION. By demonstrating the strong contribution TCF7L2 gene variants to T2DM pathogenesis among Lebanese, our data replicate similar findings established for other ethnic groups, thereby establishing TCF7L2 as a universal T2DM candidate gene.

Nothing to Disclose: RN, AE, SFW-G, ER, WYA

P3-506

Obesity and Sex Hormone-Binding Globulin (SHBG) Levels in Gestational Diabetes.

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Some studies have shown that women with gestational diabetes mellitus (GDM) have lower sex hormone-binding globulin (SHBG) compared to pregnant women with normal glucose tolerance. SHBG has further been suggested as a potential marker of GDM. The objective of the study was to determine whether SHBG concentrations are different between GDM patients and women with normal glucose tolerance (controls) and to determine whether this difference is independent of pre-pregnancy body mass index (BMI). The study included 47 pregnant women (20 women with GDM and 27 controls). GDM was diagnosed at 26.1 ± 3.7 weeks of pregnancy using a 75g oral glucose tolerance test (OGTT). Glucose and insulin responses were quantified using areas under the curves of glycemia and insulinemia over 180 minutes after the glucose load. Insulin sensitivity was also estimated by calculating the Matsuda and HOMA indices. SHBG concentrations were measured by radioimmunoassay in fasting serum obtained on the morning of the OGTT. Pre-pregnancy BMI was calculated using self-reported weight before the pregnancy. BMI was also measured at the time of GDM diagnosis. Mothers with GDM had a significantly higher pre-pregnancy BMI compared to controls (29.4 ± 7.7 vs. 24.2 ± 4.3 , $p < 0.01$). A trend was observed for significantly lower SHBG concentrations in GDM patients compared to controls (179 ± 36 vs. 195 ± 25 nmol/L, $p = 0.08$). Insulin and glycemic responses were significantly and inversely associated with SHBG concentrations ($r = -0.36$ and $r = -0.31$ respectively, $p < 0.05$). Moreover, the Matsuda and HOMA measures of insulin sensitivity were positively associated with SHBG ($r = 0.41$ and $r = 0.36$ respectively, $p < 0.05$). Pre-pregnancy BMI and BMI at the time of diagnosis were both inversely correlated with SHBG levels ($r = -0.49$ and $r = -0.53$ respectively, $p < 0.005$). In logistic regression analyses, an increase of one pre-pregnancy BMI unit increased the risk of GDM by 1.16 fold (95% confidence intervals 1.04-1.32, $p < 0.01$). Adding SHBG concentrations to the model did not improve the prediction of GDM by pre-pregnancy BMI. In conclusion, SHBG concentrations were strongly associated with markers of insulin sensitivity as well as insulin and glycemic responses to oral glucose in pregnant women. However, pre-pregnancy BMI was associated with the presence of GDM independently of SHBG concentrations at the time of diagnosis. Further studies are needed to determine whether SHBG is an independent predictor of other outcomes in GDM.

Nothing to Disclose: ASM, MCD, RD, MN, JR, SJW, AT

P3-507

Methylation and Immunodetection of 11b Hydroxysteroid Dehydrogenase Type 2 in Placental of Small for Gestational Age Infants.

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Background: Small for gestational age (SGA) birth could be associated with a higher risk for type 2 diabetes mellitus and newborns SGA have increased morbidity and mortality. In a variety of animal models, prenatal stress, glucocorticoids exposure and inhibition of 11b hydroxysteroid dehydrogenase type 2 (11bHSD-2) -the fetoplacental barrier to maternal glucocorticoids- -reduce birth weight and cause increased risk of chronic diseases at the adult life. In human products with SGA 11bHSD-2 mRNA is diminished, suggesting this process may result from down-regulation epigenetic process by DNA methylation. The investigation of epigenetic regulation of the 11bHSD-2 gene may provide information on this mechanism conducting to SGA babies.

Objective: To test difference in protein levels and methylation of 11bHSD-2 on AGA and SGA placenta.

Methods: AGA (n=10) and SGA (n=10) placental biopsies were collected. Immunodetection of 11bHSD-2 was carried out with a specific primary antibody anti-11bHSD-2 and a secondary antibody coupled to peroxidase. Detection was carried out by chemiluminiscense, using luminol as substrate. To evaluate methylation of 11bHSD-2 gene, placental DNA was treated with sodium bisulfite. After conversion of unmethylated cytosines to uracil and subsequent PCR-mediated conversion uracil to thymine, methylated and unmethylated cytosines predicted differ in thermal stability because their different GC contents. Results were analyzed with t student and U de Mann Wihtney test. Significance was considered with a p<0.05.

Results: Anti-11bHSD-2 antibodies detected a band of protein at 41 kDa in placental samples from both groups. Densitometric and statistical analysis showed lower 11bHSD-2 protein levels in SGA group (p <0.0001). Analysis of a region that includes the first CpG island of the gene of the placental enzyme 11b HSD-2 showed 2.4 °C of difference between the maximum and minimum value of Tm of groups SGA and AGA; and a difference statistically significant of 0.57 °C between the averages of the Tm of both groups. Tm of SGA group was higher than (p=0.00024).

Conclusions: These results show that lower expression of enzyme 11bHSD-2 could be associated with methylation of the gene. Suggesting that as reported in animal models, increased glucocorticoids exposure induces low birth weight products and that the enzyme 11bHSD-2 plays an important role in the fetus protection from these deleterious factors.

Sources of Research Support: CONCyTEG grant 06-16-K117-104.

Nothing to Disclose: MOS-M, GB-S, JMM, SZ, FC-A, GR-G

P3-508

Gamma-Linolenic Acid Can Increase Serum Active GLP-1, Insulin Levels and Decrease Blood Glucose in Type 2 Diabetes.

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Background

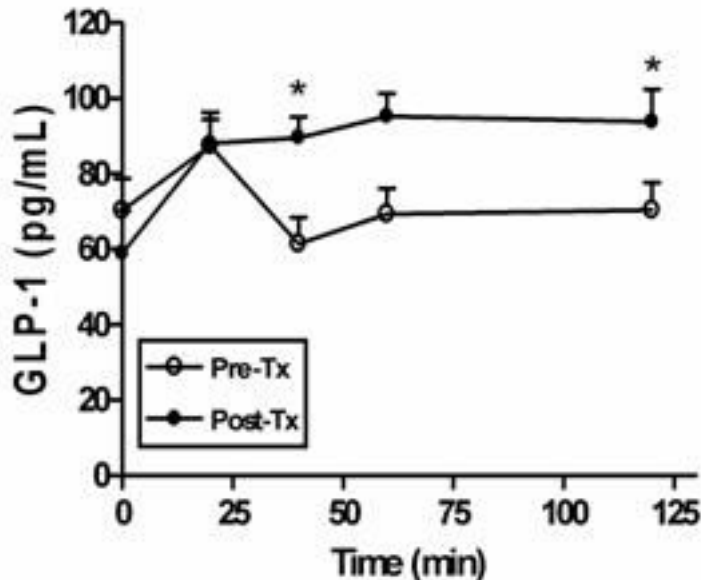
Recently, glucagon-like peptide-1 (GLP-1) is used in treatment of type 2 diabetes. Some free fatty acids (FFA) can directly stimulate GLP-1 secretion from entero-endocrine cells. Gamma-linolenic acid (GLA), a long-chain FFA, is commonly used in treatment of diabetic neuropathy. We examined the effect of oral GLA administration on blood glucose, serum insulin & active GLP-1 levels.

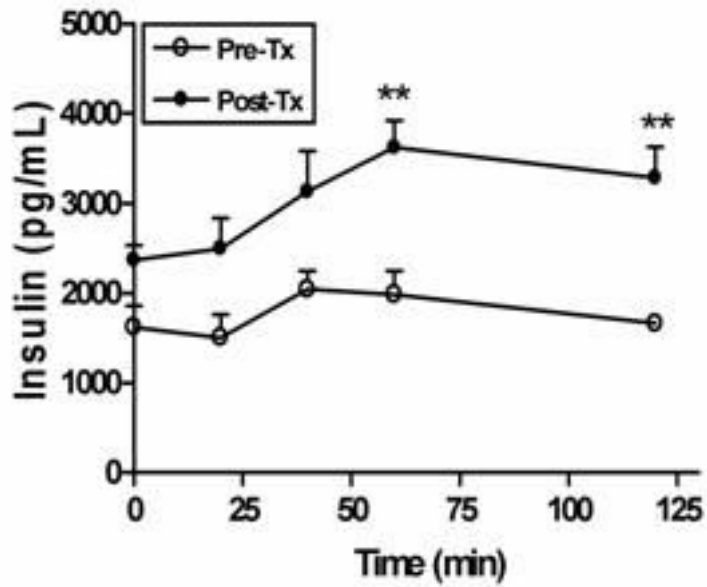
Method

We used OLETF rats (27 weeks-old, n=5) as type 2 diabetic model. We performed oral glucose tolerance test (OGTT, glucose 2 g/kg) before and after the oral administration of GLA. During administration of GLA (360 mg/kg daily) for 14 days, body weight and blood glucose level were measured. Also, we assayed blood glucose, serum insulin and active GLP-1 level before and after GLA administration.

Result

Basal mean fasting blood glucose level was 209±46.2 mg/dL and it decreased to 156.8±9.8 mg/dL after oral GLA administration for 14 days (p<0.01). Mean body weight was 637.2±11.4 g before GLA administration and it became 576±20.8 g after GLA administration. Mean serum active GLP-1 levels during OGTT were increased after oral GLA administration (p<0.05). Also mean serum insulin levels during OGTT were increased after oral GLA administration (p<0.01).





Conclusion

GLA could increase active GLP-1, serum insulin level and also could decrease blood glucose level in type 2 diabetic animal model. So, administration of GLA would be beneficial effect on blood glucose control in type 2 diabetes mellitus.

Nothing to Disclose: KWL, DML, JYK, SY, KYP, BJK

P3-509

Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of GSK1362885, a Novel Glycogen Phosphorylase Inhibitor, in Subjects with Type 2 Diabetes Mellitus.

J Lin PhD¹, LS Vasist PharmD¹, JS Stuart MS¹, RL Byerly BS¹ and RV Clark MD, PhD¹.

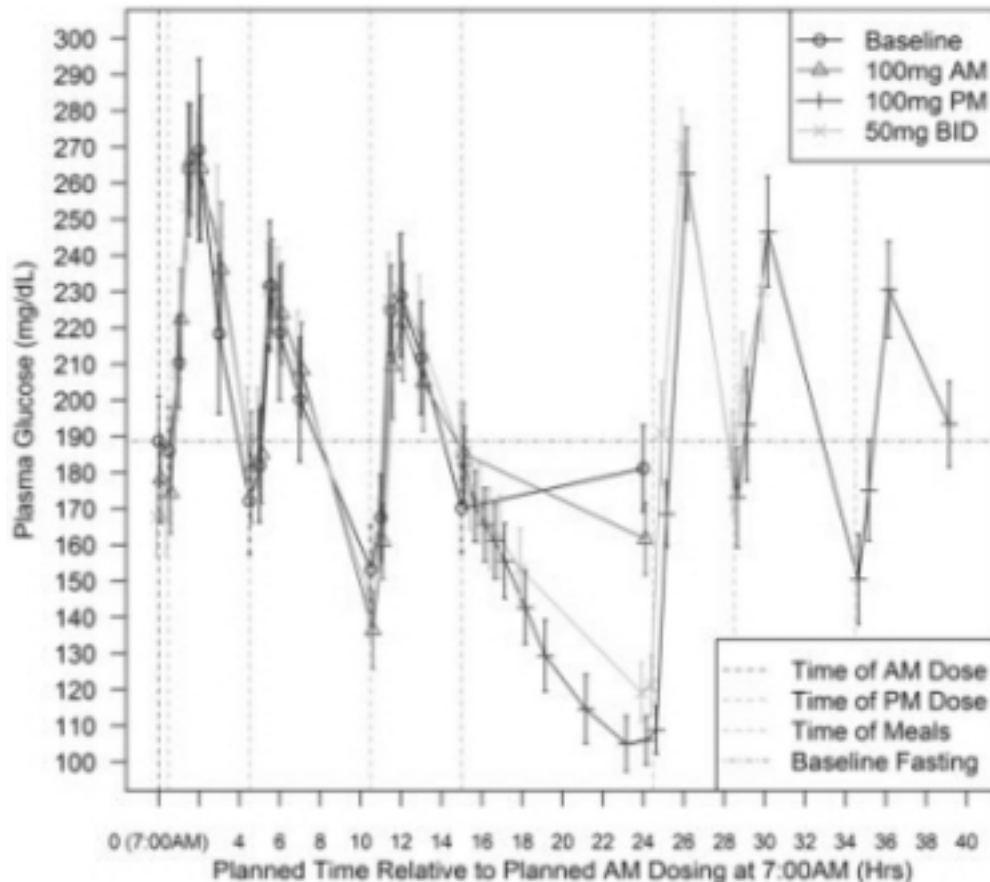
¹GlaxoSmithKline Research Triangle Park, NC.

Background: GSK1362885 is a novel glycogen phosphorylase inhibitor that reduces hepatic glucose output (HGO) by inhibiting glycogenolysis. Preclinical studies showed evidence of significant suppression of glycogenolysis with reduced fasting glucose over 3 weeks in marmosets. Reduction of glycogenolysis may offer an approach to reduce HGO that is distinct from metformin, which inhibits gluconeogenesis. In the first time in human study, GSK1362885 produced a marked reduction in glucagon induced glucose output in a dose and exposure-dependent manner, with effects reaching a plateau at 100mg.

Objectives/Methods: This was a randomized, open-label, crossover study, the first administration of GSK1362885 to T2DM subjects, to evaluate safety, PK/PD, and to compare single doses of 100mg AM, 100mg PM, and 50mg BID. T2DM subjects were enrolled (n=23 with 18 completers) with HbA1c between 5.8-11% and FPG ≤ 270mg/dL (1).

Results/Conclusions: GSK1362885 was safe and well-tolerated across the treatment groups. No clinically relevant changes in laboratory parameters, vital signs, body weight, or ECG parameters were observed. Adverse events were mild in intensity. GSK1362885 significantly reduced fasting plasma glucose compared to baseline [change from baseline (CFB) (mean±SE) = -27.1±6.6, -82.7±10.1, and -69.4±8.9 mg/dL, for 100mg AM, 100mg PM, and 50mg BID, respectively; p < 0.001 for all] (Figure 1). In addition, GSK1362885 significantly reduced the weighted mean glucose during the nocturnal period [CFB = -2.2±4.3, -43.8±8.4, and -29.0±6.8 mg/dL, respectively; p<0.001 for 100mg PM and 50mg BID] and during the 24-hour post-dose interval [CFB = -0.7±4.4, -16.5±6.8, and -8.0±5.3 mg/dL, respectively; p<0.05 for 100mg PM]. No apparent glucose reduction was seen during the post-prandial periods. These results demonstrate significant glucose lowering during the nocturnal period and support further investigation of GSK1362885 for the treatment of T2DM in repeat dose studies.

Figure 1: Mean (±SE) plasma glucose profiles (mg/dL) at baseline and following administration of GSK1362885



(1)ClinicalTrials.gov Identifier:NCT01013766

Disclosures: JL: Employee, GlaxoSmithKline. LSV: Employee, GlaxoSmithKline. JSS: Employee, GlaxoSmithKline. RLB:

Employee, GlaxoSmithKline. RVC: Employee, GlaxoSmithKline.

P3-510

Outpatient Insulin Pump Therapy Can Be Safely Continued during Hospitalization: A Review of More Than 100 Admissions.

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When diabetes patients on continuous subcutaneous insulin infusion (CSII, insulin pump) treatment require hospitalization, inpatient physicians often struggle with the appropriateness of allowing them to continue their therapy. Errors may occur unless specific hospital policies and procedures regarding the technology are in place (1). We previously developed guidelines (2) to assist inpatient practitioners on how to manage CSII users. We now have analyzed the most hospital records published to date relating to employment of inpatient CSII (3-5). We assessed adherence to our institutional policies, determined glycemic control, and examined if there were any adverse events related to inpatient pump use. From January 2006 to December 2009 we identified 129 hospitalizations involving 67 unique patients receiving CSII. Mean patient age was 55 years, diabetes duration 28 years, duration of pump therapy was 6 years, average length of hospital stay was 5 days, mean preadmission hemoglobin A1c was 7.3%; 97% were white, 88% had type 1 diabetes, and 56% were women. The number of admissions to our facility involving CSII patients has been increasing (25 in 2006, 17 in 2007, 40 in 2008, and 47 in 2009). According to institutional guidelines, use of CSII was deemed appropriate in 67% of these hospitalizations, among which endocrinology consultations were obtained in 88%; patient consent agreements were found in 83%, insulin pump order sets completed in 89%, admission glucose was checked in 100%, nursing assessments of pumps sites were documented in 89%, but bedside insulin pump flow sheets were found in only 56% of hospitalizations. The mean glucose for the entire length of stay was 176 mg/dl for hospitalizations where CSII was continued, and was comparable ($p=0.8$) to hospitalizations where CSII was not continued; hypoglycemia prevalence was similar between the two groups ($p=0.89$). There were no instances of pump site infections or fatalities related to CSII use. Our review confirms that appropriately selected patients on outpatient CSII can have therapy transitioned to the hospital safely. High compliance with inpatient CSII policies can be achieved, although there continues to be room to improve adherence. Hospital glycemic control among CSII users was similar to that achieved when pumps were discontinued, and further study is needed to determine how best to optimize glycemic control when CSII is employed in the hospital setting.

1. Cook CB. Are AHRQ WebM&M [serial online]. January 2009. Available at: <http://webmm.ahrq.gov/case.aspx?caseID=192>
2. Cook CB et. al. Diabetes Educator 2005;31:849
3. Leonhardi BJ et. al. Journal of Diabetes Science and Technology 2008;2:948
4. Bailon RM et. al. Endocrine Practice;2009 15:24
5. Noschese ML et. al. Endocrine Practice 2009;15:415

Nothing to Disclose: AAN, BJP, MEB, JCC, PBB, CBC

P3-511**LX4211, an SGLT2 Inhibitor, Shows Improvements in Cardiovascular Risk Factors over 4 Weeks in Patients with Type 2 Diabetes Mellitus.**

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Patients with type 2 diabetes mellitus (T2DM) often suffer from cardiovascular and metabolic co-morbidities, such as increased blood pressure and obesity. Small molecule inhibitors of the sodium glucose cotransporter type 2 (SGLT2) block glucose reabsorption by the kidney, resulting in glucose loss in the urine. This mechanism of action has the potential to provide not only glycemic control, but also cardiovascular and metabolic benefits for T2DM patients. Described here are the results of a four-week Phase 2 clinical study of LX4211, a once-daily orally-delivered SGLT2 inhibitor, in patients with T2DM.

Patients (N=36) with T2DM received one of two oral doses of LX4211 monotherapy, given as 150 mg or 300 mg once daily, or matching placebo, for 28 days. Preliminary data showed significant and sustained glucosuria over the 28-day dosing period for both dose levels when compared to placebo. Adverse events were generally mild and evenly distributed across all dose groups, including placebo, with no evidence of dose-limiting toxicities observed. Additionally, the following improvements in cardiovascular risk factors were observed over 28 days (table).

Table

| Change from Baseline | LX4211 150 mg (n =12) | LX4211 300 mg (n=12) | Placebo (n=12) |
|-----------------------------------|------------------------------|-----------------------------|-----------------------|
| Seated Systolic BP (mmHg) | -10.3 | -13.1 | -4.3 |
| Seated Diastolic BP (mmHg) | -5.8 | -5.3 | -2.9 |
| Serum Triglyceride (mg/dL) | -66.6 | -62.8 | -20.2 |
| Change in Weight (%) | -3.4 | -3.7 | -2.2 |

These results demonstrate that within a four-week treatment period, patients receiving LX4211 exhibited improvements in blood pressure control, weight reduction, and triglyceride levels that were associated with improvements in glycemic parameters. The observed improvements in cardiovascular risk factors associated with LX4211 treatment are particularly important given the substantially elevated risk for morbidity and mortality from cardiovascular disease seen in patients with T2DM, and warrant further evaluation of LX4211 in these patients.

Disclosures: JF: Employee, Lexicon Pharmaceuticals, Inc. DAR: Employee, Icon Development Solutions; Investigator, Lexicon Pharmaceuticals, Inc. KF: Employee, Lexicon Pharmaceuticals, Inc. KC: Employee, Lexicon Pharmaceuticals, Inc. AT: Employee, Lexicon Pharmaceuticals, Inc. MS: Employee, Lexicon Pharmaceuticals, Inc. DP: Employee, Lexicon Pharmaceuticals, Inc. JB: Employee, Lexicon Pharmaceuticals, Inc. BZ: Employee, Lexicon Pharmaceuticals, Inc. PB: Employee, Lexicon Pharmaceuticals, Inc.

P3-512

Development of Optimal Kids Insulin Dosing System (KIDS) Formulas for Young Children with Type 1 Diabetes Mellitus (T1DM).

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Background and Aims: Patients with diabetes on basal bolus insulin regimen using either multiple daily injections (MDI) or continuous subcutaneous insulin infusion (CSII) must deem both carbohydrate meal content and pre-meal blood glucose (BG) values for precise insulin dosing. Current insulin dosing formulas can only estimate insulin sensitivity or correction factor [$CF=1700/\text{total daily dose of insulin (TDD)}$] and insulin to carbohydrate ratio [$ICR=6.2*\text{body weight (BW, kg)/TDD}$] in adolescents and adults. To date predictive formulas for precise insulin dosing have not been developed for management of young T1DM children.

Materials and Methods: Consecutive one-year data from a group of 14 young patients (8F/6M) aged 3.9 ± 0.8 years with DM duration of 2.0 ± 0.8 years, transitioned from MDI to CSII was analyzed to identify parameters governing optimal insulin dosing. Basal insulin, TDD, CF, ICR, body mass index (BMI), mean amplitude of glycemic excursion (MAGE) by continuous glucose monitoring and HbA_{1c} were evaluated at baseline and every 3 months. The slopes of the CF vs. 1/TDD, bolus vs. TDD, ICR vs. 1/TDD, and CF vs. ICR were determined. Baseline patient data on MDI or non-target (NTG) and data on CSII or target (TG) were analyzed. The least squares estimates of the correlation constants and slopes were used to fit the models. Analysis of variance and F-tests were used to evaluate the differences between slopes and standard deviations, respectively.

Results: The statistical tests showed that the use of the Kids Insulin Dosing System (KIDS) slope constants in the TG was associated with MAGE as compared to the NTG ($p<0.0001$) without significant changes in BMI (16.6 ± 1.5 vs. 16.7 ± 1.4 kg/m²) and HbA_{1c} values (8.0 ± 0.50 vs. $7.8 \pm 0.40\%$). The relationship between CF and TDD changed significantly during CSII compared to baseline MDI ($p<0.0001$), whereas the coefficients for ICR and TDD relationship remained relatively unchanged. The KIDS formulas estimated $TDD = 0.71*BW$; basal insulin = $0.30*TDD$; $CF = 2800/TDD$; and $ICR = 13.5*BW/TDD$.

Conclusions: The interrelationships between ICR, CF, basal insulin and TDD remained stable on CSII and were accompanied by decreased BG excursions. The vast differences between KIDS formulas and those used in older patients are primarily due to lower BMI and higher insulin sensitivity in children. The KIDS formulas can yield consistent and easy estimates of insulin dosing factors in very young patients with T1DM.

Nothing to Disclose: RA, RH, MD, EP

P3-513

The Impact of Hispanic Ethnicity and the Titration Effect of LY2189265, a Glucagon-Like Peptide 1 Analog, on Metabolic Outcome Measures in Patients with Uncontrolled Type 2 Diabetes Mellitus (T2DM): An EGO Study Analysis.

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LY2189265 (LY) is a once-weekly, glucagon-like peptide 1 analog being developed for the treatment of T2DM. The aim of this phase 2, placebo-controlled, double-blind study was to evaluate the effect of once-weekly LY compared to placebo on metabolic outcome measures over 16 weeks in overweight/obese patients with uncontrolled T2DM. Patients (n=262) were randomized to once-weekly subcutaneous injections of either placebo or 1 of 3 LY dose regimens: 1) 1.0 mg, 16 weeks; 2) 0.5 mg, 4 weeks then titrated to 1.0 mg, 12 weeks; or 3) 1.0 mg, 4 weeks then titrated to 2.0 mg, 12 weeks. The primary metabolic outcome measure was glycemic control, measured by HbA1c change from baseline at 16 weeks. Secondary measures were body weight, test meal postprandial glucose (PPG), and PPG excursion.

Baseline characteristics for all randomized patients (n=262; 49% female; mean T2DM 8.3 years; mean BMI 33.9 kg/m²; and mean HbA1c 8.2%) were not different between treatment groups. Patients self-identified as Hispanic (H) and as non-Hispanic Caucasians (NHC) and demonstrated baseline characteristics similar to prior studies with H showing a higher baseline HbA1c (n=88, 8.44±0.97%) that was statistically significant (p=.006) when compared to NHC (n=150, 8.09±0.88%). Compared to placebo, statistically significant improvements were observed in all metabolic outcomes after treatment with LY.

| Outcome Measures | Placebo | LY 0.5/1.0 mg | LY 1.0/1.0 mg | LY 1.0/2.0 mg |
|-----------------------------------|---------|---------------|---------------|---------------|
| ΔHbA1c (%), LS Mean | -0.3 | -1.3* | -1.3* | -1.5* |
| ΔWeight (kg), LS Mean | -0.1 | -1.6* | -1.4* | -2.5* |
| Test Meal PPG AUC, mean | 36.4 | 30.7* | 32.2* | 28.2* |
| Test Meal PPG AUC Excursion, mean | 10.9 | 8.9* | 9.9* | 8.2* |

*p<0.05 vs placebo; AUC, area under the curve; PPG, postprandial glucose

With LY treatment, the H population had a larger reduction (p=0.02) in HbA1c (n=61, -1.47±0.99%) as compared to NH (n=111, -1.14±0.80%) at endpoint. Controlling for baseline HbA1c, the H group had a larger decrease (p=.003) in postprandial AUC glucose excursion (n=56, -2.82±3.78) compared to NHC (n=66, -0.05±6.17).

In conclusion, adjunctive administration of LY resulted in significant reductions in HbA1c, body weight, and both fasting and postprandial serum glucose. Additionally, reductions in HbA1c and postprandial glucose excursion were significantly greater in the H population as compared to the NHC group. Further studies of longer duration will be needed to assess possible additional benefits/risks associated with LY therapy.

Disclosures: EJB: Employee, Lilly USA, LLC. CLC: Employee, Lilly USA, LLC. JEN: Employee, Lilly USA, LLC. ABT: Employee, Lilly USA, LLC. RJT: Employee, Lilly USA, LLC. JS: Consultant, Lilly USA, LLC. LCG: Employee, Lilly USA, LLC.

P3-514

Liraglutide, a Once-Daily Human GLP-1 Analog, Reduces Systolic Blood Pressure with Minimal Impact from Weight Loss in Subjects with Type 2 Diabetes.

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Background: Hypertension is more common in individuals with type 2 diabetes (T2D) than in the general population, and it is a major risk factor for myocardial infarction and stroke. Modest weight loss can result in significant long-term reductions in blood pressure and thereby reduce the risk for hypertension.

In phase 3 clinical trials, in addition to improving glycated hemoglobin (HbA1c) by 1.0-1.5%, liraglutide also improved systolic blood pressure (SBP) by 2-6 mmHg and produced sustained bodyweight (BW) reductions of 2-3 kg. However, the precise nature of the relationship between the effect of liraglutide on SBP and BW is not well-characterized.

Methods: A meta-analysis of six randomized phase 3 clinical trials (n=3967) has been performed in the liraglutide (1.2 and 1.8 mg) and placebo arms to investigate the relationship between changes in SBP and BW from baseline at each post randomization visit up to 26 weeks using an analysis of covariance (ANCOVA) model with treatment, trial, previous OAD treatment as fixed effects, country as random effect, and change in BW from baseline as covariate. The

percentage of the change in SBP predicted by the change in BW is given by the $100 \times R^2$ (the square of Pearson correlation coefficient), a *goodness-of-fit index*. The closer the R^2 is to 1, the stronger the relationship between change in SBP and BW.

Results: The analyses show a very consistent pattern over time of a very weak correlation between change in SBP and change in BW. At week 2, up to 2% of the change in SBP could be predicted by the change in BW for both liraglutide arms and placebo. At week 26, both liraglutide doses and placebo have a percentage of R^2 below 2%. The Pearson correlation between the change in SBP and the predicted change in SBP obtained from ANCOVA model is also very weak with R^2 below 4%.

Conclusion: Only a small part of the observed effect of liraglutide on change in SBP is predicted by change in BW. Therefore, the effect of liraglutide on SBP cannot be explained by weight loss alone. Mechanistic studies will be required to better characterize the effect of liraglutide on SBP.

| Treatment | Week | LS-means of change in SBP | LS-means of change in BW | Pearson Correlation between change in SBP and change in BW | R ² =Square of Pearson Correlation between change in SBP and change in BW | Pearson Correlation between Change in SBP and predicted Change in SBP from ANCOVA model |
|-------------------|------|---------------------------|--------------------------|--|--|---|
| Liraglutide 1.2mg | 2 | -3.26 | -0.82 | 0.0823 | 0.007 | 0.115 |
| | 4 | -3.80 | -1.34 | 0.0775 | 0.006 | 0.140 |
| | 8 | -3.10 | -1.36 | 0.0907 | 0.008 | 0.120 |
| | 12 | -3.47 | -1.46 | 0.1021 | 0.010 | 0.147 |
| | 18 | -3.65 | -1.23 | 0.0773 | 0.006 | 0.098 |
| | 26 | -2.97 | -1.17 | 0.0920 | 0.008 | 0.149 |
| Liraglutide 1.8mg | 2 | -2.89 | -0.84 | 0.1293 | 0.017 | 0.165 |
| | 4 | -3.21 | -1.59 | 0.0931 | 0.009 | 0.140 |
| | 8 | -2.68 | -1.67 | 0.1246 | 0.016 | 0.173 |
| | 12 | -2.91 | -1.84 | 0.1404 | 0.020 | 0.182 |
| | 18 | -3.47 | -1.71 | 0.1161 | 0.013 | 0.132 |
| | 26 | -2.88 | -1.77 | 0.1305 | 0.015 | 0.198 |
| Placebo | 2 | -0.74 | -0.08 | 0.0428 | 0.002 | 0.140 |
| | 4 | 0.56 | 0.04 | -0.0183 | 0.000 | 0.096 |
| | 8 | -0.27 | -0.43 | 0.0932 | 0.009 | 0.080 |
| | 12 | -0.49 | -0.52 | 0.0816 | 0.007 | 0.096 |
| | 18 | -1.53 | -0.46 | 0.1161 | 0.013 | 0.125 |
| | 26 | -0.85 | -0.51 | 0.0923 | 0.009 | 0.107 |

SBP=systolic blood pressure, BW=body weight

Disclosures: RRH: Speaker Bureau Member, Novo Nordisk; Medical Advisory Board Member, Novo Nordisk. VF: Investigator, Novo Nordisk; Consultant, Novo Nordisk. RTyP: Employee, Novo Nordisk. JB: Employee, Novo Nordisk. JP: Consultant, Novo Nordisk.

P3-515

The Impact of Metformin on Vitamin B12 Levels in People with Type 2 Diabetes.

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Background: Type 2 diabetes (DM2) is a disorder of glucose metabolism that is increasing in prevalence. In the United States there are 23.5 million, or 10.7% of all people age 20 and over diagnosed with diabetes. Additionally, 1.6 million new cases are diagnosed in this age group yearly. The 2009 American Diabetes Association's Standards of Medical Care recommends metformin (MET) as a first-line therapy for DM2. Studies have suggested that MET disturbs the Ca-dependent uptake of the B12-IF complex in the ileum resulting in B12 deficiency. Because of the large population with DM2 and the high use of MET there is a significant at risk pool. B12 deficiency is clinically important due to adverse neurological and hematological effects. The purpose of this study is to characterize the relationship of MET exposure to hypovitaminosis B12 in people with DM2.

Methods: 50 male veterans with DM2 treated with MET were recruited and informed consent was performed. Demographics, medical history, and dietary information were obtained. Height and weight were measured for calculation of BMI. Subjects' length of time taking MET was recorded and blood was drawn for B12 measurements. Secondary endpoints included Ca²⁺, PO₄⁻, Mg²⁺ levels, body composition measured by bioimpedance, and metformin exposure calculated in mg/years.

Results: 50 veteran subjects, mean age 60 yrs [95% CI, 57-63] and mean time on metformin 5.4 yrs [95% CI, 0.4-12] completed the study. In this study, there was a significant difference in the primary endpoint of B12 levels in subjects taking metformin compared to a random sample of 500 veterans not taking metformin (349.1 pg/mL [95% CI, 306-393] vs. 530.9 pg/mL [95% CI, 489-573], p=0.0065) respectively. Of the 50 subjects in the study, 29% had clinically low B12 values (<250 pg/mL) compared to the 500 random subjects not taking metformin, who had only 11.5% clinically low B12 levels.

Conclusion: Vitamin B12 levels in the study population of veterans with DM2 treated with MET were 181.8 pg/mL lower compared to a random sample of the veteran population not taking MET. The percent of participants with clinically low B12 levels were also lower in the MET group versus the random sample not taking MET (29% vs 11.5%) respectively. The results of this study indicate a need for further research on the detrimental effects of metformin on vitamin B12 levels and could provide a wide impact on the management and care of people with DM2.

Nothing to Disclose: RAP, QTN, CMC, RLM, DLC, STS]

P3-516

Preliminary Results: Short-Term Safety and Efficacy of Meal Replacement and a Lifestyle Plan in Obese Low Income Hispanic Patients on Insulin.

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Background: In T2DM weight loss is known to improve glycemic control as well as overall health but few studies have evaluated safe and effective strategies in poorly controlled pts with T2DM on insulin.

Aim: To ascertain the safety and efficacy of a standardized lifestyle(LS)plan to promote weight loss in obese, poorly controlled Hispanic patients with T2DM on insulin.

Study Design: Prospective, randomized 1yr study. Target accrual: 15 patients/group. Subjects are stratified by BMI and duration of DM.

Methods: Patients are recruited from a diabetes clinic in East LA serving low-income Hispanic patients. After a 2 week run-in, subjects are randomized to LS or standard care(SC). The LS receives 3 months food as meal replacements (MR) (approx 1400 cal/day) and has weekly follow-up with the study MD. Thereafter visits are 2 weekly for 3 months with 1MR/day and then monthly with 1MR/day. Monthly lifestyle class is offered and exercise is encouraged. We compared 3month data with paired and nonpaired ttest and chi-square analysis

Results: So far 18 subjects are enrolled. [8 LS: 2 dropouts (nausea, social);10 SC: 1 dropout(colon cancer), 2<3months]. See tables. There was no significant difference between the groups at baseline. By 3 months the LS had lost significantly more weight and had a significantly lower BMI with a trend to lower A1c and daily insulin dose. When comparing changes within groups the LS had a significant decrease in weight (p=0.003), BMI(p=0.003) and total insulin dose (p=0.03) not seen in the SC. No episodes of significant hypo- or hyperglycemia were reported.

Baseline Values

| | Age | Gender | Years with known DM | A1C(%) | Weight (lbs) | BMI | Total Daily Insulin (Units) |
|---------------|-----------|--------|---------------------|----------|--------------|------------|-----------------------------|
| Standard Care | 48(8.1) | 0m:7f | 14.5(10.9) | 9.8(1.2) | 205.9(47.1) | 39.3(11.2) | 87.1(93.7) |
| Lifestyle | 48.3(4.7) | 1m:5f | 13.3(5.9) | 9.8(1.5) | 250.4(61.0) | 47.7(17.1) | 82.5(80.0) |
| p | ns | ns | ns | ns | ns | ns | ns |

Between Group Change over 3 Months

| | Weight (lb) | BMI | A1c | Total Daily Insulin (IU) |
|---------------|-------------|-------------|-------------|--------------------------|
| Standard Care | -0.13(7.43) | 0.02(1.47) | -0.41(2.04) | -10.0(2.0) |
| Lifestyle | -13.9(6.3) | -2.59(1.19) | -1.52(1.92) | -40.0(31.4) |
| p | 0.003 | 0.01 | 0.34 | 0.07 |

Conclusion: Preliminary results show that in a low-income Hispanic population with longstanding DM on insulin an intensive formal lifestyle and meal replacement program is associated with safe and significant short-term weight loss not seen with standard care.

Nothing to Disclose: EOB, W-AL, MW, ER, SS-D, AP

P3-517

A Monthly Dosed GLP-1 Analog for Treatment of Type 2 Diabetes Mellitus.

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VRS-859, a GLP-1 analog XTEN fusion protein, is projected to have a terminal half-life of 140 hr and an absorption phase of 100 hr after subcutaneous administration in humans (1) (Fig 1). Previous attempts by others to achieve monthly dosing failed due to persistent nausea and vomiting as well as lack of prolonged therapeutic effect. The low peak to trough ratios of VRS-859 after repeated dosing should reduce the gastrointestinal side effects observed with GLP-1 analogs. In normal mice, a single VRS-859 dose of 120 nmol/kg provided glycemic control against a glucose challenge of 1.5 g/kg at 48 hr post-dose. In ob/ob mice, 120 nmol/kg VRS-859 dosed every other day resulted in weight loss equivalent to 12 nmol/kg of exenatide dosed twice daily. Diabetes induced obese mice were treated with VRS-859 every 2 days (120 nmol/kg) or every 4 days (240 nmol/kg). Both treatment approaches resulted in equivalent weight loss compared to continuous infusion of exenatide (7 nmol/kg/d; 30 mcg/kg/d) and better glycemic control was noted in the every 2 day dosing of VRS-859 (Fig 2).

A single subcutaneous 100 mg/kg dose of VRS-859 in rats did not result in any acute toxicological changes except for weight loss, which is an expected pharmacological effect. 28 day GLP toxicology studies in monkeys and mice are currently ongoing and will be presented. Human clinical trials in type 2 diabetes patients will be initiated in Q2 2010 to demonstrate that a human dose of 100 mg VRS-859 administered monthly as a single 1 mL subcutaneous injection with a small gauge needle will provide therapeutic benefits comparable to currently developed weekly-dosed GLP-1 products in humans.

Figure 1: Predicted human pharmacokinetics and therapeutic window (shaded) for a monthly 100 mg dose of VRS-859

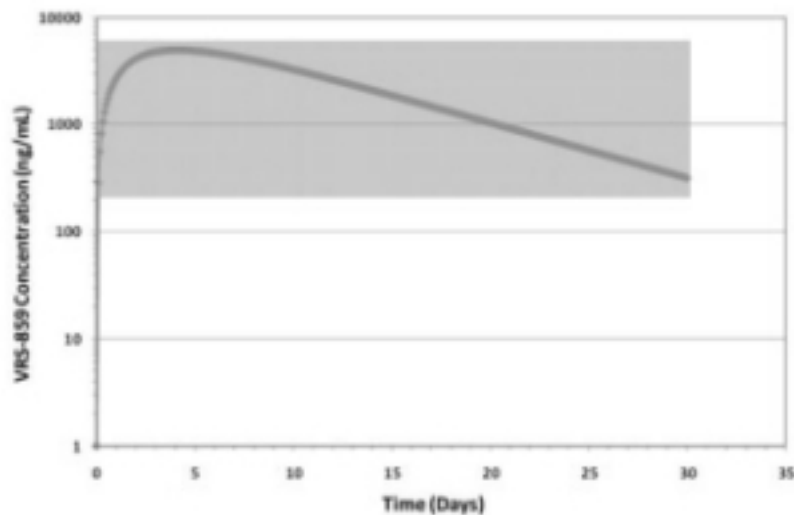
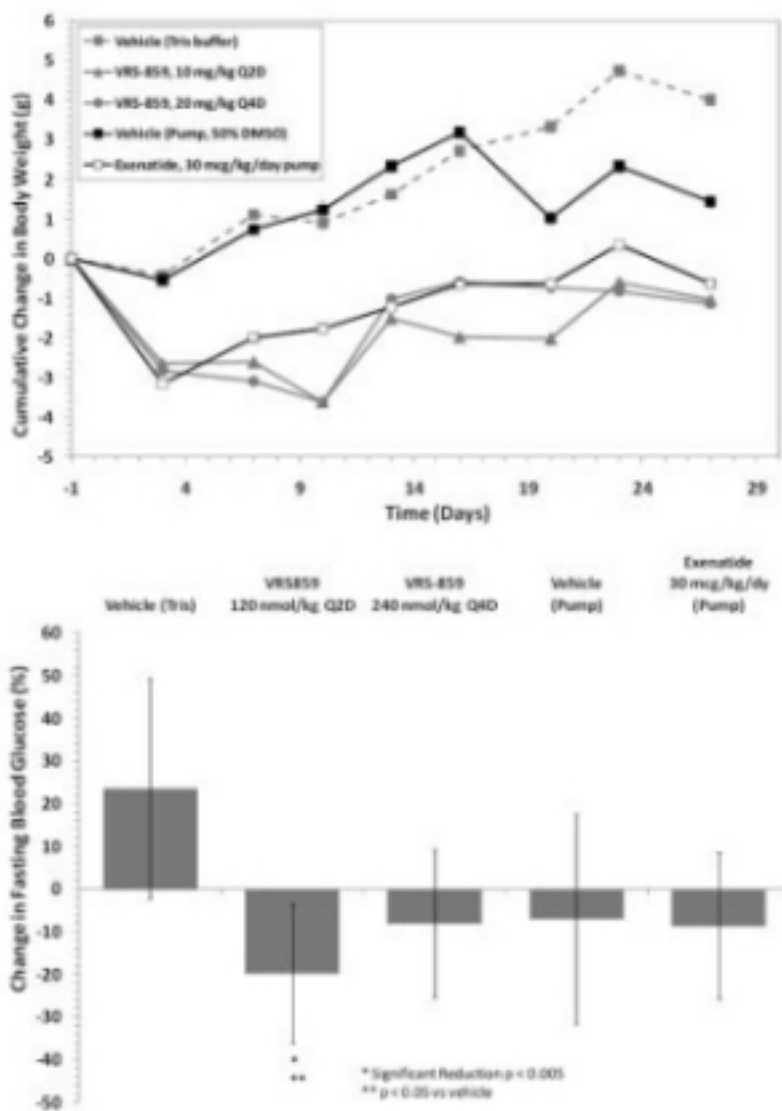


Figure 2: Weight loss and change in fasting blood glucose (Baseline vs Day 29) after treatment with VRS-859 or exenatide.



(1) Schellenberger et al., Nat. Biotech. 2009 p. 1186

Nothing to Disclose: JLC, NG, WT, BS, LL, YY, JS

P3-518

The Risk of Overall Mortality in Patients with Type 2 Diabetes Receiving Glipizide, Glyburide, or Glimepiride Monotherapy. A Retrospective Analysis.

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Background: Sulfonylureas have been associated with a higher mortality risk when compared to other oral anti-diabetic agents, specifically metformin. This may not be a class effect of sulfonylureas, but rather secondary to differences in properties inherent to the individual sulfonylureas: hypoglycemic risk, sulfonylurea receptor selectivity, and effects on myocardial ischemic preconditioning.

Purpose: To assess the relationship of individual sulfonylureas and the risk of overall mortality in a large cohort of patients with type 2 diabetes.

Methods: A retrospective cohort study was conducted using an academic health center enterprise-wide electronic health record (EHR) system to identify 11,141 patients with type 2 diabetes (4,279 initiators of monotherapy with glyburide, 4,325 initiators of monotherapy with glipizide, and 2,537 initiators of monotherapy with glimepiride), seen in the outpatient clinics, ≥ 18 years of age, with and without a history of coronary artery disease (CAD), and not on insulin or a non-insulin injectable at baseline. The patients were followed for mortality by documentation in the EHR and social security death index. Multivariable Cox models were used to compare cohorts.

Results: Baseline characteristics for the entire cohort include mean age \pm SD of 66.6 ± 13.2 years, 44.3% female, and 76.2% Caucasian. No statistically significant difference in the risk of overall mortality was observed among these agents in the entire cohort: glipizide vs. glyburide (HR 1.04; 95% CI 0.94-1.15), glipizide vs. glimepiride (HR 1.05; CI 0.92-1.19), or with glyburide vs. glimepiride (HR 1.00; 95% CI 0.89-1.14). However, we did find evidence of a trend towards an increased overall mortality risk with glyburide vs. glimepiride (HR 1.36; CI 0.96-1.91) and glipizide vs. glimepiride (HR 1.39; 95% CI 0.99-1.96), in those with documented CAD.

Conclusions: Our results did not identify an increased mortality risk among the individual sulfonylureas but did suggest that glimepiride may be the preferred sulfonylurea in those with underlying CAD. The literature contains conflicting results regarding whether an increased mortality risk accompanies the various sulfonylureas. This discrepancy would support prospective studies to determine if the difference in pharmacologic properties inherent to individual sulfonylureas translates into differences in the risk of adverse cardiovascular outcomes and overall mortality, especially in patients with preexisting CAD.

Disclosures: RSZ: Speaker, Daiichi Sankyo Inc, Merck & Co., Pfizer, Inc., Novo Nordisk, GlaxoSmithKline, Bristol-Myers Squibb.

Nothing to Disclose: KMP, MWK, CY, BJW, SA, AJ, AA

P3-519

An Innovative Method To Facilitate Frequently-Adjusted Insulin Therapy Heuristics (FAITH™).

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Insulin is the only drug for diabetes that does not have a therapeutic window. Thus, most patients should be able to achieve therapy goals ($A1C \leq 7\%$) if appropriate formulations and dosages are used. Nevertheless, two-thirds of insulin-treated patients in the USA do not achieve therapy goals and many develop complications. Many studies have demonstrated that when insulin dosages are frequently adjusted, the majority of patients realize the full benefit of their regimens. Yet, the growing mismatch between patients' needs and care-givers' availability permits only infrequent clinic adjustments. We have developed the FAITH approach capable of recommending optimized insulin dosages to patients between clinic appointments.

In a retrospective trial, 2,520 insulin dosage adjustment episodes were used as input to the FAITH algorithm and its dosage recommendations were compared to the ones made by a study-team. The clinical data was obtained from 26 older adults with suboptimally controlled type2 diabetes, treated with intensive-insulin-therapy for a year. Dosages were adjusted every 2 weeks. Therapy goals ($A1C < 7\%$) were achieved and maintained by more than 88% of the subjects. In more than 95% of the cases the FAITH algorithm provided recommendations clinically equivalent to those of the study team. In a prospective trial, 568 insulin dosage adjustments were used for comparison. The clinical data was obtained from 14 subjects with suboptimally controlled type2 and type1 diabetes, treated with intensive-insulin-therapy for 12 weeks. The study-endocrinologist adjusted dosages weekly without direct contact with the subjects. All subjects responded to therapy with $1.96 \pm 1.5\%$ average improvement in A1C without excessive hypoglycemia. The FAITH algorithm and the study-endocrinologist agreed more than 99% of the time.

Hygieia INC. plans to provide the FAITH approach embedded in a state-of-the-art blood glucose meter. After being initiated by the care-giver, this device will routinely optimize patients' insulin dosage recommendations without any additional effort or cost. We foresee that FAITH will enable patients to make frequent insulin dosage adjustments and achieve and sustain glycemic control.

Disclosures: IH: Speaker, Hygieia INC. EB: Founder, Hygieia INC.

P3-520

Vascular Endothelial Impairment and Bone

Marrow-Derived CD34⁺/133⁺ Cells in Diabetic Subjects with Erectile Dysfunction.

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The present study was undertaken to determine vascular endothelial impairment and erectile dysfunction (ED) in subjects with type 2 diabetes mellitus. One hundred type 2 diabetic men were enrolled in the present study. Endothelial function and exercise capacity were evaluated by flow-mediated dilatation (FMD) and cardiopulmonary exercise stress test (CPX). Also, endothelial progenitor cells (EPCs) were determined by FACS. In the 42 ED diabetic subjects (64 ± 8 years, mean ± SD) FMD and anaerobic threshold (AT) after exercise tolerance were significantly less than those in the 58 subjects with normal erectile function (62 ± 14 years) (FMD: 2.8 vs. 3.8 %, p=0.038, and AT: 11.2 vs. 12.7 ml/kg/min, p=0.022). Basal levels of bone marrow-derived CD34⁺/133⁺ cells were comparable in both the diabetic subjects with and without ED, but exercise tolerance significantly increased the number of CD34⁺/133⁺ cells in both groups (49 to 60 cells/100ml, p=0.015, and 72 to 99 cells/100ml, p=0.0003). The diabetic subjects were subgrouped according to the development of autonomic neuropathy. In the subjects having the coefficient of variation of the R-R interval (CVRR) more than 2 %, FMD was significantly reduced in the ED subjects than those without ED (2.6 vs. 3.8 %, p=0.029). In response to exercise tolerance the number of CD34⁺/133⁺ cells increased in both the diabetic subjects with ED (57 to 76 cells/100ml, p=0.003) and without ED (92 to 125 cells/100ml, p=0.007). On the contrary, in the diabetic subjects with autonomic neuropathy (CVRR <2.0 %), FMD was reduced and there was no difference in FMD between the subjects with and without ED. The exercise tolerance increased the number of CD34⁺/133⁺ cells in the subjects without ED (45 to 60 cells/100ml, p=0.023), but it disappeared in those with ED (37 to 40 cells/100ml, ns). Similar results were obtained with the ED diabetic subjects according to the progression of nephropathy. These findings may indicate that ED diabetic subjects have endothelial impairment during the early period of diabetic complications, whose deranged endothelial function is concomitantly repaired by promoting bone marrow-derived endothelial progenitor cells.

Nothing to Disclose: MM, HT, TS, AI, HT, MK, SI

P3-521

Gastric Dysfunction and Glucose Control.

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Post-prandial glucose (PPG) plays a significant role in glucose homeostasis. We studied the relationship of PPG and gastric emptying time in 27 male diabetics. CGMS (Medtronic) was used to determine 72 hr glucose profile and gastric emptying time (GE time) was assessed with ⁹⁹Tc-labeled solid food. In diabetics with gastroparesis (group A, N=16) and without gastroparesis (group B, N=11) the clinical data (MeanSD), i.e., age, BMI, DM duration (DDM), HbA1c, serum creatinine clearance (cr cl) and total daily insulin dose (TDD) were not different between groups statistically; actual values were 66±10.9 vs. 61±10.4 yrs age, 7.6±1.4 vs. 7.9±1.2 % HbA1c, 31±4.8 vs. 30±7.4 BMI, 65±32.1 vs. 69±24.1 ml/min cr cl, 15±11.1 vs. 17±11.5 yrs DDM, 87±63.6 vs. 60±54.2 units TDD respectively. The CGMS data were used to calculate the percentage area under the curve (AUC) of glucose for every hr for 4 hrs following breakfast; peak glucose values after breakfast and time to reach the peak glucose were calculated.

PPG Profile in Diabetics with or without gastric dysfunction (GD)

| | With GD (N=16) | Without GD (N=11) | p-value |
|---|----------------|-------------------|---------|
| GE Time (t _{1/2} minutes) | 198 ± 110.5 | 49 ± 11.8 | 0.00 |
| Average % AUC 1st Hour Post-Breakfast (% of four hours) | 25 ± 3.5 | 21 ± 4.5 | 0.02 |
| Time to Peak Glucose (minutes) | 99 ± 47 | 161 ± 94.4 | 0.03 |
| % Increase to Peak Glucose After Breakfast | 34 ± 19.2 | 92 ± 61 | 0.001 |
| % Time of Nocturnal Hypoglycemia | 3.5 ± 6.0 | 9 ± 7.9 | 0.05 |

Average 24 hr glucose profile was analyzed as percentage time distribution of glucose level for hyperglycemia (glucose >140 mg/dL, 65±22.8 vs. 60±16.4%), normoglycemia (glucose 70-140 mg/dL, 32±21 vs. 35±14.2%) and hypoglycemia (glucose <70 mg/dL, 3±3.6 vs. 5±3.8%) in subjects with or without gastroparesis and the data did not reflect any statistical significance.

Bivariate correlation analyses in group A subjects showed that gastric emptying times negatively correlates with nocturnal hypoglycemia (r^2 0.15, $p < 0.05$), negative correlation with peak glucose excursion post-prandially (r^2 0.34, $p < 0.001$), and a negative correlation with time to peak glucose (r^2 0.17, $p < 0.03$). In contrast, there was a positive correlation of gastric emptying time with % of AUC of glucose in the first hour (r^2 0.21, $p < 0.02$). Conclusion, male type 2 diabetics with delayed gastric emptying time have early, but diminished glucose increment postprandially and it correlates negatively with nocturnal hypoglycemia.

Nothing to Disclose: PZ, BT, SPS

P3-522

Renal Insufficiency and Correctional Insulin Use Increase the Risk of Inpatient Hypoglycemia in a Large Multi-Hospital Health System.

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Severe hypoglycemia in hospitalized patients has been associated with increased mortality and has been added to the list of "never" events by the CMS 2009 Inpatient Prospective Payment Rule. The aim of this study was to identify factors in hospitalized patients that increase the risk for severe hypoglycemia. We conducted a case-control study looking at patients at 6 acute care community hospitals. Severe hypoglycemia was defined as a blood glucose of <41 mg/dL. 10 cases were identified at each hospital along with 25 controls using the hospitals' information and laboratory systems. In 2008, 11,500 hypoglycemic fingerstick glucoses were recorded in the laboratory systems of the 6 hospitals. There was significant variation among the 6 hospitals. At hospital "A" the proportion of hypoglycemic point-of-care blood glucoses was 0.30% as compared to hospital "B" where the proportion was 0.76%. None of the patients at hospital "A" were prescribed correctional insulin, whereas over half of the patients at hospital "B" received this form of insulin therapy. In the case control study, cases and controls were well matched for A1C, race, age, length of stay, hemoglobin, and leukocyte count. Cases of hypoglycemia were more likely to be male ($p=0.02$), admitted with a principle diagnosis of an infectious disease ($p=0.003$), and less likely to have been admitted for surgery. The mean creatinine was significantly higher in cases of hypoglycemia (2.88 vs. 1.82, $p=0.002$). The admission glucose and median glucoses were similar in cases and controls. The lowest glucoses during the admission was, of course, lower in the cases, but the highest glucoses during the admission was higher in the cases than the controls. In reviewing data from the pharmacy database, cases were more likely to have been treated with correctional insulin (odds ratio 2.0, 95% CI 1.0 to 4.0) and basal insulin (odds ratio 2.8, 95% CI 1.5 to 5.3). The risk of hypoglycemia was greatest in patients with renal insufficiency who received correctional insulin (odds ratio 3.3, 95% CI 1.3 to 8.0). In conclusion, severe hypoglycemia is common in hospitalized patients. The incidence may differ widely between institutions and may be due to differences in prescribing correctional insulin. It may be prudent to avoid the use of correctional insulin in patients with renal insufficiency.

Nothing to Disclose: SKS, JJ, MFM, LM, MES

P3-523

Analysis of Elevated Amylase and Lipase Levels in Type 2 Diabetes Patients Assessed during a Randomized Clinical Trial: The EGO Study.

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Elevations in serum lipase and amylase are a critical component in the diagnosis of pancreatitis, but, the prevalence of elevated enzyme levels in T2DM is still not well characterized.

The purpose of this analysis is to assess serum lipase and amylase levels in a cohort of 440 T2DM patients who were screened for the EGO study. This randomized, double-blind clinical trial compared the glucagon-like peptide-1 analog LY2189265 (LY) versus placebo in overweight and obese patients with suboptimal control of T2DM despite therapy with 2 oral antihyperglycemic agents.

Study participants were administered once-weekly subcutaneous injections of either placebo or 1 of 3 LY doses: 1.0 mg, 0.5 mg titrated to 1.0 mg, or 1.0 mg titrated to 2.0 mg. Amylase and lipase levels were obtained at screening and after 16 weeks of treatment. Simple linear regression analyses tested associations between baseline and endpoint factors and ranked change in lipase during treatment (logistic regression examined associations between factors and categorical endpoint lipase level). Factors ($p < .10$) from the bivariate analysis were analyzed by multiple linear or logistic regression using backward selection (type I error of .10).

Of 440 patients screened [48% female; 57 ± 11 years; baseline HbA1c $8.3 \pm 1.6\%$], 13% had elevated lipase (≥ 60 IU/dL), 5% had elevated amylase (≥ 112 IU/dL), 16% had either enzyme elevated, and 3% had both enzymes elevated.

Comparable frequencies of elevations were observed for randomized study participants [$n=262$; 49% female; 57 ± 12 years; HbA1c $8.2 \pm 0.9\%$]. At study endpoint in randomized patients, increases in both amylase and lipase were observed. There were no significant differences ($p > .05$) between placebo and LY treatment groups. Multiple linear and logistic regression analyses identified 2 common factors, higher baseline lipase and lower HbA1c, as significantly associated with increase in lipase and abnormal lipase at endpoint.

Table 1. Categorical Lipase and Amylase at Baseline and Endpoint

| | | Post-baseline Result | | |
|--------------|--------------|----------------------|------------|------------|
| | | Baseline Result | Normal | Elevated |
| Lipase | Placebo | Normal | 51 (82.3%) | 8 (12.9%) |
| | | Elevated | 1 (1.6%) | 2 (3.2%) |
| | LY 0.5/1.0mg | Normal | 45 (73.8%) | 13 (21.3%) |
| | | Elevated | 1 (1.6%) | 2 (3.3%) |
| | LY 1.0/1.0mg | Normal | 40 (66.7%) | 9 (15.0%) |
| | | Elevated | 5 (8.3%) | 6 (10.0%) |
| LY 1.0/2.0mg | Normal | 38 (62.3%) | 11 (18.0%) | |
| | Elevated | 5 (8.2%) | 7 (11.5%) | |
| Amylase | Placebo | Normal | 58 (93.5%) | 4 (6.5%) |
| | | Elevated | 0 (0%) | 0 (0%) |
| | LY 0.5/1.0mg | Normal | 54 (88.5%) | 4 (6.6%) |
| | | Elevated | 1 (1.6%) | 2 (3.3%) |
| | LY 1.0/1.0mg | Normal | 53 (88.3%) | 5 (8.3%) |
| | | Elevated | 0 (0%) | 2 (3.3%) |
| LY 1.0/2.0mg | Normal | 51 (83.6%) | 6 (9.8%) | |
| | Elevated | 1 (1.6%) | 3 (4.9%) | |

In conclusion, elevations in lipase and amylase occur at greater frequency in this population than may be expected. These findings support the need for further analysis to explore their potential for clinical significance.

Disclosures: EJB: Employee, Lilly USA, LLC. CLC: Employee, Lilly USA, LLC. ABT: Employee, Lilly USA, LLC. JS: Consultant, Lilly USA, LLC.

P3-524

Comparison of Thrice Daily Biphasic Human Insulin Versus Basal Detemir and Bolus Aspart in Patients with Poorly Controlled Type 2 Diabetes Mellitus.

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OBJECTIVE— To compare the efficacy and safety of thrice-daily biphasic human insulin (BHI) versus basal detemir and bolus aspart (BB) in type 2 diabetic patients insufficiently controlled by biphasic human insulin twice a day and insulin sensitizers (metformin and pioglitazone).

RESEARCH DESIGN AND METHODS— This is a open labeled randomized pilot study in 50 patients with type 2 diabetes on twice-daily BHI and insulin sensitizers. Patients were randomized to BHI thrice-daily or BB for 12 weeks. Insulin dose were titrated according to predefined preprandial and postprandial plasma glucose (PG) targets. HbA1c, mean PG, mean fasting and postprandial PG, increment in insulin dose, weight gain were compared between the two treatment groups at the end of the study. The incidence of hypoglycaemic episodes and adverse events was evaluated.

Results: Mean HbA1c (± SD) decreases from 8.96±0.9 % at randomization to 7.88±0.8 % in BHI and from 9.36±1.3 % to 8.2±1.0 % in BB regimen after 12 weeks of treatment. The difference in HbA1c at the end of the study between the group was not significant (p 0.83). Similar improvements in glycaemic control in both groups were confirmed by 6-point PG profiles, mean PG, average fasting and postprandial PG values. Weight gain was similar in the both groups. Insulin dose increment at 12 weeks was significantly more in BB regimen (p <0.01). The incidence of hypoglycaemic episodes was more in the BHI thrice-daily but without statistical significance (p 0.07)

CONCLUSIONS— A thrice daily BHI regimen is a suitable alternative to an intensified insulin regimen in people with inadequately controlled type 2 diabetes mellitus, and requires fewer daily injections than a basal-bolus therapy without compromising efficacy and safety.

Nothing to Disclose: AB, GS, PD, NS

P3-525

Patient and Provider Factors Associated with Initiation of Novel Diabetes Therapies, 2006-2008.

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Treatment intensification is often needed to achieve adequate glycemic control in patients with type 2 diabetes, but the role of agents new to the market is often unknown. This study describes patient and provider factors associated with initiation of exenatide, pramlintide, or sitagliptin in the Translating Research into Action for Diabetes (TRIAD) study, a prospective, observational study of diabetes care in managed care. In 2006, a random sample of 1,257 patients with diabetes completed a written survey and had their medical records reviewed, and 491 primary care providers of these patients were surveyed. In 2008, 857 patients completed follow-up surveys. Data from patients on diet/exercise therapy or oral medications in 2006 who also responded in 2008 were analyzed (n=540). Of these, 179 patients added at least one traditional medication (insulin, metformin, a secretagogue, a thiazolidinedione, or an alpha-glucosidase inhibitor), and 52 patients added at least one novel therapy (exenatide, pramlintide, or sitagliptin). Patient characteristics in 2006 such as age, gender, race/ethnicity, income, education, duration of diabetes, body mass index, hemoglobin A1c, and Charlson comorbidity index did not differ significantly between those who added traditional vs novel therapies. Among providers, 120 added only traditional therapies to their patients' regimens, of whom 55 (45.8%) answered the provider survey, and 44 providers added a novel therapy, of whom 25 (56.8%) answered the survey. Between providers who added traditional vs novel agents, there were no significant differences in age, years since internship, gender, specialty, presence of a diabetes educator or registered nurse in the office, or opinions on the efficacy or safety of new medications. Providers who agreed with the statement, "my patients often request newer medications," tended to be more likely to prescribe novel therapies [16/40 (40.0%)], while providers who disagreed were more likely to prescribe traditional therapies [31/39 (79.5%), p=0.06]. In addition, university-based practitioners tended to be more likely than community-based (solo or group) practitioners to prescribe novel therapies [university: 13/29 (44.8%) vs. community: 12/50 (24.0%), p=0.06]. Thus, patient requests and practice settings, rather than patient characteristics, appear to be the most important determinants of novel medication use.

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Nothing to Disclose: VCH, LNM, DB, RB, WHH

P3-526

Glycemic Control Achieved with Insulin Glargine Analyzed by Cardiovascular Risk Factors in Patients with Type 2 Diabetes.

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Few data examine the likelihood of achieving glycemic goals with specific diabetes treatments based on CV risk factors. Patient-level data from 9 prospective, randomized, controlled trials (N=2938) were analyzed to evaluate the likelihood of achieving glycemic control with insulin glargine (GLAR)(n=1462) vs comparators (C)(n=1476) based on the existence at baseline of common CV risk factors (hypertension, dyslipidemia, history of CV disease, any combination of these). Patients were insulin naive, on oral antidiabetic agents (OADs), and randomized to the addition of GLAR or C (OADs, NPH, lispro, premix, or lifestyle measures). Insulin titration algorithms were utilized to target fasting glucose ≤ 100 mg/dL. Groups were similar at baseline (mean age 57.2 y, 56% male, 83.5% white, 8.6 y duration, A1C 8.73%, 69% hypertension, 57.6% dyslipidemia, and 24.6% with history of CV disease). Compared to all C at week 24, A1C reduction was greater, more patients achieved A1C $\leq 7.0\%$, and more patients had a $\geq 1\%$ decrease in A1C with GLAR treatment overall. Patients with hypertension, dyslipidemia, or any of these CV risk factors were also more likely to achieve A1C $\leq 7.0\%$ and have an A1C decrease $\geq 1\%$ with GLAR (table). There were no differences in weight. Overall rates of symptomatic and severe hypoglycemia were similar with GLAR vs C, while confirmed hypoglycemia was lower with GLAR (29.6% vs 35.2%, $P=0.0008$). In summary, glycemic benefits of GLAR vs C are maintained without excess hypoglycemia in patients with CV risk factors.

A1C with Insulin Glargine (GLA) vs Comparators by Baseline CV Risk Factors

| Treatment | Δ in A1C From BL to Week 24, % | Week 24 A1C $\leq 7.0\%$, % | A1C Reduction $\geq 1.0\%$, % |
|--|---------------------------------------|------------------------------|--------------------------------|
| Overall | | | |
| GLA, n=1462 | -1.72 | 57.7 | 76.5 |
| Comparators, n=1476 | -1.54 | 51.4 | 70.3 |
| Odds ratio (95% CI) | | 1.375 (1.178, 1.606) | 1.458 (1.217, 1.747) |
| P value | <0.0001 | <0.0001 | <0.0001 |
| Patients with Hypertension | | | |
| GLA, n=988 | -1.69 | 58.9 | 76.0 |
| Comparators, n=1038 | -1.53 | 51.3 | 69.5 |
| Odds ratio (95% CI) | | 1.419 (1.178, 1.710) | 1.503 (1.208, 1.869) |
| P value | | 0.0002 | 0.0003 |
| Patients with Dyslipidemia | | | |
| GLA, n=797 | -1.72 | 58.3 | 76.3 |
| Comparators, n=833 | -1.51 | 52.9 | 69.0 |
| Odds ratio (95% CI) | | 1.335 (1.084, 1.644) | 1.488 (1.169, 1.895) |
| P value | | 0.0064 | 0.0012 |
| Patients with CVD History | | | |
| GLA, n=339 | -1.68 | 55.2 | 75.5 |
| Comparators, n=383 | -1.51 | 50.9 | 70.5 |
| Odds ratio (95% CI) | | 1.280 (0.938, 1.747) | 1.393 (0.958, 2.027) |
| P value | | 0.1195 | 0.0831 |
| Patients with any CVD Risk Factor | | | |
| GLA, n=1186 | -1.70 | 58.7 | 76.4 |
| Comparators, n=1250 | -1.53 | 51.8 | 69.7 |
| Odds ratio (95% CI) | | 1.400 (1.181, 1.660) | 1.512 (1.239, 1.845) |
| P value | | 0.0001 | <0.0001 |

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Nothing to Disclose: RZ

P3-527

How To Detect and Manage Hyperglycemia during Steroid Therapy?.

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Aim To use continuous glucose monitoring (CGM) to evaluate the timing and magnitude of glucose excursions in patients receiving oral prednisolone. This information could assist in determining optimal diagnosis and treatment.

Method Fifteen patients {aged 51±12yrs, BMI 26.7±4.3kg/m², prednisolone dosage 22.8±9.5mg/day given in the morning for 1.1[0.6-1.4]months} had CGM for 65[range40-80]hrs. Ten subjects were kidney transplant recipients receiving additional immunosuppression. Five patients had known diabetes, two diet controlled and three using a sulfonylurea only. The CGM data were divided into 4 time periods (Morning 6am-12pm; Afternoon 12pm-6pm, Evening 6pm-12am and Night 12am-6am). BGLs were determined every 5 minutes by CGM and hourly means were used to determine glucose^{AUC} (G^{AUC}) of each period and its % increase above the Night values. Results were expressed as mean ±SD or median [IQR] and compared by Kruskal-Wallis test. Multivariate analysis was used to assess which factors determined the magnitude of rise in each of the three day time quarters. Independent variables examined included prednisolone dosage, history of diabetes, additional immunosuppressive treatment, fasting BGL and Night G^{AUC}.

Results

Magnitude of G^{AUC} and its % increase above Night G^{AUC}

| Time | G ^{AUC} (mmol/L) | Increase from Night(%) |
|----------------------|---------------------------|------------------------|
| 12am-6am (Night) | 31.5 [29.3-37.6] | N/A |
| 6am-12pm (Morning) | 33.4 [29.4-39.3] | 10.5 [-7.1-23.3] |
| 12pm-6pm (Afternoon) | 44.2* [34.3-59.5] | 40.3†[16.3-64.7] |
| 6pm-12am (Evening) | 38.2 [32.8-47.7] | 27.9 [11.9-34.3] |

(* vs Night , † vs Morning, p<0.005)

The presence of established diabetes was a significant determinant of the magnitude of rise in G^{AUC} in the Morning and Afternoon Periods (p<0.02) whilst Night G^{AUC} was determinant for each of the three day time periods.

Conclusion

Patients receiving oral prednisolone in the morning exhibited a maximal increase in blood glucose during the afternoon. In patients receiving mane prednisolone, BGL measurement in the afternoon would be more efficient in detecting hyperglycemia and guiding anti-hyperglycemic therapy, with fasting BGL an inadequate measure. Treatment should target improving insulin availability, particularly in the afternoon. In subjects with pre-existing diabetes and overnight hyperglycemia, additional measures to provide more insulin in the morning and evening may also be necessary. Further studies addressing the impact of various therapies on BGL control, as well as the steroid regimen used, are warranted.

Nothing to Disclose: LRS, LM, SJC, ELC, DKY

P3-528

Efficacy and Patient Satisfaction of Human U-500 Insulin Administered by Multiple Daily Injections Versus Continuous Subcutaneous Insulin Infusion Regimen in Obese, Severely Insulin Resistant Patients with Type 2 Diabetes.

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Rationale: Severe insulin resistance in obese patients with type 2 diabetes markedly increases insulin requirements in insulin-treated patients. Thus, delivering the correct volume of insulin can be technically difficult despite using multiple daily injections (MDI). Alternatively, delivery of appropriate doses and volume of insulin to achieve better glycemic control with less daily injections may be best accomplished by using the U-500 insulin (a 5-fold more concentrated insulin than U-100 insulin) delivered via continuous subcutaneous insulin infusion (CSII).

Objective: To compare the efficacy and patient satisfaction of U-500 insulin delivered by MDI *versus* CSII regimens to obese severely insulin resistant patients with type 2 diabetes (mean 24-hour U-100 insulin requirement: 3.25 U/kg/day).

Methods: Three obese patients (2M, age 45.3 ± 3.3 yrs, BMI 50.5 ± 8.1 kg/m²) with type 2 diabetes (A1c $9.37 \pm 1.14\%$) and severe insulin resistance were studied. The patients were commenced on the MDI regimen, which consist of U-500 insulin injected thrice daily pre-meal with either detemir, premixed NPH with insulin aspart and insulin lispro. After 13.7 months on the MDI regimen, the patients were switched over to the U-500 insulin CSII regimen for 8 months and discontinued all U-100 insulins. A1c, weight, blood pressure, total daily insulin dose and telephone patient satisfaction questionnaires were determined at the end of the MDI and CSII regimens.

Results: The MDI regimen decreased A1c by 1.83%, insulin requirements by 112.3 U/day and increased body weight by 8.1 kg, while the CSII regimen further decreased A1c by 0.33% and insulin requirements by 16.0 U/day without inducing further weight gain. No changes were noted with blood pressure readings for both regimens. All 3 patients reported higher satisfaction with the CSII over the MDI regimen stating convenience, less daily injections and better glycemic control as the main reasons, while mild hypoglycemic episodes were marginally more common with the CSII regimen.

Conclusion: The U-500 insulin administered by CSII is an effective therapeutic regimen for obese patients with severely insulin resistant type 2 diabetes who have failed to meet glycemic goals using the standard intensive MDI regimen and improved quality of life. However, with tighter glycemic control, patients should be made aware that the U-500 insulin CSII regimen may potentially induce more frequent hypoglycemic episodes compared to the MDI regimen.

Nothing to Disclose: KCJY, LRL, SBH, AJA

P3-529**Effect of Long-Acting Sandostatin Analogs on Blood Glucose Profiles in Patients with Acromegaly.**SK Wangnoo M.D., D.M.¹, MA Siddiqui M.D.¹ and M Gupta M.D.¹.¹Indraprastha Apollo Hosp New Delhi, India.**Objective:**

To determine the abnormalities in blood glucose profiles in patients' of Acromegaly on long acting Somatostatin analogs (SSA).

RESEARCH DESIGN AND METHODS—

8 patients (6 males; 2 females) of surgically treated Acromegaly with elevated growth hormone (GH) and Insulin-like growth factor 1 (IGF-1) on follow up were included in the study. They were initiated on SSA for biochemical remission at 20 mg SSA and in 6 patients the dose of SSA was increased to 30 mg depending upon biochemical response. Fasting (F) and 2-hour 75 gm Post prandial (PP) sugars, Glycosylated haemoglobin (HbA1c) and fasting insulin (FI) & Aspartate and Alanine transaminases (AST & ALT) levels were measured at baseline (before initiation of SSA), at 6 months and at 12 months. None of them had any family history of diabetes.

Results:

Results

| | Baseline | 6 months 20 mg SSA | 6 months 30 mg SSA | 12 months 20 mg SSA | 12 months 30 mg SSA |
|----------------------------------|------------|-----------------------|-----------------------|------------------------|------------------------|
| Fasting sugars (mg%) | 85±4 | 87±3 | 90±4 | 89±4 | 92±3 |
| 2-hrs. Post 75 gm sugars (%) | 123±6 | 124±7 | 126±8 | 126±6 | 129±7 |
| Fasting Insulin | 14.36±3.12 | 14.21±2.71 | 13.11±3.66 | 13.91±2.21 | 13.01±2.19 |
| Glycosylated Haemoglobin (%) | 5.2±0.2 | 5.3±0.1 | 5.3±0.2 | 5.3±0.1 | 5.4±0.3 |
| Aspartate Tranaminase (IU/mL) | 13±4 | 16±3 | 18±5 | 14±4 | 18±6 |
| Alanine aminotransferase (IU/mL) | 16±5 | 18±6 | 21±3 | 17± 5 | 22±4 |

Conclusions:

Treatment of Acromegaly with long acting SSA (20 mg & 30mg) did not have any significant changes on Fasting & 2 hrs.Post 75 gm Glucose, HbA1c, Fasting Insulin, and ALT and AST levels. More changes in biochemical parameters, although, not significant, were observed in 30 mg group compared to 20 mg group.

Nothing to Disclose: SKW, MAS, MG

P3-530

The Trend of Medication Use Since 2000 in a Cohort of Medium to Long Term Diabetes Patients.

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Introduction: Diabetes mellitus is increasingly common and costly. In the last decade, there is strong promotion of more intensive intervention in order to achieve treatment targets and to reduce the risk the long term complications. Therefore, patterns of drug treatment should reflect this. This study evaluates whether the patterns of antihyperglycemic medications used by diabetes patients has changed significantly since 2000.

Methods: We used the patient data collected during patient diabetes assessment to evaluate the trend of diabetes patient medication use between 2000 and 2009. This cohort of patients has been assessed at a specialist diabetes clinic after being referred by their GPs. They are managed by their GPs. Only patients who have been diagnosed at least 1 year at the assessment were included in the study. The dataset included 2054 patients, 784 diagnosed within 1 to 5 years and 1270 diagnosed more than 5 years before the assessment.

Results: The proportion of diabetes patients who have been on antihyperglycemic medication increased from 52% to 65% for patients diagnosed between 1 to 5 years (significant trend, $P < 0.01$), mostly due to the increased use of metformin, from 35% to 52% ($P < 0.01$) (Figure 1). Medication use increased from 78% to 88% for those diagnosed > 5 years (significant trend, $P < 0.05$), with insulin use increasing from 6% to 33% ($P < 0.001$) (Figure 2). The increase in medication use was significantly greater in men, increasing from 49% to 79% for those diagnosed 1-5 years and from 76% to 90% for those diagnosed > 5 years. Younger patients (< 50 yrs old) also had greater increase, from 50% to 80% and 75% to 95% for those diagnosed 1-5 years and > 5 years respectively.

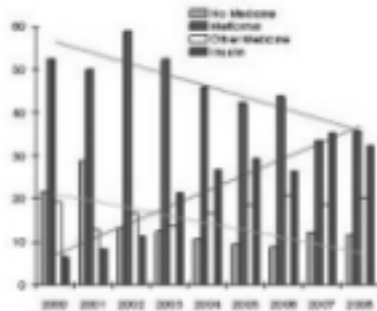


Fig 1 Antihyperglycemic medication use by patients (%) diagnosed in 1 to 5 years

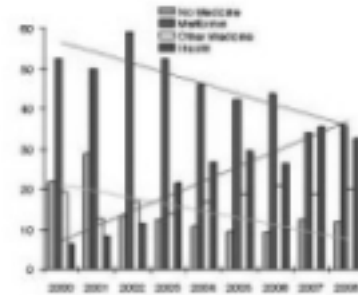


Fig.2 Antihyperglycemic medication use by patients (%) diagnosed > 5 years ago

Conclusion: the promotion of more intensive management of diabetes patients has been associated with an increased prescription of antihyperglycemic medication for diabetes patients managed by their GPs. The increase is particularly significant in men and in younger patients.

Nothing to Disclose: PJP, JW

P3-531

Exenatide with and without Insulin; Effects on the Weight and Glycemic Control.

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Exenatide is the GLP-1 analog approved for use in the type-2 DM, alone or with oral antidiabetic drugs. Its use with insulin has not been studied, but exenatide has been used with insulin by various practitioners treating diabetes mellitus.

We examined the medical records of 85 consecutive patients treated with exenatide in last 4 years in our department, to determine effects of exenatide in patients treated with and without insulin in addition to exenatide. We focused on the glucose control and body weight within the first year of the treatment. Longer observation was thwarted by the small number of patients using the exenatide for longer period of time.

Our population was 31-77 years in age, 47 males and 38 females, 64 Caucasian 18 African American and 3 of unknown ethnicity. At the start of the exenatide therapy 6 patients were overweight, 25 obese, 53 were morbidly obese and 1 was normal weight.

The 11 patients were treated only with exenatide, while 48 had exenatide combined with insulin. The TZD-s were used in 33 patients, metformin in 55 and sulfonylurea in 22 patients. Four patients could not tolerate exenatide, all because of severe nausea and have only used it for few weeks. The 6 patients started therapy less than 3 months prior to analysis and they had to be dropped from the analysis.

Of the rest of the patients, in the 3-6 months period after the initiation of exenatide treatment 56 patients (75%) have had some weight loss, while 19 (25%) gained weight. Of the 44 patients who lost weight initially and were followed at least a year, 6 (14%) have regained the weight at the end of the first year. The Hba1c data were available for 69 patients and 42 (61%) had improvement in glycemic control, 7 (10%) had stable control and 20 (29%) had worsening control. Neither of the patients had developed pancreatitis.

Patients treated with insulin in addition with exenatide had average weight loss of 3% while those not treated with insulin lost 4.5% of the weight. Patients treated with insulin in addition with exenatide lowered Hba1c by average of 0.8% while those treated without insulin had 0.5% lower Hba1c. Neither difference was statistically significant.

Combining exenatide with insulin appears to be safe treatment strategy with tendency for less weight loss with this combination but trend toward better glycemic control. Longer observation period and larger patient group will be necessary to further characterize effects of this treatment strategy.

Nothing to Disclose: MS, MS, BM

P3-532

Correlation Factor of Autonomic Neuropathy and Vestibular Dysfunction in Diabetic Patients.

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Background : Diabetic autonomic neuropathy(DAN), the subtype of diabetic neuropathy (DN) is a common complication of diabetes. The dizziness of diabetic patients is occurred from not only DAN, but hypoglycemia, side effect of medication to DN, cerebrovascular insufficiency, and others. There are reports that vestibular dysfunction (VD) is combined with about from 60% to 70% of diabetic patients. So the evaluation to VD of diabetic patient with dizziness may have clinical importance. We examined the frequency and related factor of DAN and VD in diabetic patients with a diagnosis of DN.

Method : Thirty-five diabetic patients with a diagnosis of DN from August 2008 to July 2009 were included. All subjects underwent tilt test, valsalva test, heart rate response to deep breathing, and quantitative sudomotor axon reflex test as autonomic function test. and then We diagnosed DAN and evaluated the severity of autonomic deficit with the objective, laboratory-based composite Autonomic Scoring Scale. Nineteen of them (54.3%) underwent videonystagmography and vestibular evoked myogenic potential as vestibular function test.

Results : Twenty-eight (80%) patients had DAN. Eighteen of them (64.3%) had mild autonomic failure and 10 of them (35.7%) had moderate autonomic failure. The significantly related factor to autonomic neuropathy was diabetic nephropathy ($p=0.032$), degree of chronic kidney disease (CKD) ($p=0.003$), and duration of diabetes ($p=0.044$). Degree of CKD($p=0.036$) and duration of diabetes ($p=0.009$) were also related to degree of DAN. Eleven (57.9%) patients had vestibular dysfunction (VD) but no significant related factors. The major otoneurologic complaint of them was dizziness (79.0%). Dizziness was related to duration of diabetes ($p=0.017$). VD was not related to DAN ($p=0.636$). Those with VD and DAN were 8 (42.1%) patients and without VD and DAN were 3 (15.8%) patients.

Conclusion : Diabetic nephropathy, CKD, and duration of diabetes are significantly related to DAN. VD is observed over half (57.9%) and major complaint of them is dizziness. Dizziness is related to duration of diabetes. Therefore a diabetic patient with dizziness, especially with longstanding diabetes, need to evaluate DAN. And vestibular function test may be helpful for differential diagnosis, treatment, and education of them.

1. Vinik, A.I., Maser, R.E., Mitchell, B.D., Freeman, R. 2003. Diabetic autonomic neuropathy. *Diabetes care* 26 : 1553-1579
2. Vinik, A.I., Erbas, T. 2001. Recognizing and treating diabetic autonomic neuropathy. *Cleve Clin J Med* 68: 928-930, 932, 934-944
3. Klagenberg, K.F., Zeigelboim, B.S., Jurkiewicz, A.L., Martins-Bassetto, J. 2007. Vestibulocochlear manifestations in patients with type I diabetes mellitus. *Braz J Otorhinolaryngol* 73 : 353-358
4. Gawron, W., Postpiech, L., Orendorz-Fraczkowska K., Noczynska, A. 2002. Are there any disturbances in vestibular organ of children young adults with Type I diabetes? *Diabetologia* 45 : 728-734
5. Li, J., Jhang, T., Shen, J., Gong, J., Wang, H., Zhang, J., Pang, Y. 2008. The changes in vestibular function in patients with diabetes mellitus and its clinical significance. *Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi* 22 : 10-13
6. Low, P.A. 1993. Composite autonomic scoring scale for laboratory quantification of generalized autonomic failure. *Mayo Clin Proc* 68 : 748-75
7. Veves, A., King, G.L. 2001. Can VEGF reverse diabetic neuropathy in human subjects? *J Clin Invest* 107 : 1215-1218
8. Cameron, N.E., Cotter, M.A. 1997. Metabolic and vascular factors in the pathogenesis of diabetic neuropathy. *Diabetes* 46 Supp 12 : S31-37
9. Hoeldtke, P.D., Bryner, K.D., McNeil, D.R., Hobbs, G.R., Riggs, J.E., Warehime, S.S., Christie, I., Ganser, G., Van Dyke, K. 2002. Nitrosative stress, uric acid, and peripheral nerve function in early type 1 diabetes. *Diabetes* 51 : 2817-2825
10. Horrobin, D.F. 1997. Essential fatty acids in the management of impaired nerve function in diabetes. *Diabetes* 46 Supp 12 : S90-93
11. Brownlee, M. 1992. Glycation products and the pathogenesis of diabetic complications. *Diabetes Care* 15 : 1835-1843
12. Ziegler, D., Gries, F.A., Spuler, M., Lessmann, F. 1992. The epidemiology of diabetic neuropathy. Diabetic Cardiovascular Autonomic Neuroopathy Multicenter Study Group. *J Diabetes Complications* 6 : 49-57
13. 1998. The effect of intensive diabetes therapy on measures of autonomic nervous system function in the Diabetes Control and Complications Trial (DCCT). *Diabetologia* 41 : 416-423
14. Kennedy, W.R., Navarro, X., Sutherland, D.E. 1995. Neuropathy profile of diabetic patients in a pancreas transplantation program. *Neurology* 45 : 773-780
15. Ewing, D.J., Clarke, B.F. 1982. Diagnosis and management of diabetic autonomic neuropathy. *Br Med J (Clin Res Ed)* 285 : 916-918
16. Ryder, R.E., Hardisty, C.A. 1990. Which battery of cardiovascular autonomic function tests? *Diabetologia* 33 : 177-179 ; discussion 180-171
17. Low, R.A., Benrud-Larson, L.M., Sletten, D.M., Opfer-Gehrking, T.L., Weignad, S.D., O'Brien, P.C., Suarez, G.A., Dyck, P.J. 2004. Autonomic symptoms and diabetic neuropathy : a population-based study. *Diabetes Care* 27 : 2942-2947
18. Shimada, H., Kihara, M., Kosaka, S., Ikeda, H., Kawabata, K., Tsutada, T., Miki, T. 2001. Comparison of SSR and QSART in early diabetic neuropathy - the value of length-dependent pattern in QSART. *Auton Neurosci* 92 : 72-75
19. Itoh, H., Uebori, S., Asai, M., Kashiwaya, T., Atoh, K., Makino, I. 2003. Early detection of orthostatic hypotension by quantitative sudomotor axon reflex test (QSART) in type 2 diabetic patients. *Intern Med* 42 : 560-564
20. Rybac, L.P. 1995. Metabolic disorders of the vestibular system. *Otolaryngol Head Neck Surg* 112 : 128-132
21. Raynor, E.M., Carrasco, V.N., Prazma, J., Pillsbury, H.C. 1995. An assessment of cochlear hair-cell loss in insulin-dependent diabetes mellitus diabetic and noise-exposed rats. *Arch Otolaryngol Head Neck Surg* 121 : 452-456
22. Smith, T.L., Raynor, E., Prazma, J., Buening, J.E., Pillsbury, H.C. 1995. Insulin-dependent diabetic microangiopathy in the inner ear. *Laryngoscope* 105 : 236-240

Nothing to Disclose: JRH, JHB, TSJ

P3-533

Automatic Snacking Prevents Hypoglycemia among Inpatient and Outpatient Type 2 Diabetic Patients on Intensive Insulin Therapy.

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BACKGROUND

Hypoglycemia is a limiting factor in type 2 diabetic patients on intensive insulin therapy both in inpatient and outpatient settings. The ideal approach for optimum glycemic control without fear of hypoglycemia is therefore in order.

OBJECTIVES

To demonstrate that automatic snacking offsets the problem of hypoglycemia among admitted and outpatient diabetic patients on intensive insulin therapy.

METHODS

Charts of type 2 DM patients (inpatient; Group A, outpatient; Group B) with elevated postprandial blood glucose (BG>140 mg/dL) and HbA1c (>7%) respectively, despite on maximum oral anti-diabetic agents, insulin therapy or in combination were included in the study. Intensive insulin regimens were given in all, as follows: combination of premixed 70/30 twice daily or glargine/detemir OD and aspart/lispro thrice daily premeals. In all, blood glucose levels were measured using capillary blood glucose (CBG) 2 hours after each main meal. Insulin was started at 0.6units/kg/day and increased by 10-20% every 2 days in the admitted patients and each clinic visit (every 4-6 weeks) in the outpatient cases. In Group A, the target postprandial BG levels were between 140-180 mg/dL and HbA1c of <7% in Group B. Twenty-four hour calorie supplementation was distributed into 3 main meals and 3 snacks taken automatically 2 hours after each meal, even the patient was not hungry. Hypoglycemia monitoring was done accordingly.

RESULTS

In Group A (n=20), initial postprandial BG levels were elevated at 260-400 mg/dL. Improvement in the postprandial BG levels of 112 to 172 was achieved after a mean duration of 5.25 days (range: 3-10 days). In Group B (n=118), mean baseline HbA1c was 9.2 ± 1.96 % (range: 7.9 to 14%). Improvement in HbA1c with a mean of 7.17 ± 1.10 % was observed on follow-up within 6 months with a significant mean decrease of 2.03 ± 1.66 % (range: 0.3 to 5.95%, p value <0.0001). A total of 103 (88%) patients reached target HbA1c of < 7%. Of the 88%, 46% reached target HbA1c after 3 months while the remaining 54% after 6 months. Of note, no one had significant hypoglycemia in both groups.

CONCLUSION

Automatic snacking addressed the problem of hypoglycemia among diabetic patients in intensive insulin therapy.

Nothing to Disclose: ZGL, LBM-A

P3-534

Protocol for Insulin Cessation in Post Cardiothoracic Surgery Patients.

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Introduction

Euglycemia is associated with better postoperative outcomes in cardiothoracic surgery (CTS). Previous retrospective study in our institute indicated that more than half of the patients admitted for CTS had inpatient hyperglycemia with blood sugars >200mg/dl. Even amongst those with HbA1C<7% some were discharged on insulin as a new medication. To address this, Yale insulin infusion protocol is now initiated in all patients for 48 hours post surgery. Since it is difficult to predict how to transition a patient from high doses of insulin infusion to a regimen that will ensure euglycemia, we designed a protocol to safely titrate post operative insulin in previously insulin naïve patients with HbA1C<7%.

Methods

In a prospective study, we enrolled 19 patients post CTS with preoperative hemoglobin A1C < 7%. Patients requiring more than 1 unit of IV insulin per hour 48 hours after the surgery, were started on glargine dose calculated using our protocol. Meal time aspart dose was decided based on patient's oral intake. Every subsequent day, the glargine dose was decreased by half if fasting blood glucose (BG) was less than 140 mg/dl. Glargine was discontinued if the calculated daily dose was less than 10 units. Patients were asked to monitor fasting and three post prandial BG every day for 2 weeks post discharge. We considered all fasting BG levels of > 130mg/dl or postprandial > 180 mg/dl as being out of target.

Results

Out of 19 patients, only 4 had known history of diabetes. The mean age of the patients was 61.6 ± 12.6 years. There were 15 males and 4 females. The mean pre-operative HbA1C was 5.9 ± 0.6. The total drip requirement on the second day post CTS was 73 ± 32units. There were no episodes of hypoglycemia during the titration period. Only one patient needed to be discharged home on insulin. The average period required to titrate patients completely off insulin in the hospital was 2.8 ± 1.1 days. During the two weeks post discharge, only one patient had more than 10% of documented BGs out of target range.

Conclusion

Using this protocol we have concluded that patients with HbA1C < 7%, previously not on insulin or secretagogues with high insulin requirements post CTS, can be safely and quickly titrated off insulin before discharge with minimal post discharge hyperglycemic episodes.

Nothing to Disclose: GS, HH, DB, DR, LG

P3-535

Perioperative Hypoglycemia in Patients with Diabetes: Comparing the Incidence after Low Normal Fasting Preoperative Blood Glucose to That after High Fasting Preoperative Blood Glucose Treated with Correctional Insulin.

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To avoid perioperative hypoglycemia (PH), anesthesia literature advocates conservative treatment for patients presenting with hyperglycemia. Little has been published on hypoglycemia prevention for diabetes patients with low normal preoperative fasting blood glucose (PFBG).

Retrospectively, in a single institution between 2005-2009, 2 PFBG subsets were identified by glucometrics. Patients who presented with PFBG of 70-89 mg/dl (n=308) were labeled as low normal group (LN). Patients who presented with PFBG >249 mg/dl and then treated with correctional insulin (n=279) were labeled as hyperglycemic-treated group (HT). Included were patients with self-reported or documented diabetes diagnosis prior to surgery. Excluded were patients with <2 perioperative blood glucose (BG) measurements. LN was compared to HT for subsequent development of PH, defined as BG <70 mg/dl.

Results: In HT subset, mean PFBG was 324.0 ± 59.9 mg/dl. 230 patients were treated with bolus(es) of intravenous (IV) or subcutaneous (SQ) insulin. Mean total insulin given was 8.1 ± 5.5 units. 30 patients received IV insulin infusions only, 18 received SQ or IV insulin bolus followed by IV insulin infusions, and 1 was treated by insulin pump. In HT patients who developed PH (n=10), mean PFBG was 391.5 ± 87.1 mg/dl and mean total insulin given was 10.2 ± 4.6 units.

In LN subset, PH increased with surgery start time of 12:00 PM or later (22.6% incidence) compared to before 12:00 PM (12.9%) (p=.024). Presence of renal disease, type 1 diabetes, insulin use, beta blocker use, general anesthesia, or existing 5% dextrose IV infusion was not associated with significant difference of PH in LN subset.

LN and HT groups were similar in BMI, diabetes type, insulin presence in regimen, and surgery start time. LN (M=61.3 yrs) was older than HT (M=56.3 yrs)(p<.001). LN had higher incidence of beta blocker use than HT (48.1% vs. 38.4% respectively)(p=.018). LN was 7.5 times more likely to develop PH than HT (p<.0001).

Perioperative Hypoglycemia Incidence

| Preoperative FBG Group | BG < 70 mg/dl† | |
|------------------------|----------------|-----|
| | Yes | No |
| LN (70-89 mg/dl) | 53 | 255 |
| HT (>249 mg/dl) | 10 | 269 |

†p-value based on logistic regression analysis <0.0001, 95% C.I. for O.R. = 1/0.261 = 3.83, 1/0.068 = 14.71.

Patients with diabetes undergoing surgery who present with low normal PFBG are at greater risk for development of hypoglycemia than those who receive insulin treatment for preoperative hyperglycemia. In LN subset, morning surgery time is supported.

Nothing to Disclose: SR, FO, SB, HD, CE, AT, TD, BH, RH, AH, LM

P3-536

Sterility and Stability of Expired Glucagon.

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Introduction: Glucagon, a 29 amino acid polypeptide, promotes rapid glycogenolysis and gluconeogenesis. Canadian diabetes guidelines support ready access to glucagon for treatment of severe hypoglycaemia ¹. Our local patient surveys suggest that expired glucagon is not promptly replaced. A study by the US FDA found that 88% of drugs in military storage were stable to an average of 66 months post expiration². There is no data on the quality of expired *non-reconstituted* glucagon.

Objectives:(a) To determine the sterility of expired glucagon and accompanying saline; (b) To use magnetic resonance and mass spectrometry to analyze new glucagon and compare it to randomly selected expired samples (1-8 yrs post exp.).

Design: (a) 98 glucagon samples (Eli Lilly Inc) and 103 accompanying diluent samples, ranging in age from 1 to 12 yrs post expiration, were incubated in thioglycolate broth for 14 days and analyzed for bacterial growth. (b) 2 new samples and 5 expired samples (1-8 yrs post exp.) were subjected to HPLC using an acetonitrile/water gradient on a reverse phase column. Mass spectrometry was carried out using Voyager-DE STR MALDI-TOF and Waters/Micromass Quattro Micro ESI-MS/MS instruments. Proton NMR spectroscopy was acquired at 400.13 MHz on a Bruker Avance-400 spectrometer at 25 °C.

Results: (a) The broth culture study found no bacterial growth in any of the samples; (b) Only minor differences were observed in the NMR spectra, mostly in the older samples in the region corresponding to the tryptophan signals. Mass spectra obtained on the MALDI and ESI-MS/MS systems found evidence for oxidation of methionine and tryptophan. Methionine sulphoxide formation occurred relatively quickly and was evident in the non-expired samples. There was a 32u increase observed due to addition of 2 oxygens to the tryptophan residue to form N-formylkynurenine. These changes increased with age such that the older the drug, the greater the ratio of oxidized to original intact glucagon.

Discussion: There is a knowledge deficit among clinicians as to how expiration dates of drugs are determined. This study found continued sterility in glucagon as old as 12 yrs. The spectroscopy studies, however, suggest a rapid onset of, and progressive oxidative changes near the C-terminal ends of the peptide. As this region participates in peptide receptor binding, the likelihood that the changes have biological significance is increased. Our study supports timely replacement of old glucagon.

1. Canadian Diabetes Association. 2008 Clinical practice guidelines for the prevention and management of diabetes in Canada. Can J Diabetes. 2008 Sep;32(Supp 1).

2. Lyon RC, Taylor JS, Porter DA, Prasanna HR, Hussain AS. Stability profiles of drug products extended beyond labeled expiration dates. J Pharm Sci. 2006 Jul;95(7):1549-60.

Sources of Research Support: Stewart Diabetes Research Fund.

Nothing to Disclose: JMD, DS, OV, DDM, HJV

P3-537

Effect of *Lepechinia Caulescens* (Labiatae) in Subjects with Type 2 Diabetes Mellitus.

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Objective: To investigate the effect hypoglycemic of the Labiatae (*Lepechinia caulescens*) in type 2 diabetic subjects with recently diagnosed pathology, according to the popular practice.

Methodology: 20 subjects were divided in two groups. One, control group included healthy subjects and the other group, patients with type 2 diabetes. Both groups were overloaded with 75 g of glucose for the tolerance test. Glucose and insulin were assayed for a period of four hours. Both groups had another glucose tolerance test (75 g) plus 200 mL of a 5 g infusion of *L. caulescens* dry leaves. Glucose and insulin were assayed again for a period of four hours.

Results: The significant differences found when comparing both groups: the levels of glycemia, glyated hemoglobin A1c, urea and systolic arterial pressure. The lowest levels were found in the group control ($p < 0.05$). In the test of tolerance with 75 grams of glucose, when we compared the effects of the *L. caulescens* administration, we observed a significant diminution in the levels of insulin at 30 minutes of the test in patients with diabetes.

Discussion: With the presented results, it is evident that although the plant has hypoglycemic properties, the unique dose with the used concentration did not get to have statistically significant effect. On the other hand, still does not know the time of impregnating of the components of this plant, as well as the possible routes of their metabolism; reason why it is required to evaluate the answer to the administration of this type of infusions per prolonged periods of time, and also greater doses and different concentration and furthermore in different stages from the disease. The significant diminution in the levels of insulin at 30 minutes of the test in subjects with diabetes in the presence of the Labiatae, could explain by the effect of the components of the *L. caulescens* on the modification of sensitivity to the insulin of peripheral tissues.

Conclusions: In this work we found that the unique dose of watery extracts of Labiatae preparations by means of infusion of 5 g of dry leaves in 200 ml of water, in agreement with the popular practice, does not induce hypoglycemia in subjects with diabetes type 2 of recent diagnosis. Although this plant has been reported as having hypoglycemic properties, according to this study design there was not a significant difference either with or without the infusion of *L. caulescens* leaves using described doses.

Sources of Research Support: FOFOI Grant 2002-114 Instituto Mexicano del Seguro Social.

Nothing to Disclose: ERV, MdRAV

P3-538

Circadian Variation of Pancreatic Polypeptide Responses to Identical Meals.

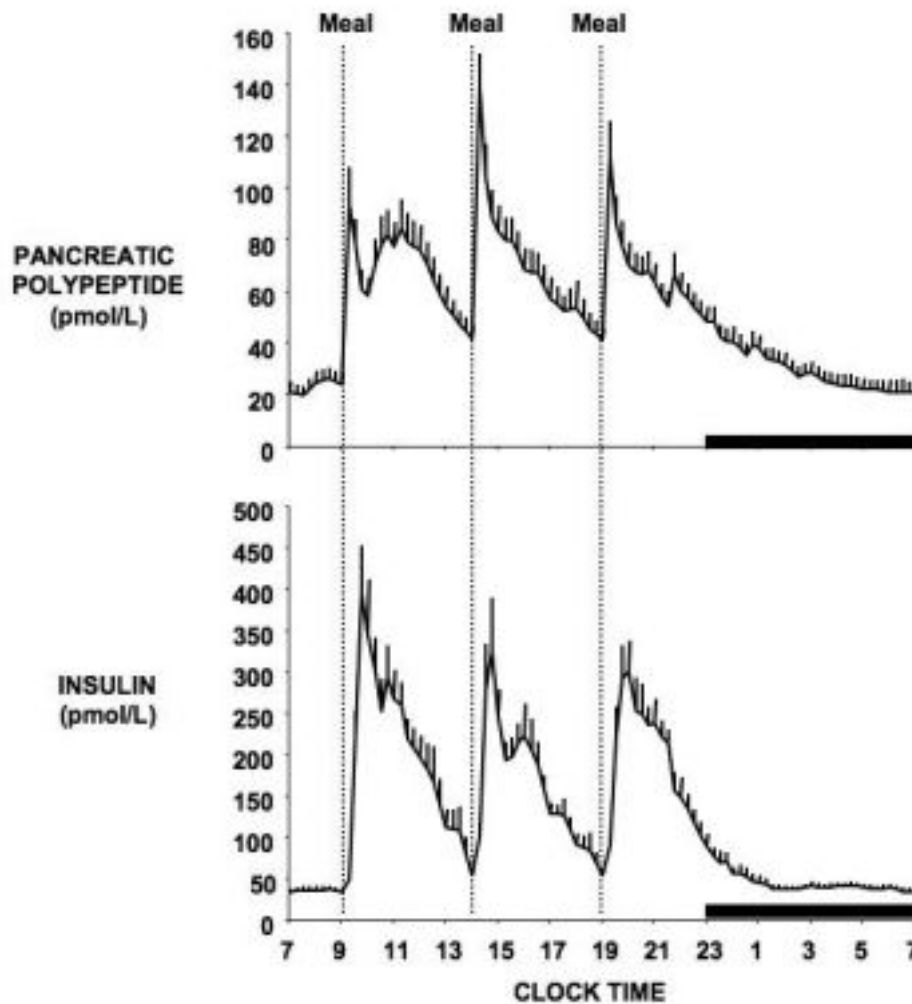
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Pancreatic polypeptide (PP) is a gut hormone that plays a role in appetite regulation. The circulating levels of PP reflect caloric intake and may remain elevated for up to 6hr post-prandially. PP infusion in healthy adults reduces both appetite and food intake. Whether the PP response to meal ingestion is modulated by time of day is not known.

Thirteen healthy subjects (age:24±4yrs; BMI:23±1kg/m²) were studied in the laboratory with controlled caloric intake and energy expenditure. Time in bed was from 2300h to 0730h. They all consumed identical carbohydrate-rich meals presented at 5-hour intervals at 0900h,1400h, and 1900h. Blood sampling was performed for 24 hours starting at 2130h. Samples were collected at 15-min intervals after each meal and every 30min at other times. Each sample was centrifuged at 4°C and kept frozen at -20°C until assay. Plasma PP levels were measured using an RIA assay (ALPCO, Salem, NH).

Irrespective of time of day, meal ingestion was associated with an acute sharp PP response culminating 15- to 30-min post-meal presentation. The response to the morning meal was clearly biphasic whereas the decline in PP levels following the afternoon and evening meals was monophasic. The post-meal PP response, as estimated by the area under the curve above the pre-meal level, was largest in the morning (11375± 1906 pmol.min/L), intermediate in the afternoon (7589± 1379 pmol.min/L) and minimal in the evening (6484± 997 pmol.min/L; p<0.0001 for time of day). In the morning, PP concentrations did not return to pre-meal levels prior to the following meal in all but one subject. In contrast, in the afternoon and evening, PP levels returned to pre-meal levels in 217± 21 min and 218± 18 min, respectively (P<0.001 relative to morning meal). This diurnal variation of PP responses to identical carbohydrate-rich meals is similar to the diurnal variation in meal-related insulin responses, which are typically larger in the morning than in the evening. These findings support the concept that feeding schedules matching the circadian organization of pancreatic cells responsiveness may optimize glucose tolerance and appetite regulation.



Sources of Research Support: NIH Grant R01HL086459; NIH Grant R01HL086459-03S1; P01AG-11412; P60-DK20595;

RR024999.

Nothing to Disclose: ET, FD, JB, AD, VA, EVC

P3-539

Efficacy and Safety of DBCare®, a Food Supplement, in Patients with Type 2 Diabetes Mellitus and Inadequate Glycemic Control: A Randomized, Double-Blind, Placebo-Controlled Trial.

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Background: Despite current oral hypoglycemic agents, most patients with type 2 diabetes mellitus (T2DM) do not maintain treatment goals, i.e. glycated hemoglobin level (A1C) <7%. Over time, uncontrolled diabetes may lead to microvascular and macrovascular complications. DBCARE® is a traditional Indian herbal food supplement that contains 11 ingredients, some of which have been shown to have hypoglycemic properties in anecdotal animal and human studies. We report the first prospective, randomized, placebo-controlled study that evaluated the effect of DBCARE® on patients with T2DM.

Aim: To evaluate the efficacy and safety of DBCARE® in patients with inadequately controlled T2DM despite oral hypoglycemic treatment.

Methods: A prospective, 12-week, randomized, double-blind, placebo-controlled trial was conducted in a secondary referral center in Israel. Patients (>18 yrs of age) with T2DM on oral hypoglycemic agents, with A1C level >7.5%, were randomized to receive DBCARE® (2 tablets three times daily) or placebo.

Results: 41 patients (26M/15F; mean age 60.8±9.4 years) received DBCARE® (N=22) or placebo (N=19). Baseline clinical and biochemical characteristics of both groups were not statistically different (glucose 155.8±46.1 mg/dL; A1C 7.7±0.72%). From baseline to week 12, A1C levels declined 0.3±0.68% in the DBCARE® group (p=0.051) and 0.23±0.73% in the placebo group (p=0.224). In parallel, fasting plasma glucose (FPG) decreased 6.1±31.0 mg/dL in the DBCARE®-treated group (p=0.405) and 9.6±44.7 mg/dL in the placebo group (p=0.405). Subgroup analyses of patients with baseline BMI>30 or <10 years duration of diabetes did not reveal significant glucose or A1C reductions at week 12 vs. baseline. Other parameters, including body mass index (BMI), the homeostasis model assessment for insulin resistance and for insulin secretion, and C-reactive protein were not statistically different at 12 weeks vs. baseline. DBCARE® was generally well tolerated. Four patients withdrew from the study, one from the treatment group and 3 from the placebo group.

Conclusions: DBCARE® treatment was not effective in improving glycemic control in our cohort of patients with inadequately controlled T2DM, despite oral hypoglycemic treatment. Further studies are needed to evaluate the effect of DBCARE® treatment on specific groups of patients, i.e. those with BMI>30, FPG>200mg/dL, A1C >8%, and recent onset diabetes at baseline.

Trial Registration: Clinicaltrials.gov Identifier NCT0056004

Nothing to Disclose: PR-P, RN-A, GC, AR, YL

P3-540

Visual and Odorous Food Cues Do Not Modulate the Hypothalamic Response to Fasting in Healthy Humans.

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Objective

Calorie restriction extends life span and prevents aging-related disease like cancer and diabetes in a variety of species. Odorants from live yeast restrain the beneficial effects of calorie restriction in *Drosophila Melanogaster*. Odorants are perceived by the vomeronasal organ, an evolutionary conserved part of the (mammalian) olfactory system, primarily responding to nonvolatile cues and relaying environmental information to the hypothalamus. We hypothesized that food odorants and images impact the neuroendocrine and metabolic response to starvation in humans.

Aim

To evaluate the impact of attractive visual and odorous food cues on the hypothalamic and metabolic adaptations to nutrient deprivation in healthy humans.

Patients & Methods

In this randomized, controlled, cross-over intervention study 12 healthy, young, normal-weight and normoglycemic men fasted twice for 60-hours; in the presence or absence of food-related visual and odorous stimuli. At baseline and on the last morning of each starvation intervention, an oral glucose tolerance test (OGTT) was performed. During the OGTT, blood was sampled and the hypothalamic neuronal activity was measured by functional MRI from time -8 till +28 minutes.

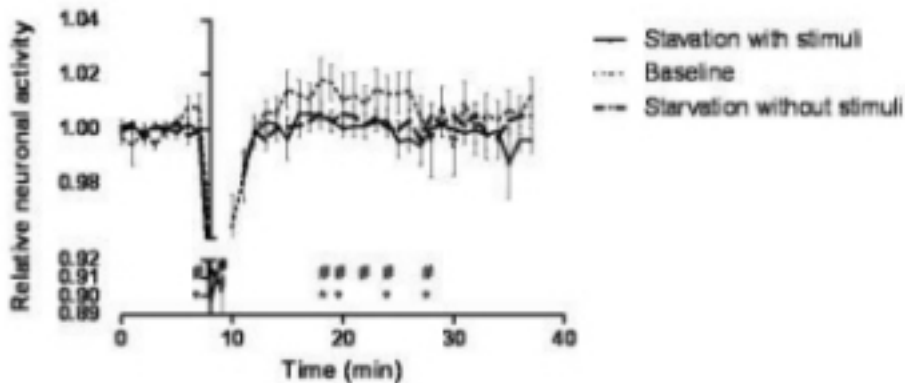
Results

The main effects of starvation on metabolism were: 1) decreased plasma TSH and T3 levels, while T4 levels remained unchanged; 2) reduced fasting serum glucose and insulin concentrations, while the areas under the curves during the OGTT were increased; 3) reduction of the signal produced by the hypothalamus in response to the glucose load (particularly during the first 20 minutes after ingestion); 4) increased resting energy expenditure. Exposure to food stimuli affected none of these parameters.

Conclusions

This study shows that 60h of starvation in healthy young men 1) alters the hypothalamic BOLD (blood oxygenation level dependent) signal in response to glucose ingestion; 2) induces profound glucose intolerance; 3) increases resting energy expenditure; 4) down regulates the pituitary-thyroid axis. Exposure to visual and odorous food cues did not alter any of these metabolic and neuroendocrine adaptations to nutrient deprivation.

Figure 1 Hypothalamic neuronal activity as measured by functional MRI ± SEM



* significantly ($p < 0.013$) different between starvation with stimuli compared to baseline (minutes 8, 19, 20, 24 and 27)

significantly ($p < 0.013$) different between starvation without stimuli compared to baseline (minutes 8, 19, 20, 22, 24 and 27)

Nothing to Disclose: MAW, MS, MBB, JvdG, IMJ, JAR, WMT, HP

P3-541

Body Composition Changes in Newly Diagnosed Type 1 Diabetic Children.

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Children with new onset type 1 diabetes mellitus (T1DM) often present with weight loss beyond dehydration; weight is typically regained after insulin treatment. Historically, T1DM was considered a protein catabolic disease [1-3]. Studies in adults have shown insulin results in gain of fat mass [4-7]; insulin's inhibitory regulation of lipase is a critical step in the catabolism of fat. During insulin deficiency prior to diagnosis of T1DM, a child would be expected to lose fat mass and then regain it after treatment. Our aim was to determine body composition changes in children with new onset T1DM.

METHODS

9 new onset T1DM children were studied (Table 1). Dual-energy x-ray absorptiometry (DXA), a validated method of estimating body composition [8-13], was obtained at discharge and then 1 and 3 mo.

RESULTS

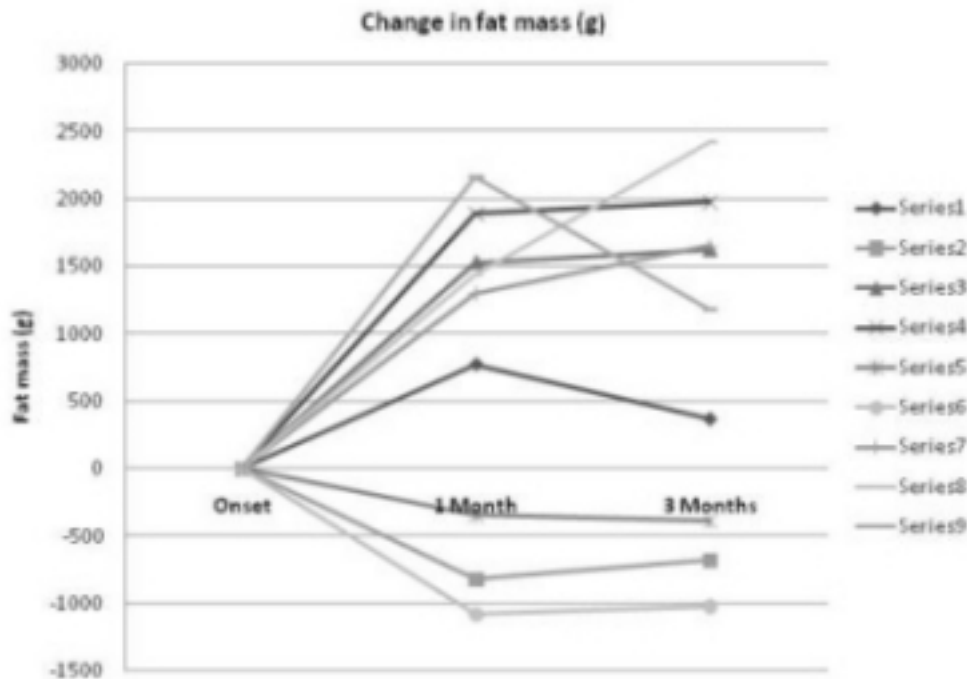
All subjects gained weight. Gain in fat mass was not consistent (fig 1) however gain in lean mass was (fig 2). At 3 mo, change in fat mass was 0.79 kg (+/-1.3)(p=0.064) while lean mass was 2.48 kg (+/- 1.2)(p<0.001).

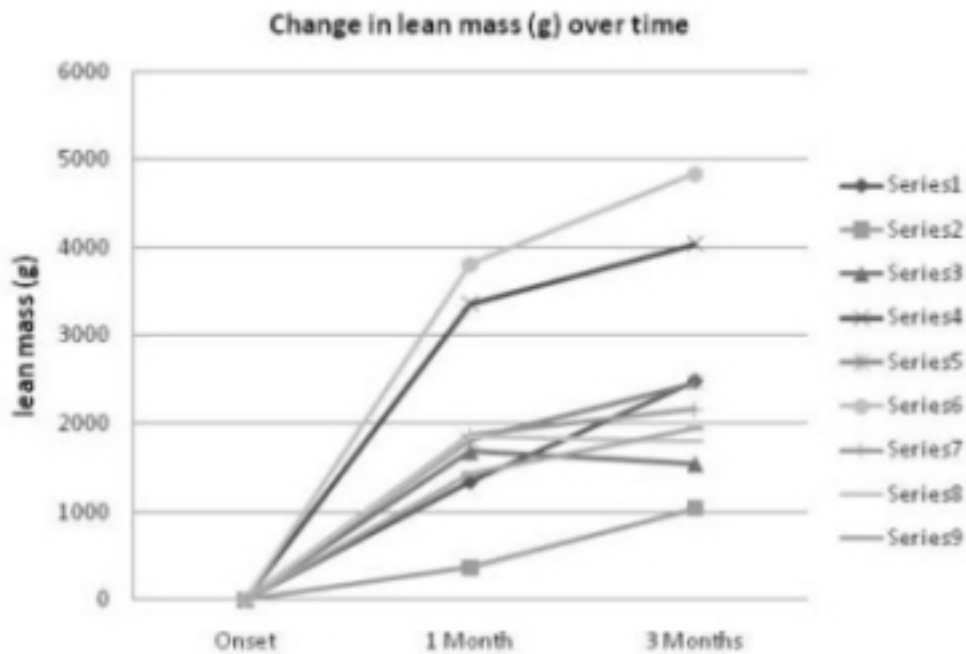
CONCLUSIONS

Insulin replacement in new onset T1DM resulted more consistently in a protein anabolic state than a fat anabolic one. This suggests that insulin deficiency prior to diagnosis of type 1 diabetes results predominantly in protein catabolism. This information will be useful in counseling patients about the nature of weight changes following diagnosis.

| Patient | Age | Sex | Tanner stage (onset) | Tanner stage (at 3m) | Weight gain at 3m (kg) | % Weight change at 3m | Change in BMI SDS at 3m |
|----------|------------|-----|----------------------|----------------------|------------------------|-----------------------|-------------------------|
| 1 | 13 | M | 3 | 4 | 4.7 | 11 | 0.3 |
| 2 | 13 | M | 5 | 5 | 2.5 | 5 | -0.2 |
| 3 | 9 | M | 1 | 1 | 5.4 | 20 | 1.9 |
| 4 | 12 | M | 4 | 4 | 7 | 15 | 0.7 |
| 5 | 14 | M | 4 | 4 | 2.4 | 4 | -0.2 |
| 6 | 13 | M | 4 | 4 | 7.9 | 14 | 0.2 |
| 7 | 12 | M | 2 | 2 | 2.1 | 6 | 0.4 |
| 8 | 10 | M | 2 | 2 | 6.2 | 18 | 0.7 |
| 9 | 10 | F | 2 | 3 | 6.2 | 18 | 0.3 |
| Mean(SD) | 11.8 (1.7) | | 3 (1.3) | 3.2 (1.3) | 4.9 (2.2) | 12.3 (6.1) | 0.5 |

Table 1





- (1) Atchley, D.W., et al., J Clin Invest, 1933. 12(2): p. 297-326.
- (2) Boddy, K., et al., Clin Sci Mol Med, 1976. 50(6): p. 455-61.
- (3) Walsh, C.H., et al., Q J Med, 1976. 45(178): p. 295-301.
- (4) Sinha, A., et al., Diabet Med, 1996. 13(1): p. 40-6.
- (5) Rosenfalck, A.M., et al., Diabet Med, 2002. 19(5): p. 417-23.
- (6) Ingberg, C.M., et al., Diabet Med, 2003. 20(12): p. 1005-11.
- (7) Carlson, M.G. and P.J. Diabetes, 1993. 42(12): p. 1700-7.
- (8) Rosenfalck, A.M., et al., Scand J Clin Lab Invest, 1995. 55(8): p. 691-9.
- (9) Margulies, L., et al., J Clin Densitom, 2005. 8(3): p. 298-304.
- (10) Kim, J., et al., Am J Clin Nutr, 2006. 84(5): p. 1014-20.
- (11) Plank, L.D., Curr Opin Clin Nutr Metab Care, 2005. 8(3): p. 305-9.
- (12) Fields, D.A. and M.I., J Appl Physiol, 2000. 89(2): p. 613-20.
- (13) Sopher, A.B., et al., Pediatrics, 2004. 113(5): p. 1285-90.

Sources of Research Support: General Clinical Research Center Grant MO1RR10710.

Nothing to Disclose: NSS, KT, JO, TAW, AHL

P3-542

Maternal Fasting Plasma Active GLP-1 Level Is a Negative Predictor of Fetal Abdomen Circumference and Maternal Weight Change in Normal Pregnancy.

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Objective: Maternal weight in pregnancy contributes to glycemic environment affecting fetal growth. Gut peptides (GLP-1; GIP; ghrelin; PYY) have been related to insulin sensitivity and secretion, weight control and adipose tissue metabolism. This study aimed at examining the associations of gut hormones during pregnancy with maternal glucose homeostasis, maternal weight and fetal growth.

Methods: 55 pregnant non-obese non-diabetic Caucasian women were examined during the three trimesters of pregnancy and had anthropometric measurements, evaluation of fasting maternal plasma GLP-1(active), ghrelin (active), total PYY, total GIP and a 75g oral glucose tolerance test. Homeostasis model assessment (HOMA-R), insulin sensitivity index (ISI), and indices of insulin secretion were calculated. Fetal growth was estimated by ultrasound.

Results: Fasting GLP-1 increased significantly from second to third trimester ($p < 0.05$). Fasting GLP-1 correlated positively with HDL-C ($r = 0.52$, $p = 0.04$). At second trimester fasting GLP-1 levels correlated negatively with fetal abdomen circumference ($r = -0.55$, $p = 0.034$), birthweight ($r = -0.50$, $p = 0.040$), HOMA-R ($r = -0.65$, $p = 0.001$), insulin secretion and triglycerides [figure 1]. By backwards multiple regression analysis, first trimester GLP-1 levels were the best negative predictor of second trimester fetal abdomen circumference ($\beta = -0.96$, $p = 0.009$). By longitudinal regression model, maternal fat and HOMA-R were positive and fasting GLP-1 levels negative predictors of maternal weight change during pregnancy [Table 1].

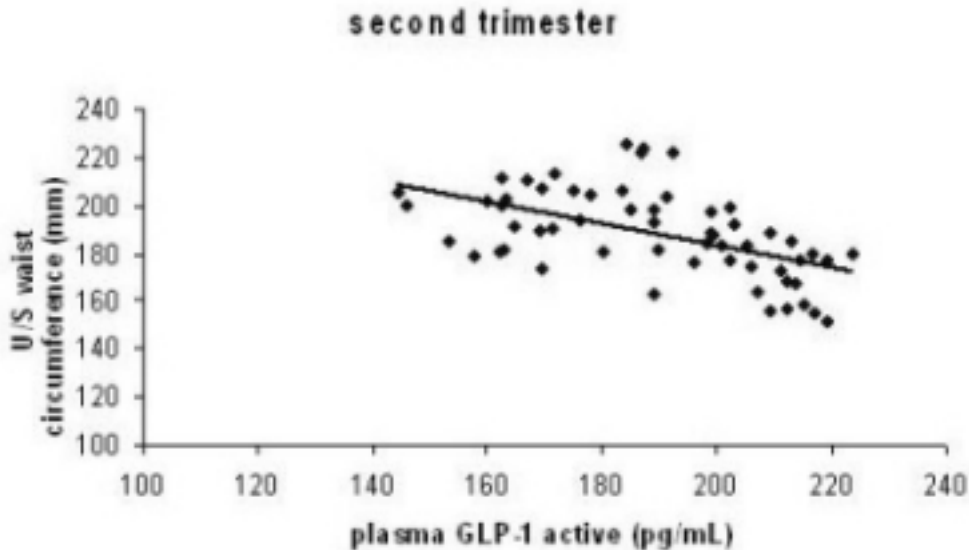


Table 1

| | t-value | p-value |
|--------------------------------------|---------|---------|
| N=55 | | |
| Maternal total body percent fat | 3.12 | 0.005 |
| Maternal HOMA-R | 2.79 | 0.013 |
| Maternal fasting plasma active GLP-1 | -2.71 | 0.015 |

Predictors of maternal weight change during all trimesters of the pregnancy by longitudinal regression model

Conclusions: During pregnancy, maternal fasting GLP-1 might be involved in mechanisms compensating for the pregnancy-related increase of glycemia and insulin resistance, suggesting a role of this peptide in maternal metabolism and weight and fetal growth.

Nothing to Disclose: GV, AM, NV, EGS, GP, DB, IP, GC, GM

P3-543

Peripheral Metabolic Effects of Intra-Arterial Somatostatin Infusion in Healthy Young Men.

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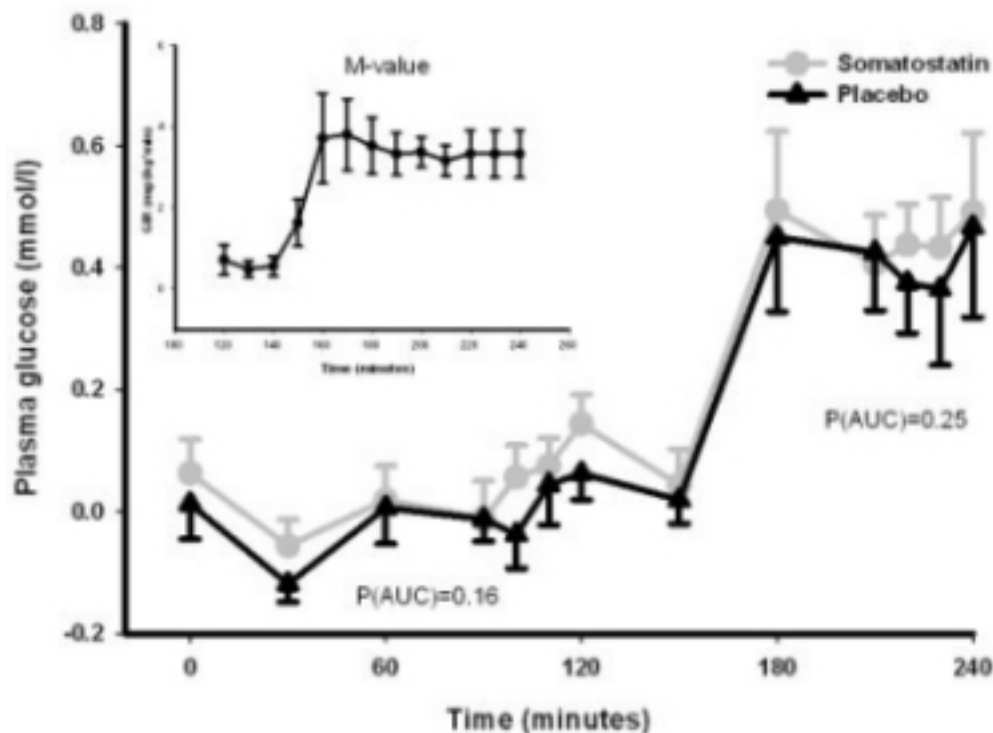
¹Aarhus Univ Hosp Aarhus, Denmark.

Background: Somatostatin (SRIF) analogs (SA) provide safe and effective therapy for acromegaly. Previous SRIF perfusion studies provide evidence that somatostatin increases skeletal muscle glucose uptake. This effect is an added benefit for patients suffering from acromegaly and elucidating the molecular mechanisms subserving the direct, peripheral effects of somatostatin would bring new insight to diseases like obesity and type 2 diabetes.

Materials and Methods: Healthy young men (n = 8, age 25.3 [21.9; 29.4] years, BMI 24.2 ± 0.7 kg x m⁻²) in the post-absorptive state received 240 min of intra-arterial SRIF infusion (150 µg x hour⁻¹) into one femoral artery and intra-arterial placebo infusion into the contra lateral femoral artery in a blinded and randomized design. Furthermore, each participant received a constant infusion of growth hormone in an antecubital vein throughout the study day. The study consisted of a basal period (120 min) followed by a hyperinsulinemic euglycemic clamp period (insulin 1.0 mU x kg⁻¹ x min⁻¹). Simultaneous blood samples were drawn every 20 min from both femoral veins and muscle biopsies were obtained from both legs during both the basal and the clamp period. Arterial plasma glucose was measured every 10 min during the clamp. Leg blood flow was determined by Doppler ultrasound.

Preliminary results: Limb blood flows were similar during both the basal (P = 0.78) and the clamp period (P = 0.88). The glucose infusion rate during the clamp period was 3.3 ± 0.3 mg x kg⁻¹ x min⁻¹. Arteriovenous glucose differences, measured as area under the curve (AUC), were similar in the basal (1.59 ± 4.85 [mM x min] (SRIF) vs. -3.06 ± 2.71 [mM x min] (placebo), P = 0.16) and in the clamp period (37.57 ± 5.21[mM x min] (SRIF) vs. 33.23 ± 6.43 [mM x min] (placebo), P = 0.25).

Arteriovenous glucose difference



Conclusions:

- 1) SRIF has no significant impact on the arteriovenous glucose difference
- 2) Muscle blood flow is unaffected by somatostatin.

Nothing to Disclose: TK-H, ETV, MB, RN, NJ, NM, JOLJ

P3-544

Hypercaloric High Fat Fast Food Feeding for 5 Days Does Not Affect Oral Glucose Tolerance or Gut Hormone Levels in Healthy Young Men.

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¹Leiden Univ Med Ctr Leiden, Netherlands.

Objective

Understanding the pathophysiological changes that occur during the development of obesity is of utmost importance for the prevention and treatment of obesity and its complications. Gut hormones are of special interest, since plasma levels of these hormones were found to be altered in both obesity and type 2 diabetes mellitus. We hypothesized that temporary overfeeding would change gut hormone levels in response to glucose ingestion in healthy young men.

Aim

To establish if overfeeding affects gut hormone levels or glucose tolerance in healthy young men.

Patients & Methods

In this randomized, cross-over intervention study 10 healthy, young, normal-weight and normoglycemic men consumed a fast food diet containing 40% more calories than required to maintain energy balance for 5 days. Participants were instructed to buy 3 meals of predetermined composition per day in auxiliary branches of a large fast food chain and were asked to bring in their receipts to check compliance. Energy percentages of macronutrients were: carbohydrates 51.7%, fat 37%, protein 11.5%. At baseline (in randomized order either before or after a "wash out" period of 6 weeks after intervention) and directly after the dietary intervention, blood sampling was performed at t=-10, t=0, t=10, t=20, t=30, t=60, t=90 and t=120 during an oral glucose tolerance test. Areas under the curves (AUCs) were calculated for all serial measurements.

Results

Plasma concentrations in fasting condition and AUCs of glucose, insulin, cortisol, PYY, ghrelin, GLP-1 and GIP in response to glucose ingestion were unaffected by hypercaloric high fat feeding.

Conclusion

This study is the first to show that overfeeding healthy young men for 5 days does not affect gut hormone levels in fasting condition and in response to glucose ingestion. Moreover, this short term intervention does not appear to affect glucose tolerance in these subjects.

Table 1 Fasting levels of glucose and insuline and AUCs of gut hormone levels during OGTT at baseline and after the hypercaloric intervention

| | Baseline# | After hypercaloric diet |
|--------------------------|--------------|-------------------------|
| Fasting Glucose (mmol/l) | 4.9 ± 0.10 | 5.1 ± 0.16 |
| Fasting Insulin (mU/l) | 3.6 ± 0.83 | 3.5 ± 0.86 |
| AUC GIP | 13777 ± 1259 | 14954 ± 1281 |
| AUC GLP-1 | 1254 ± 454 | 1073 ± 280 |
| AUC PYY | 16151 ± 1358 | 15842 ± 1484 |
| AUC Ghrelin | 92350 ± 9954 | 93561 ± 10283 |

All data depicted as mean ± SEM

Nothing to Disclose: MAW, MAvB, JvdG, KWvD, HP

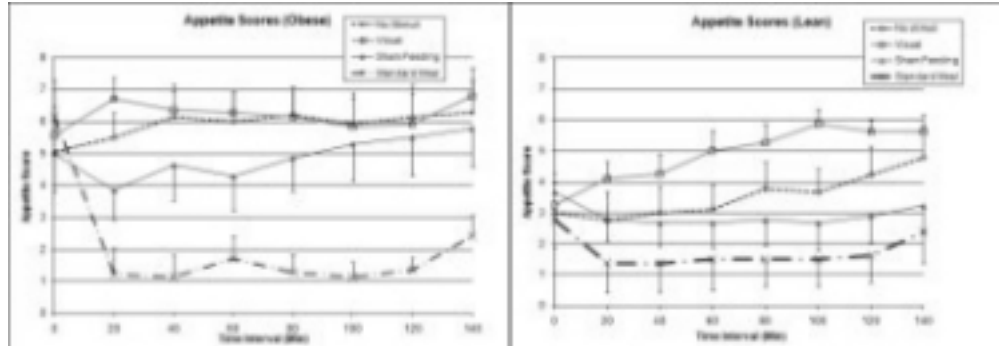
P3-545

The Role of the Cephalic Phase of Feeding in the Regulation of Gastrointestinal Peptides Related to Appetite.

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Pancreatic polypeptide (PP), a gut peptide synthesized mainly by the pancreatic islets, is felt to be involved in appetite and weight regulation. PP is low during fasting and rises postprandially. Secretion is under vagal control and when administered, it causes a sustained decrease in appetite and food intake. Little is known about the physiology; studies suggest that food intake is not the only regulating factor and that PP also increases in response to the modified sham feeding test (MSF) (1). We postulated that PP secretion is regulated by the cephalic phase of feeding and that this mechanism is altered in obese subjects. Methods: Nine normal-weight healthy subjects and 6 obese, otherwise healthy subjects had PP, appetite and glucose levels measured in the fasting state at baseline and at 20-minute intervals for 140 minutes on 4 different occasions: 1) Standardized meal with fixed calories from fat, protein, and carbohydrate; 2) With visual and olfactory stimulation from the same meal (VS); 3) MSF; and 4) Without stimuli related to food. Results: Obese subjects had significantly higher appetite at baseline which later suppressed to the same extent as the lean. During MSF, appetite scores remained stable for both. Appetite in the obese group increased with VS at 20 min. On the other hand, within the lean group, VS increased appetite significantly at 100-140 minutes showing that appetite seemed to increase later.



Within the lean group, sham feeding increased PP significantly from baseline at 20, 40, and 140 min. Within the obese group, SF significantly decreased PP from baseline at 80 min. Between the two groups, SF increased percent change of PP significantly in the lean compared to the obese at 60-100 min. In conclusion: The obese have a higher appetite during fasting but it is suppressed to the same extent with a meal compared to the lean. In spite of having the perception of increased appetite at baseline, the obese group started with the same PP levels. Although sham feeding seems to have no effect on appetite, it did stimulate PP in the lean group. Therefore, MSF seems to stimulate vagal tone in the lean group, and not in the obese.

(1) Simonian HP et al., *Neurogastroenterol Motil.* 2005 Jun;17(3):348-54.

Sources of Research Support: National Institutes of Health, M01-RR00188, GCRC SLEH.

Nothing to Disclose: SMT, SMP, GRC, JMG

P3-546

Women with Type 2 Diabetes and past Gestational Diabetes Are More Likely To Be Materially Deprived Than Women with Type 2 Diabetes Alone.

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¹McGill Univ Montreal, Canada.

Aims: Material deprivation has emerged as an independent risk factor for type 2 diabetes, particularly in women (1,2). There is also evidence that (GDM) may occur more often among the disadvantaged (3-5). We sought to determine whether women with type 2 diabetes and past GDM were materially deprived compared to women with type 2 diabetes alone.

Patients and Methods: Using Canadian Community Health Survey data, we constructed three cohorts: women without diabetes, women with type 2 diabetes and past GDM, and women with type 2 diabetes without past GDM. Through logistic regression, we assessed for associations with income separately for diabetes with and without past GDM vs. no diabetes and for past GDM among both groups of women with diabetes. Models were adjusted for age, body mass index, immigrant and marital status, smoking history, physical activity, and hypertension.

Results: Diabetes, both with or without past GDM, was associated with lower income; but a stepwise risk increase with lower income levels was more evident in diabetes with past GDM (high middle OR 1.44, 95% CI 0.97 to 2.13; low middle OR 1.95, 95% CI 1.28 to 2.96; lowest OR 2.56, 95% CI 1.63 to 4.10). Among those with diabetes, odds of past GDM were higher for both lower middle (OR 1.62, 95% CI 1.00-2.63), and lowest income (OR 1.78, 95% CI 1.42 to 3.06).

Conclusions: Among women with type 2 diabetes, past GDM is associated with greater material deprivation. This subset of women thus has a pronounced risk of cardiovascular disease and represent a potential public health burden. Our findings support the need for identifying materially deprived women with GDM for diabetes prevention.

Summary of associations of income between study groups.

| | Type 2 DM with GDM vs. no diabetes | Type 2 DM without GDM vs. no diabetes | Type 2 DM with GDM vs. type 2 DM without GDM |
|--------------|------------------------------------|---------------------------------------|--|
| | Odd Ratio (95% CI)* | | |
| Income level | | | |
| Lowest | 2.56 (1.63-4.10) | 1.37 (1.07-1.75) | 1.94 (1.15-3.27) |
| Lower middle | 1.95 (1.28-2.96) | 1.17 (0.96-1.44) | 1.71 (1.06-2.74) |
| High middle | 1.44 (0.97-2.13) | 1.27 (1.07-1.51) | 1.10 (0.71-1.70) |
| Highest | 1 | 1 | 1 |

* Model adjusted for income, age, ethnicity, immigrant status, marital status, BMI, hypertension, physical activity, smoking status.

- (1) Robbins et al., Am J Public Health 2001;91:76-83
- (2) Dasgupta et al., Diabet Med, in press
- (3) Feig et al. CMAJ 2008;179:229-234
- (4) Anna et al. Diabetes Care 2008;31:2288-2293
- (5) Joseph et al. CMA 2007;177:583-590

Nothing to Disclose: ICL, NAR, LZ, KD

P3-547

A Novel Biallelic Mutation in the Insulin Binding Domain of the Insulin Receptor Causing Severe Insulin Resistance Syndrome in Five Unrelated Saudi Families; a Possible Founder Mutation.

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Insulin resistance is an integral part of the pathogenesis of type 2 diabetes. Its underlying mechanisms are largely unknown. On the other hand, severe insulin resistance syndromes are rare and frequently associated with gene mutations in the insulin signaling pathway. In this study, we have identified 5 unrelated families with extreme insulin resistance syndrome type A characterized by normal to mild overweight (BMI <27.5), severe acanthosis nigricans, mild diabetes mellitus or impaired glucose tolerance, normal lipid profile, severe hyperinsulinemia (median fasting insulin 997 pmol/l, range 332-3230 pmol/l), high C-peptide (median 1.08 nmol/l, range 0.74-1.27), high HOMA-IR, acromegaloïd features with low serum level of IGF-1, severe skin hyperpigmentation and hyperandrogenism in female members. The number of patients per family ranged between 1 to 3 patients of either gender and the median age (range) was 20 years (12-35). Most patients were treated with pioglitazone 15-30 mg QD and showed significant reduction in their insulin resistance markers and some improvement in their clinical manifestations. The clinical and biochemical profiles of these cases shed light on the complex pathophysiologic aspects of insulin action. To investigate the underlying genetic defect, the entire coding region and intron-exon boundaries of the insulin receptor gene were PCR-amplified and sequenced from peripheral leukocyte DNA isolated from the patients and their healthy relatives. A biallelic R118C mutation in the binding domain of the insulin receptor was found in all the patients. A monoallelic R118C or wild type was present in healthy subjects in a pattern consistent with autosomal recessive inheritance. The wild type and mutant R118C insulin receptor were cloned and expressed in CHO cells for functional analysis. As expected, no insulin binding was observed in mutant R118C transfected CHO cells. Western blot analysis showed that both wild type and mutant R118C are expressed equally in CHO cells. Confocal microscopy demonstrated that the mutant R118C are expressed on the cell surface and not retained in the endoplasmic reticulum. We conclude that the severe insulin resistance syndrome is caused by biallelic germline R118C mutation in the insulin receptor gene, which renders the receptor unable to bind insulin to transmit the signal. Given that the same mutation is present in 5 unrelated families, it is likely that this is a founder mutation present in Arab population.

Nothing to Disclose: ASA, MZ, NA-K, JK, EYB, HR, RA-R, AA, RSP, FAA-M, BM, YS

P3-548

Severe Insulin Resistance, Diabetes Mellitus, Hypertriglyceridemia, and Pseudoacromegaly Due to Novel Homozygous Mutation in the Acyl-3-Phosphate O-Acyltransferase 2, (AGPAT2) Gene.

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Introduction: The defining feature of congenital generalized lipodystrophy (CGL) is near total absence of adipose tissue, usually complicated by insulin resistance (IR), dyslipidemia, and hepatic steatosis. We report two sisters presenting first with pseudoacromegaly - acromegaloid features with normal GH/IGF1- before later diagnosis of CGL, caused by homozygosity for a novel missense mutation in the *AGPAT2* gene.

Clinical Cases: Patients A (age 14 years) and B (age 17-yrs) both presented with markedly acromegaloid facial appearance, oligomenorrhea, paucity of subcutaneous fat, and acanthosis nigricans. Growth hormone levels were appropriately suppressed after oral glucose challenges, and IGF-1 levels were normal. However both patients showed extreme fasting hyperinsulinemia, severe hypertriglyceridemia and hyperandrogenemia (Table 1). Characteristically for post receptor IR, serum SHBG levels were low. Bone age was advanced.

Patient A developed insulin-resistant diabetes at 15 years age with poor glycemic control, complicated by proliferative retinopathy, nephropathy, neuropathy and severe hepatic steatosis, despite therapy with large doses of insulin, pioglitazone, gemfibrozil and simvastatin. Patient B developed diabetes at 18 years age and required metformin and pioglitazone.

Genetic studies revealed both sisters to be homozygous for the novel p.Cys48Arg mutation in the *AGPAT2* gene, a rare example of a pathogenic missense mutation in a poorly characterized domain of the enzyme.

Conclusion: These cases emphasize the importance of being alert to the possibility of severe insulin resistance and lipodystrophy in patients presenting with pseudoacromegaloid soft tissue overgrowth but without GH excess. Characterization of function of the novel mutant *AGPAT2* identified may yield new insights into the molecular regulation of *AGPAT2* activity.

| Analyte (all fasting) | Patient | | Reference Range |
|-----------------------|---------|--------|-----------------|
| | A | B | |
| blood glucose, mmol/l | 4.2 | 4.6 | <6.9 |
| insulin, pmol/l | 1, 700 | 309 | 18-173 |
| C-peptide, pmol/l | 6, 900 | 1, 400 | 380-650 |
| IGF-1, µg/l | 700 | 575 | 261-1096 |
| Triglyceride, mmol/l | 25 | 14.8 | <1.7 |
| Testosterone, nmol/l | 9.2 | 4.7 | 0.2-2.9 |
| SHBG, nmol/l | 9 | 24 | 18-114 |

Nothing to Disclose: MA, HR, ER, RKS

P3-549

A Case of HNF-1- β MODY Misdiagnosed as T1DM and Unnecessarily Treated with Insulin.

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BACKGROUND: Heterozygous mutations in HNF-1- β are associated with a phenotype of cystic kidney disease, liver dysfunction, pancreatic aplasia and urogenital malformations. Some patients with HNF-1- β mutations develop early onset diabetes (<25 years), and these patients usually require insulin for glucose control. This autosomal dominant syndrome is called either HNF-1- β MODY (maturity onset diabetes of the young), formerly MODY5, or RCAD (renal cysts and diabetes). **CLINICAL CASE:** A 21 year old woman was referred to the endocrinology service for evaluation of "atypical" diabetes during pregnancy. She had been diagnosed at age 16 with T1DM, reportedly after an episode of DKA. She was using CSII and reported a history of good blood glucose control and periods of several weeks without requiring insulin for years after her diagnosis. She had been incidentally diagnosed with multiple bilateral renal cysts and "half of a pancreas" on imaging. Her family history was significant for T2DM in father and renal cysts in father and 2 siblings. At 23 weeks gestation, HbA1C was 5.7% (nl 4-6), C peptide was 3 ng/mL (nl 1.0-7.1) and GAD65 antibodies were negative. ALT and AST were normal. Ultrasound confirmed simple bilateral renal cysts and no genitourinary malformations. She developed premature rupture of membranes at 27 weeks and delivered a pre-term baby boy at 30 weeks. The newborn had renal cysts and transient neonatal hyperglycemia requiring insulin. Genetic testing of the patient showed a truncation mutation in exon 3 of transcription factor-2 gene, confirming a diagnosis of HNF-1- β MODY. Given the patient's history of periods of insulin independence and her residual C-peptide, she was started on metformin in combination with insulin at 2 weeks postpartum. Two weeks later, insulin was discontinued and she was maintained on metformin alone, during which time her mean glucose was 104 mg/dL with a standard deviation of 34 mg/dL. One week after discontinuing insulin, HbA1C was 5.7% (nl 4-6), C peptide was 1.1 ng/mL (nl 1.0-7.1), random glucose was 97 mg/dL (nl 62-125), fructosamine was 213 μ mol/L (nl 200-285), and 1,5-anhydroglucitol was 12.4 mcg/ml (nl 6.8-29.3). **CONCLUSION:** HNF-1- β MODY should be suspected in patients with diabetes and renal cysts, and genetic testing can prevent misdiagnoses of T1DM or T2DM. While most patients with HNF-1- β MODY are insulin dependent, this patient did not require insulin for blood glucose control, even five years after her diagnosis.

Nothing to Disclose: AT, EH, ZAB, LKG

P3-550

Discordant Occurrence of Diabetes in Monozygotic Twins with Suggestive History of MODY - Possible Influence of Weight Gain.

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INTRODUCTION: MODY (Maturity Onset Diabetes of Youth) is characterized by precocious appearance of non-insulin dependent diabetes mellitus (NIDDP) and an important family history of DM. MODY also involves a dominant inheritance and the presence of precocious microangiopathic complications, mostly in MODY 1, MODY 3 and MODY 5. We will describe a case of a patient with a clinical history of DM suggestive of MODY who has a monozygotic twin without DM.

CASE: 32 years old patient, male, diabetic since 18 years, negative auto-antibodies to DM1, now controlled only with sulphonylurea after a ten year period receiving insulin, presents with diabetic nephropathy (CrCl=32,3 mg/ml/1,73m², 24 hours proteinuria of 4,5 g) and severe retinopathy. The father's patient is alive, with diabetes discovered at 24 years, with severe retinopathy and nephropathy and with one brother and five aunts who also presents with diabetes. The grandfather's patient died in an accident at 37 years old and his morbid history is not known. The monozygotic twin of this patient doesn't have DM and have a normal oGTT. Looking for ambiental influences that could be responsible for the appearance of DM in only one of the twins, it was found that the diabetic patient achieved 96 kg (BMI 33,2 kg/m²) when he was 18 years old and his brother's maximal weight was 86 kg/m² (BMI 29,7 kg/m²).

As MODY was highly suggested by the patient's history, we realized DNA analysis looking for mutations in HNF1A gene - the most prevalent gene involved in MODY cases in literature, also associated with severe microangiopathy.

DNA ANALYSIS: We looked forward three possible alterations in HNF1a gene in both siblings. We couldn't find any mutation, only five polymorphisms, one of those which was firstly associated with the appearance of atypical diabetes in Africans and afro-americans but, in a preliminary study in our center, appears to be very frequent in Brazilian controls without diabetes.

CONCLUSION: We hypothesized that monozygotic twins with a very suggestive familiar history of MODY could be discordant in the appearance of DM. This discordance must be due to ambiental differences in their lives; in the cases here described possibly the weight gain was responsible for the appearance of DM and its posterior complications in the patient affected by the disease.

Nothing to Disclose: BH, MLC-G, AH

P3-551

Effect of Exenatide on Postprandial Hyperinsulinemic Hypoglycemia in Two Gastric-Bypass Patients.

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Background: Severe post-prandial hypoglycemia is a problematic complication of gastric bypass (GB) surgery. Elevated levels of GLP-1 following GB surgery have been considered to be involved in the pathogenesis of this condition, but a direct causative relationship has not yet been established. Some patients with post GB surgery hypoglycemia will develop transient post-prandial hyperglycemia followed by severe hyperinsulinemic hypoglycemia, suggesting loss of first phase of insulin secretion. Exenatide is a GLP-1 analogue that reduces post-prandial hyperglycemia by enhancing first phase insulin secretion, decreasing glucagon production, and delaying gastric emptying. These properties make exenatide a potential agent for treating post-GB hypoglycemia.

Here, we present two cases of severe post-GB hypoglycemia effectively managed by exenatide.

Clinical Case: Two patients with post-GB hypoglycemia refractory to conventional therapy, underwent serial blood samplings prior and after a mixed meal. The measured parameters were glucose and insulin. In patient 2, glucagon levels were also determined. On a separate day both received exenatide 5 mcg subcutaneously prior to the meal and the same parameters were again evaluated.

Prior to exenatide both patients had significant postprandial hyperglycemia (17.0 and 16.38 mmol/l, n < 7.8 mmol/l) and hyperinsulinemia (1207.7 and 2250.9 pmol/l, n 48-763 pmol/l) followed by symptomatic hypoglycemia (2.72 and 3.0 mmol/l). In addition, patient 2, had relative hyperglucagonemia (38 ng/l, n < 24ng/L). After exenatide treatment, both subjects had lower postprandial blood glucose levels (10.60 and 11.94 mmol/l), lower insulin peaks (393.1 and 1100 pmol/l), and less prominent and more transient hypoglycemia (3.4 and 3.2 mmol/l), with no symptoms. In addition, there was reduction in measured glucagon levels (25 ng/l). Patient 2 elected treatment with exenatide, which resulted in resolution of her symptoms.

Conclusion: In this report of two cases, exenatide administration was associated with decreased postprandial hyperglycemia, decreased postprandial insulin peak, reduction of late hypoglycemia and improvement of patient's symptoms. To our knowledge, this is the first case report demonstrating the benefit of GLP-1 analogue therapy in patients with hyperinsulinemic hypoglycemia after gastric bypass. Further randomized clinical trials are required to fully evaluate the potential beneficial effect of GLP-1 analogues in this population.

Nothing to Disclose: SECF, RP, UK, VM

P3-552

A Case Report of Utilizing Incretin Therapy To Achieve Diabetes Remission in a Late Stage, Morbidly Obese Diabetes Patient.

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Background: Patients with longstanding diabetes and high insulin burden generally have a very low chance of diabetes remission due to a significantly low beta cell mass. Despite this, similar patients undergo bariatric surgery and attain diabetes remission. The incretin system has been hypothesized as a possible mechanistic role in this reversal process.(1)

Purpose: We present a late stage morbidly obese, diabetes patient who achieved diabetes remission using incretin therapy without bariatric surgery.

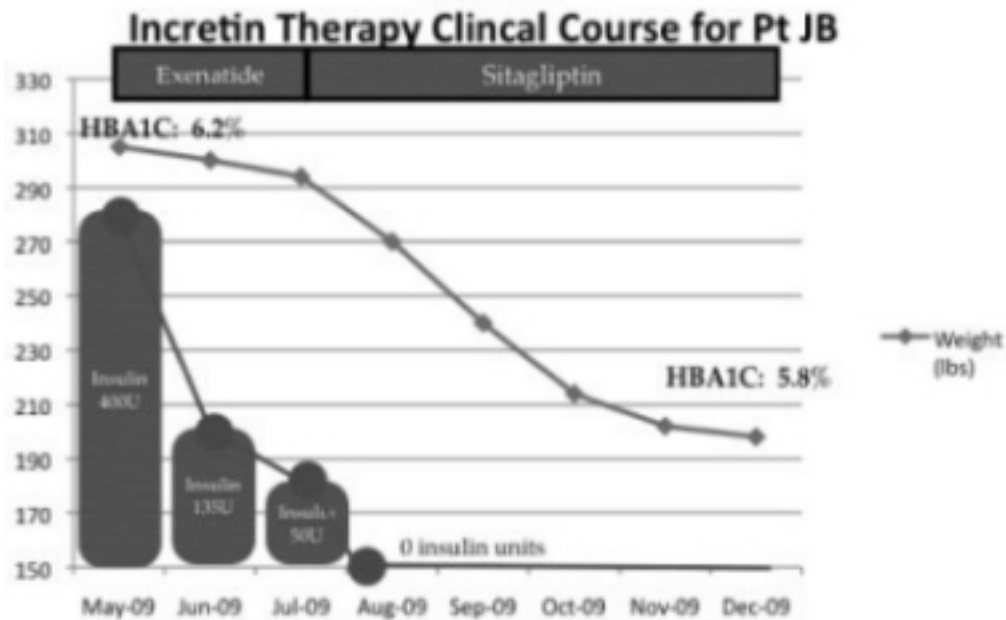
Clinical Case:

Mr. JB is a 45 year old Caucasian male with type 2 diabetes for 16 years. Over the years, his diabetes had progressed with worsening weight gain. He eventually transitioned from pills to insulin therapy. He had attempted numerous dietary programs with limited success. On presentation, he was morbidly obese with the insulin equivalent dose of 400units daily. The table below shows his baseline metabolic characteristics.

| | Baseline Metabolic Profile | 3 months | 6month Post intervention |
|------------------------|----------------------------|----------|--------------------------|
| Duration of DM2 | 16 years | 16 years | 16 years |
| Weight(lbs) Ht (5'11') | 305 | 270 | 202 |
| BMI | 42.5 | 37.5 | 28.2 |
| Insulin(Total Units) | 400units | 0 | 0 |
| Metformin | 1800mg | 0 | 0 |
| Exenatide | Start 20mcg | 0 | 0 |
| Sitagliptin | | 100mg | 100mg |
| Simvastatin | 40mg | 40mg | 40mg |
| Niaspan ER | 500mg | 500mg | 500mg |
| Fenofibric Acid | 150mg | 0 | 0 |
| HBA1C | 6.2 | | 5.8 |
| Fasting Blood Sugar | 206 | | 110 (w/o meds) |
| 2 hour 75gm OGTT | | | 181 (w/o meds) |
| Tchol | 246 | | 121 |
| TG | 911 | | 89 |
| HDL | 25 | | 43 |
| LDL | 102 | | 61 |
| LDL-Size | Small | | Large |

He was initiated on exenatide with an intensive lifestyle plan. He was able to rapidly weaned off insulin After two months, he was transitioned from exenatide to sitagliptin. At six months, patient had withheld his hyperglycemic medication for 1 week prior to the lab tests below.

Chart 1.



Patient's fasting blood sugar was 110mg/dl. 2 hour oral glucose tolerance was 180mg/dl. HbA1C was 5.8%. Patient had been able to maintain his weight loss pass 6 months.

Conclusion: Despite having late stage diabetes with a tremendous amount of insulin resistance, this patient was able to achieve diabetes remission with minimal beta cell reserve. In addition, patient's medication burden for blood pressure and lipids decreased dramatically. This is the first documented case report of the remission of a late stage, morbidly diabetes patient using incretin therapy.

(1) Rubino F, Schauer PR, Kaplan LM, Cummings DE. Annu Rev Med. 2010;61:393-411.

Nothing to Disclose: WAL

P3-553

The Value of Retrospective Continuous Glucose Monitoring in Patients with Adequate Diabetes Control.

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Introduction

Diabetes control is considered adequate if HbA1c <7% (ADA) or <6.5 % (AACE). Yet patients with good diabetes control may exhibit glycemic excursions and variable duration of hyperglycemia that may contribute to glucose – mediated vascular damage.

Background

No studies have been published to demonstrate the benefit of retrospective CGM use in patients with adequate diabetes control by HbA1c.

Clinical cases

3 patients with similar diabetes control by HbA1c have undergone a retrospective CGM for 5 days. Their 24 hour sensor data are presented in figure 1 and table 1.

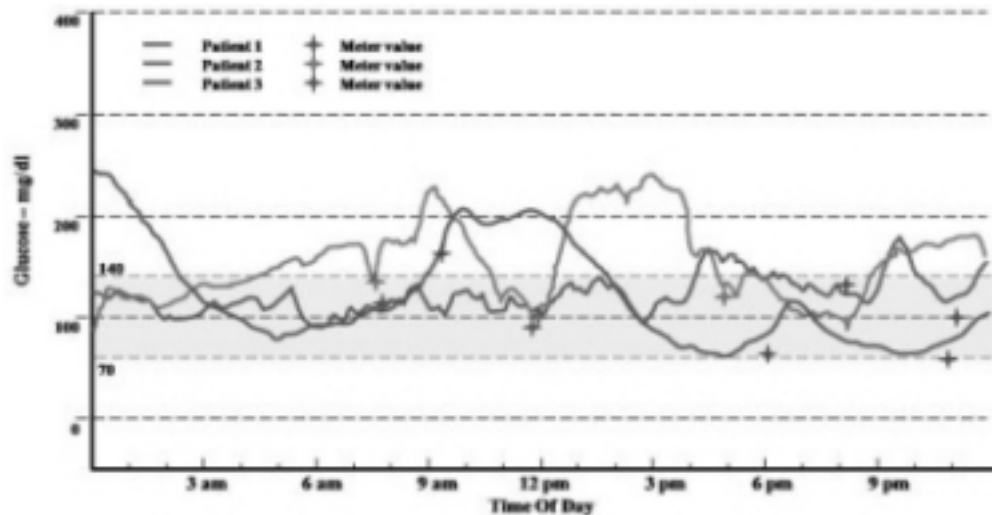
Patient 1 is a 55 year old female with type 2 diabetes, on metformin 500 mg twice daily. HbA1c 6.2%.

Patient 2 is a 50 year old female with type 2 diabetes, on Lantus 40 units subq Q12 hr and Novolog carbohydrate ratio 1:5. Hb A1c 6.1%.

Patient 3 is a 57 year old female with type 1 diabetes, on Lantus 20 units subq at bedtime and Humalog, carbohydrate ratio 1:10. Hb A1c 6.3%.

24 hour CGM sensor data summary of 3 patients

| | Patient 1 | Patient 2 | Patient 3 |
|--|-----------|-----------|-----------|
| Duration between blood glucose 70-140 mg/dl, % of time/24 hr | 86% | 41% | 70% |
| Duration above blood glucose 140 mg/dl, % of time/24 hr | 14% | 59% | 30% |
| Blood glucose range, mg/dl | 93-179 | 87-245 | 70-238 |



Clinical lessons

3 patients with similar HbA1c yet demonstrated different glycemic profiles by CGM. Patient 1 exhibited the best glycemic control with only 14% of the time spent >140 mg/dl in a 24 hour period. Patient 2 spent >50% of the time >140 mg/dl. Patient 3 spent 30% of time (7.2 hr /day) >140 mg/dl. Because of considerable duration of hyperglycemia in patient 2 and 3 they may benefit from additional therapy changes. The evaluation of duration of hyperglycemia in the above patients would not be possible with fingerstick tests alone.

Conclusion

Retrospective CGM in patients with adequate glycemic control by HbA1c helps to identify those with wide glycemic excursions and considerable duration of hyperglycemia. It is a valuable tool that may assist in diabetes therapy changes, specific diet and behavior modifications in order to attempt to reduce glycemic excursions and duration of hyperglycemia.

(1)BW Bode, TM Gross, KR Thornton, JJ Mastrototaro. Continuous glucose monitoring used to adjust diabetes therapy improves glycosylated hemoglobin: a pilot study. Diabetes Research and Clinical Practice 1999;46:183-190.

(2)IB Hirsch, M Brownlee. Should minimal glucose variability become the gold standard of glycemic control? Journal of

Diabetes and Its Complications. 2005;19;178-181.

Disclosures: GS: Expert Opinion, Medtronic Minimed.

P3-554

Insulin Edema - A Rare Presentation.

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Introduction -Insulin edema is a rare and benign complication of insulin therapy. A syndrome of unidentified origin with exclusion of all other causes of edema. It occurs in patients with either type 1 or type 2 diabetes after the introduction or intensification of insulin treatment. It is characterized by weight gain, mild to moderate edema and rarely generalized edema.

Case report - A thirty two year old Caucasian female diagnosed with Type 1 Diabetes Mellitus eight years prior with an initial weight of 250lbs. She was previously on and off insulin therapy. She has not taken her insulin for five months , no hospitalization .She had a glycosylated hemoglobin of 17.4%.

Physical Examination - BMI of 20kg/m² and a weight of 125lbs. BP-120/70 , PR -82 .

Subsequent course -Basal and bolus insulin was restarted and was increased on the succeeding days. There was significant improvement in her blood glucose levels. One week after insulin treatment, she noted significant swelling in her feet that ascended to her legs and abdomen. She had a significant weight gain of 60 pounds two weeks after the initiation of Insulin when she presented to the emergency department. Physical examination revealed a puffy face and bilateral lower extremity pitting edema up to the abdomen. Lung, cardiovascular and abdominal examination was normal.

Investigations - K-3.5 , Na - 135 , Glucose -138 , creatinine 1.0 Chest X-ray, echocardiogram, abdominal ultrasound, liver function, thyroid function tests were normal

Treatment and Follow up -The patient was placed on a sodium and fluid restriction diet, continued her insulin and was mildly diuresed with Furosemide. Her edema significantly lessened and she lost 15 pounds on the third hospital day. The patient remained on Insulin therapy and was seen at the clinic two months later. Her weight was stable at 150lbs with no recurrence of edema.

Discussion- The pathogenesis of insulin edema involves two effects of insulin treatment - anti-natriuresis and increased capillary permeability. For our patient, she has been on a severe catabolic state given her insulin non-compliance with the majority of her weight loss from protein breakdown that resulted in a hypo-proteinemic state and capillary wall damage. When insulin was re-introduced, there was sodium and therefore fluid retention directly caused by insulin. Treatment is usually conservative with continuation of insulin therapy and occasionally diuretics.

The Edematogenic Properties of Insulin: AJKD Vol44, No 4, October 2004 - Kalambokis et al
Case Report - An unusual presentation of insulin Oedema: Diabet Med 24,1282-1285 (2007)
A.Chelliah et al Journal of Investigative Medicine vol 52 March 2004
P.Lee et al , diabetic medicine, 24,1282-1285 , April 2007

Nothing to Disclose: MK, JA, MR

P3-555

Acute Bilateral Cataracts in Newly Diagnosed Diabetes.

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Introduction:

Acute cataracts are rarely reported in diabetic patients, although chronic cataracts are a frequent complication of gradual painless vision loss. Our review of the literature revealed about 26 case reports (10 adults, 16 children) of acute cataracts that developed in diabetic patients in 14 articles.

Clinical case:

A 49 year old male presented to the walk-in clinic with a 6-week history of gradual onset painless blurry vision. A metabolic panel and ophthalmology evaluation were ordered. His random glucose was 419 mg/dL (normal 70-100 mg/dl) and A1C 14.1% (normal <6%); he was begun on glipizide and referred to diabetes clinic. Ophthalmology evaluation, the next day, revealed a bilateral visual acuity of 20/40, with mild cortical lens changes with a swollen lens in the right eye, described as early cataract changes. Fundoscopic exam revealed no evidence of any retinopathy or macular edema. After initial therapy with insulin, he was treated with oral agents (metformin and sulfonylureas), and his glycemic control improved. Despite improved blood sugars, he experienced worsening vision. Two weeks later, eye evaluation revealed no acute changes. However, repeat evaluation a week later (3 weeks after diagnosis) showed a best corrected visual acuity of 20/400 in the right and 20/150 in the left eye with nuclear sclerosis and star-shaped posterior subcapsular cataracts in the visual axis bilaterally. He underwent surgical extraction of the right eye cataract with intra-ocular lens implantation 2 weeks later. Visual acuity improved to 20/25 immediately and 20/40 one month after surgery. He continues to work as a computer analyst. Cataract surgery is scheduled for the left eye. Also, glycemic control has improved significantly with a recent A1C of 5.0%.

Conclusion: The differential diagnosis of acute vision loss in diabetes typically includes infections/inflammation, hemorrhage, central retinal artery/vein occlusion, hyphema, retinal detachment, and hyperglycemia causing changes in refractive power of the lens. Cataracts have not been considered part of this differential. We urge our fellow colleagues to consider an acute development of cataract in the differential diagnosis of acute worsening of vision loss. Also, such cases are more common in adolescents than in adults. The mechanism is unknown but may be related to the conversion of glucose to sorbitol in the lens by aldose reductase in the setting of hyperglycemia.

Nothing to Disclose: AHK, AT, RC, MAB

P3-556

Type II Diabetes Mellitus Diagnosed after Workup for an Episode of Prolonged Post-Prandial Hypoglycemia.

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A 2009 Clinical Practice Guideline (1) states, "An oral glucose tolerance test should never be used for the evaluation of suspected postprandial hypoglycemia." We present a case of an 83-year-old male with no history of diabetes found slumped over on the toilet shortly after lunch. EMS found his capillary blood glucose (CBG) was in the 30s (mg%), he was given glucose and became alert. In the emergency room CBG again was in the 30s and he was admitted, treated with IV D10W and food. Serum glucose was documented =41 mg% at 5 pm, 35 mg% at 7:20 pm and 30 mg% at 9 pm. on that day. Drug screen for sulphonylureas, nateglinide and repaglinide was negative. At 4:15 pm insulin=108.30 uIU/mL, C-peptide=20.7 ng/mL and proinsulin=82.1 pmol/L. MRI and CT of pancreas with contrast showed no convincing mass lesion. Liver and kidney function were normal. Blood sugars normalized on the following day and he was discharged from the hospital without recurrent episode of hypoglycemia over the following 4 months.

Outpatient workup included 14 hour fasting glucose=102 mg% with C-peptide=2.8 ng/mL (n:0.9-7.1), insulin=11.62 uIU/mL (n:1.90-23.00) and proinsulin=36.1 pmol/L (n:2.1-26.8). Post-cosyntropin cortisol=27 mcg/dL, HbA1C=5.7. After 75-gram oral glucose: serum glucose fasting=111 mg%, 0.5 hour=176 mg%, 1 hour=228 mg%, 1.5 hour=234, 2 hour=224 mg%, 3 hour=159 mg%, 4 hour=105 mg%. Anti-GAD and anti-insulin antibodies were absent.

This case demonstrates that Type II diabetes mellitus may present with an episode of postprandial hypoglycemia. Although a common belief, it is difficult to find documentation of such in the medical literature. The persistence of hypoglycemia, in this case, was consistent with prolonged exposure to endogenous insulin. We propose the hypothesis that an episode of inflammation in the beta cells following a meal resulted in a biphasic dysfunction similar to thyroiditis, first with leakage of excess hormone from the pancreas causing prolonged hypoglycemia and then, insufficient hormone production causing diabetes.

(1) Cryer PE et al., J Clin Endocrinol Metab 2009;94:709-728

Nothing to Disclose: FS, CRZ

P3-557

Persistent Hypoglycaemia in a Type-1 Diabetes Patient under Haemodialysis Treatment and Treated with an Insulin Analogue.

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Background: Diabetic patients with chronic kidney disease present several anomalies in the glucose and insulin metabolism that enhance their risk to develop hypoglycaemia. The beginning of haemodialysis treatment makes it easier to happen, therefore making insulin analogues a more frequently option in some patients. Nevertheless, there's little scientific information about the metabolic stability with the insulin analogues in haemodialysis patients.

Clinical case: The authors report a case of a 41-years old woman with chronic C hepatitis and long-standing type-1 diabetes, with generalized microvascular disease and end-stage renal disease under haemodialysis treatment. She was being treated with insulin glargine for the last 3 months because of frequent hypoglycaemia with de NPH-insulin. She was admitted into our hospital with non-specific symptoms and developed hypoglycaemic coma, for about 7 hours after she had injected the 37 insulin units that she was doing for the last 3 days (although she was only prescribed 27 units). The hypoglycaemic coma was very difficult to treat, though she was treated with several glucagon injections, hypertonic glucose, continuous infusion of 5% glucose and one dialysis session. Forty-eight hours after the last insulin injection the insulin serum levels were still measurable (7.2uU/mL, N=2.6-24.9) and C-peptide was low (0.51ng/mL, N=1.1-4.4) and about 72 hours after hospitalization she was still having glycaemic levels under 60mg/dL whenever the iv glucose was stopped. Adrenal insufficiency and thyroid dysfunction were excluded. The results of anti-insulin antibodies are not available yet. The authors consider that persistent hypoglycaemia was probably caused by an excessive dosage of the insulin glargine she was taking and also caused by a longer half-life of that long-acting insulin analogue in a haemodialysis patient with some degree of failure of the counterraregulatory hypoglycaemic mechanisms. When discharged she was with a lower dose of insulin detemir (18 units) but, although she had no new hypoglycaemic episodes, she had hyperglycaemic levels, making it necessary to frequently reevaluate her treatment.

Conclusion: Diabetic patients under haemodialysis treatment have a greater risk of glycaemic instability and hypoglycaemic episodes. Hypoglycaemia is not usual with insulin glargine but, as it can occur, health care professionals should be aware of it and may need to reduce the insulin dosage or change the insulin type.

Nothing to Disclose: AMS, ST, MA, AG, GR, AC, CA, JD, CF

P3-558

Pegylated Interferon α -Related Type 1 Diabetes.

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Background: Interferon alpha (IFN- α) is widely used for its anti-viral, antiproliferative, and immunomodulatory activities. IFN- α treatment for chronic hepatitis C virus (HCV) infection is associated with the development of autoimmune disorders in 2.5-20% of patients (1). First case of IFN- α associated type 1 diabetes (T1DM) was reported in 1992, and since then, 31 cases have been reported; however, the exact incidence is unknown (1).

Clinical Case: 56 year-old Hispanic male with history of chronic HCV (genotype 1) infection, who presented with malaise, nausea, vomiting, and polydipsia 6 months after starting pegylated IFN- α and ribavirin treatment. Initial tests were consistent with DKA: glucose of 743 (70-110mg/dL), bicarbonate 13 (24-31 mmol/L), sodium 136 (135-145mmol/L), chloride 95 (95-106mmol/L), anion gap of 28, positive serum ketone 1:2 (neg), hemoglobin A1c 9.3 (4.8-6.2%). His arterial blood gas showed a pH of 7.27 (7.35-7.45), pCO₂ of 16.8 (35-45 mmHg), bicarbonate of 7.8 (18-23 mmol/L). His GAD antibody level was 92.6 (1-1.45 U/ml), and c-peptide level was 0.1 (1.1-4.6 ng/ml). Upon review of the patient's medical record, it was noted that he had an elevated random glucose level of 300 mg/dL 3 weeks prior to his DKA presentation, which was not intervened.

He was started on insulin therapy, with excellent glycemic control; his A1c went from 9 to 5.9 over the ensuing months, only on 16 units of insulin a day. IFN- α and ribavirin therapy was continued, and patient finished a one year course. Despite no changes in his medical condition, he gradually required increasing dose of insulin, with difficulties in maintaining glycemic control. He is currently on 40 units of insulin a day, with hgb A1c of 8.8%. His GAD level one year after the diagnosis of T1DM is still elevated at 104 ng/ml.

Conclusion:

T1DM is a known, albeit rare, complication of IFN- α therapy. Most recently published prospective study reports an incidence of IFN- α associated T1DM as 2.6% (1). Currently, there is no guideline screening for pancreatic autoimmunity or monitoring of glycemic values in patients undergoing interferon therapy. Our patient was noted to have an elevated random glucose of 300mg/dL on routine labs done a few weeks prior to onset of DKA. Given the potential morbidity of DKA, we suggest increasing awareness of risk of diabetes (especially T1DM) during IFN- α treatment, and frequent monitoring of glycemic values should be advocated.

(1) Schreuder TCMA et al. High incidence of type 1 diabetes mellitus during or shortly after treatment with pegylated interferon alpha for chronic hepatitis C virus infection. *Liver International* 2007; 39-46

Nothing to Disclose: JYC, SE

P3-559

Initial Presentation of Type 1 Diabetes as Hyperglycemic Hyperosmolar Syndrome along with Severe Hypothyroidism in a 7 Year Old Boy with Down syndrome.

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Background: Hyperglycemic Hyperosmolar Syndrome (HHS) is a very rare presentation of type 1 diabetes mellitus. The purpose of this report is to highlight the clinical course, complications associated with the hyperosmolar state and successful management in a young boy with Down syndrome. This case is quite unique for the associated severe hypothyroidism diagnosed at the same time.

Clinical Case: A 7 year old boy with Down syndrome presented with 3 days of polyuria, polydipsia and one day of altered mental status and heavy breathing. He was being treated with antibiotics for a presumed sinus infection. He was intubated electively due to lethargy and altered mental status and dopamine drip started for inotropic support. The initial blood glucose was 1905 mg/dl, serum osmolality 418 mosm/kg (275-295), 1+ ketones, venous pH 7.3, HCO₃ 18 mmol/l. HbA_{1c} 10.5%, c-peptide 0.3 ng/ml (0.8-8.5), GAD 65 antibody >250 IU/ml (<5), islet cell antibody positive. Hyperglycemia was managed with IV hydration and a low dose insulin drip at 0.05 units/kg/hr facilitating a slow fall in blood glucose. On the second day of admission, he was found to be profoundly hypothyroid, TSH 1470 mcunit/ml, free T₄ 0.85 ng/dl (1.1-1.6), Total T₄ 0.6 mcg/dl (5.7-10.8), thyroid peroxidase antibody 450 IU/ml (<35 IU/ml). L-thyroxine was started NG at 50 mcg/day. Despite improvement in blood glucose and mental status, there was continue need for dopamine over the next 48 hours at which point IV l-thyroxine 62.5 mcg/day was given instead of oral l-thyroxine and he was quickly weaned off inotropic support. As mental status improved, he was transitioned to subcutaneous insulin. Cincial course was complicated by rhabdomyolysis (CK 6427 n: 31-152) and thrombosis of the left iliac and popliteal vein at the site of the central line axis as well as right brachial and subclavian vein.

Conclusion: Hyperglycemic Hypersomolar syndrome is a very rare presentation of type 1 diabetes. A literature review suggests that young children with Down syndrome and those with psychomotor delay have an increased frequency of HHS. There is a high degree of morbidity and mortality associated with this state and complications like cerebral edema and venous thrombosis are to be expected. This report also highlights that the severe hypothyroidism may have prolonged the need for inotropic support after rehydration and correction of hyperglycemia.

Nothing to Disclose: PV, PK

P3-560

Latent Autoimmune Diabetes in Adults (LADA) with High Titers of GAD 65 Antibodies but Lack of Long-Term Insulin Dependence.

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Objective: To present cases of LADA with high titers of GAD 65 antibodies but long duration of insulin independence.

Presentation: First patient is a 72 year old male with diabetes for 10 years. At diagnosis HbA1c was 9.8%, C-peptide was 0.8 ng/mL, anti-GAD 65 titer was 6,836 IU/mL (reference <176), ICA was negative, and anti-IA2 was negative. After five months of insulin therapy, C-peptide was 2.1 ng/mL and HbA1c was 6.1%. Treatment was then changed from insulin to metformin and glimepiride, and the patient has been on this regimen since 2000. Most recently, HbA1c is 6.6%, C-peptide 1.4 ng/mL, and GAD 65 antibody >250 IU/mL. Second patient is a 28 year old male diagnosed with diabetes and Graves' disease 9 years ago. He was initially prescribed insulin, which was discontinued after three months due to hypoglycemia. The patient remained off insulin for eight years until he was admitted for hyperglycemia. HbA1c was 9.3%, C-peptide was 0.6 ng/mL, GAD 65 antibody >250 IU/mL, and ICA was negative. He was prescribed glargine and aspart and HbA1c improved to 6.9%. Recently, he experienced hypoglycemia necessitating discontinuation of aspart. Discontinuation of glargine is being considered. Third patient is a 50 year old female diagnosed with diabetes 6 years ago. Initial HbA1c was 8.0%, C-peptide was 0.6 ng/mL, GAD 65 antibody titer was 1125 U/mL (ref <1.0), and ICA was negative. She was started on oral hypoglycemic agents, and three months later HbA1c improved to 5.3% and C-peptide to 2.0 ng/mL. Three years later her GAD 65 antibody continued to be detectable at >30 U/mL. Currently she is on metformin, rosiglitazone, and exenatide, and continues to maintain good glycemic control.

Discussion: Autoimmune diabetes mellitus results from autoimmune mediated destruction of the pancreatic beta cells. The rate of destruction is variable, with the slowly progressive form generally occurring in adults (LADA). By definition, LADA patients do not require insulin initially after diagnosis, but have faster decline in beta cell function compared to those with antibody negative type 2 diabetes. Multiple studies have shown a positive correlation with the presence of islet antibodies and relative insulin requirement, and that high concentrations of islet antibodies predict future beta-cell failure.

Conclusion: Some patients with LADA can remain mostly insulin independent for up to a decade. More investigation is needed to determine factors favoring insulin independence.

Nothing to Disclose: SC, RP, JK, EPL, LP

P3-561

A Case of Spontaneous Pneumomediastinum and Diabetic Ketoacidosis in Patient with Diabetes Mellitus Which Was Undiagnosed before.

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Introduction:

Pneumomediastinum is a rare complication of diabetic ketoacidosis (DKA). The pathophysiology of DKA commonly associated with changes in pressure gradients in the pulmonary alveoli secondary to vomiting and/or Kussmaul respiration. We report a 21-year-old male with DKA who have no history of diabetes mellitus before and was found to have pneumomediastinum and subcutaneous emphysema.

Case:

A 21-year-old male without previous history of diabetes mellitus was admitted via ER with complaints of general weakness and throat pain on swallowing. His BMI was 19.6 kg/m², white blood count 18,480 /mm³, haemoglobin 16.5 g/dL, BUN/Cr 23.5/2.8 mg/dL. His serum glucose was 771 mg/dL, sodium/potassium/chloride/bicarbonate 138/45/92/15.2 meq/L. His calculated anion gap was 31 with an arterial pH of 7.31. Serum ketone was elevated to 5.7 mmol/L. Initial chest radiograph showed mediastinal air along left cardiac border but this finding was ignored by ER doctor. On 2nd day of admission subcutaneous emphysema was detected by physical examination, and initial chest X-ray film was re-examined. After then chest CT and gastrograffin swallowing study was followed. Chest computerized tomography (CT) demonstrated the air in the soft tissues of the neck, nasopharynx and within the anterior mediastinum. There was no evidence of esophageal tear or extravasation of gastrograffin into the mediastinum. His serum C-peptide was 0.7 ng/mL and HbA1c 16.9%. Test about anti-GAD antibody and anti-islet cell antibody showed negative results. His pneumomediastinum and subcutaneous emphysema resolved spontaneously during the admission with oxygen therapy using facial mask, and he was discharged in improved condition with the prescription of multiple daily insulin injection.

Nothing to Disclose: D-MK, HN, MGC

P3-562

Successful Pregnancy in a Patient with Type I Diabetes and Prior Ischemic Heart Disease.

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BACKGROUND

The coexistence of myocardial ischemic heart disease and diabetes (White Class H) has been considered a formal contraindication for pregnancy due to the high fetal and maternal mortality(1). Only 10 cases of pregnancy in diabetic women with prior history of ischemic heart disease are documented in the literature(2). We report a pregnancy in a woman 6 years after myocardial infarction with good maternal and fetal outcome.

CLINICAL CASE

A 29 year old woman with a twenty year history of diabetes type I on an insulin pump was initially evaluated at NY Medical College faculty practice at 8 weeks gestation for glycemic management. Pertinent past medical history included coronary artery disease, myocardial infarction requiring stenting of left circumflex artery in 2003, hyperlipidemia, hypertension, retinopathy and nephropathy. Patient's HBA1c was suboptimal in the range of 7 - 9 %, with improvement during pregnancy to 6.5%.

The patient presented to labor and delivery at 33 weeks gestation with progressive proteinuria, hypertension and edema. Cesarean section was performed for worsening pre-eclampsia. The neonate was large for gestational age and initially had APGAR scores of 9/9 but subsequently required intubation for respiratory distress and remained in neonatal intensive care 26 days. Patient was discharged home on post operative day 4 without complication with her infant requiring an additional ICU stay of 22 days.

CONCLUSION

Coronary artery disease in women with diabetes of child-bearing age is likely to be encountered more frequently in the future due to the increasing incidence of type II diabetes. The timing of myocardial infarction in relation to pregnancy onset is important for both maternal and fetal outcomes. Decreased time interval between myocardial infarction and pregnancy may be associated with maternal and fetal morbidity(3).

Currently there is limited experience with Class H Pregnancy and outcomes. Good ventricular performance and patent coronary vessels are good prognostic indicators for diabetic women who conceive after myocardial infarction(2).

Pre-conception counseling, adequate glycemic control, evaluation of cardiac function and optimal treatment of coexisting medical conditions may improve maternal and fetal outcomes.

(1)Gordon MC, Landon MB, Boyle J, Stewart KS & Gabbe SG. Coronary artery disease in insulin-dependent diabetes mellitus of pregnancy (class H) : A review of the literature. *Obstetrical and Gynecological Survey* 1996 51 437-444.

(2)R. Darias, L. Herranz, M T Garcia-Ingelmo and L F Pallardo. Pregnancy in a patient with type 1 diabetes mellitus and prior ischemic heart disease.

(3) W. Bagg, et al. Pregnancy in Women with Diabetes and Ischaemic Heart Disease. *Aust NZ J OBSTET GYNAECOL* 1999; 39:1:99.

Nothing to Disclose: RPA, CK, RA, MDS, GV, IAW

P3-563

Hyperglycemic Hyperosmolar State at the Onset of Type 2 Diabetes Mellitus in an Adolescent Male.

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Objective: To describe the clinical presentation of hyperglycemic hyperosmolar state (HHS) in an adolescent with new onset Type 2 Diabetes Mellitus (T2DM).

Introduction: Hyperglycemic hyperosmolar state is rare in the pediatric population; however, this is changing as the incidence of T2DM increases. HHS has a high mortality and morbidity rate. The diagnosis and management of HHS presents a unique challenge in pediatric patients who may present with a mixed picture of HHS and diabetic ketoacidosis (DKA). Males, African-Americans, and patients with developmental delay have been reported at highest risk of presenting in HHS. Evidence for optimal treatment of HHS in the pediatric population is sparse.

Clinical Case: A 15-year-old African-American male with developmental delay was brought to the emergency department after a two-day history of feeling generally unwell. Upon presentation to hospital, the patient was noted to be obtunded, hypotensive, and tachypneic. He had acanthosis nigricans on his posterior neck. His weight was approximately 100kg with a body mass index (BMI) of 32.5 kg/m² (>95th percentile). Initial investigations revealed the following: pH=6.97 (N=7.35-7.41), HCO₃⁻= 5 mEq/L (N=20-25 mEq/L), glucose=1636 Mg/dL (90.9 mmol/L, N=2.4-6.3 mmol/L) serum osmolality=454 mOsm/kg (N=275-295 mOsm/kg), Na=141 mEq/L (N=135-145 mEq/L), K=8.4 mEq/L (N=3.5-5.0 mEq/L), urea 45.93 mg/dL(16.4 mmol/L, N= 3.0-7.0 mmol/L), creatinine 4.88 mg/dL (432µmol/L , N=65-121µmol/L), and urinalysis revealed 1+ ketones and 4+ glucose. CT and MRI studies of the brain showed no evidence of cerebral edema. Normal saline was started at 4-6 ml/kg/hr. Insulin infusion was started (0.03-0.08 units/kg/hr) with dextrose 4hrs after fluids began. The patient's clinical course was complicated by severe hyperkalemia, acute renal failure, refractory status epilepticus, rhabdomyolysis, pancreatitis, and hypertension. On the sixth day of admission, the patient was transferred out of the intensive care unit in stable condition. He was started on subcutaneous insulin and metformin prior to discharge. He did not develop any long-term sequelae secondary to HHS.

Conclusion[bold]: Our case emphasizes the complexity in managing patients with a mixed DKA/HHS presentation and associated morbidities. Furthermore, it highlights the importance of disseminating and implementing screening guidelines for T2DM, so as to prevent this potentially devastating complication.

Nothing to Disclose: SLT, MNN

P3-564

Clinical Controversy: The Use of Pramlintide Acetate in Gastroparesis.

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Background: Gastric emptying time is an important determinant of the rise in blood glucose levels following a meal. Patients with gastroparesis have delayed gastric emptying and decreased intestinal motility, which can lead to erratic blood glucose levels. Since pramlintide acetate slows gastric emptying time, it is contraindicated in patients with gastroparesis. The use of pramlintide acetate in patients with gastroparesis has been abandoned as a result of the potential for further delay of release of food from the stomach.

Case 1: A 34 year-old white male with uncontrolled type 1 diabetes mellitus and diabetic gastroparesis presented to the hospital with acute, constant epigastric abdominal pain associated with nausea and vomiting. The patient had additional complaints of chronic diarrhea for years prior to admission. He did have a gastric pacemaker placed six months prior to admission. The patient was begun on an insulin drip at 2.5 units per hour and pramlintide acetate was begun at 2 units with each meal. The patient's insulin drip was stopped with development of postprandial hypoglycemia within 12 hours and his insulin requirements adjusted downward with less erratic glycemic control, less subjective nausea and report of abdominal pain.

Case 2: A 47 year-old African American female with a history of pancreatectomy for insulinoma who developed post-surgical diabetes with subsequent gastroparesis development presented with nausea, vomiting, and right upper quadrant abdominal pain in diabetic ketoacidosis. She was begun on an insulin drip at 4.5 units per hour and pramlintide acetate was begun at 2 units with each meal. The patient's insulin drip was stopped with development of postprandial hypoglycemia within 12 hours and his insulin requirements adjusted downward with less erratic glycemic control, less subjective nausea and report of abdominal pain.

Conclusion: The significance of the aforementioned cases with similar outcome is two-fold. Pramlintide acetate should not be abandoned as an adjunctive treatment of the diabetic patient suffering from gastroparesis. This agent can be used to stabilize blood sugar readings without worsening symptoms of hypoglycemia or gastroparesis. Furthermore, post-prandial hypoglycemia does occur in a predictable fashion and this eliminates the erratic glycemic pattern of the diabetic patient with gastroparesis. To assist in management of this subset of patients, it is instead the insulin regimen that needs modification.

Disclosures: EAC: Clinician, Amylin Pharmaceuticals; Advisory Group Member, Novo Nordisk, Amylin Pharmaceuticals; Speaker Bureau Member, Amylin Pharmaceuticals, Abbott Laboratories, Lilly USA, LLC, Takeda, Novo Nordisk, Merck & Co., Pfizer, Inc., Novartis Pharmaceuticals.

Nothing to Disclose: DPH, MG

P3-565

Severe Insulin Resistance and Hypertriglyceridemia with Acquired Partial Lipodystrophy after Allogenic Bone Marrow Transplant.

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Background

Partial lipodystrophic syndromes are underdiagnosed, except for the most common ones found in HIV/AIDS patients receiving antiretroviral therapy.^{1,2,3,4}

Case Report

We describe an unfamiliar case of acquired partial lipodystrophy in a 37 year old woman. She had received whole body radiation and allogenic bone marrow transplant (BMT) for acute myeloid leukemia (AML) at age 17. She also suffered from chronic sclerodermatous graft vs. host disease (GVHD) following BMT. Nine years after BMT, she developed diabetes with severe insulin resistance (7.2 units insulin/kg/day at weight 47kg and BMI 20), marked hypertriglyceridemia (>3000 mg/dl), hepatic steatosis (enzymes, US, CT) and profound loss of subcutaneous fat in buttocks and extremities, sparing face, neck and trunk.



Currently she is in complete remission from AML and free of GVHD symptoms, but the metabolic changes remain. Addition of pioglitazone reduced A1C by 4% at the same insulin dosage, but no change in subcutaneous fat. Her phenotypic pattern of subcutaneous fat loss is different to most of the widely described familial or acquired lipodystrophies.

Phenotypes of Various Partial Lipodystrophies

| Lipodystrophy Type | Areas of Fat Loss | Areas Spared | Abdominal Obesity |
|--|-------------------------|---------------|-------------------|
| Acquired | | | |
| HIV/AIDS Rx with Protease Inhibitors | Face, arms, legs | Neck | Yes |
| Acquired partial lipodystrophy | Face, neck, arms, trunk | Hips and legs | Yes |
| Inherited Familial partial lipodystrophy | | | |
| Kobberling | Arms and legs | Face and neck | Yes |
| Dunnigan | Arms, legs, trunk | Face and neck | Yes |
| PPARG mutations | Arms, legs, face, neck | Trunk | Yes |

| Patient in this Case | | | |
|----------------------|---------------|-------------------|----|
| Post GVHD | Arms and legs | Face, neck, trunk | No |

Conclusions

Literature review reveals only one other reported case⁴ similar to this case in age of onset, phenotypic/biochemical patterns, and predisposing factors (i.e., following BMT/GVHD; but not HIV infection/Protease Inhibitors therapy, nor a viral-like illness, not congenital, nor familial).

We conclude that these cases may represent an entirely separate entity of acquired partial lipodystrophy related to sclerodermatous GVHD after bone marrow or stem cell transplantation. Further research to establish the association is warranted.

(1) Karen L. Herbst et al., *Diabetes Care* 2003;26:1819

(2) Anoop Misra et al., *Medicine* 2004;83:18

(3) Abhimanyu Garg., *N Engl J Med* 2004;350:1220

(4) D. P. Rooney and M. F. Ryan; *Diabetic Medicine* 2006;23:436

Nothing to Disclose: NMS, KCC, MH, EI

P3-566

Type B Insulin Resistance Syndrome: Hyperglycemia Switching to Hypoglycemia - Case Report.

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Background: Type B insulin resistance syndrome is a rare cause of hypoglycemia. It is caused by polyclonal IgGs antibodies directed against the insulin receptor that block the binding of insulin and inhibit its effects, or may have an insulin-mimicking effect, which can cause spontaneous hypoglycemia. **Clinical case:** A 33-year-old Brazilian woman was diagnosed with mixed connective tissue disease (MCTD) in 1999. In December 2008, her disease became clinically silent. After 9 months there was recurrence of MCTD associated with development of acanthosis nigricans affecting axillae, groin and neck, hypertrichosis, acne and amenorrhea. Laboratory evaluation revealed anti-dsDNA >1000 UI/mL (NR <100UI/mL), anti-Sm 26 UI/mL (NR <10UI/mL), complement consumption, CK 330 U/mL (NR 26-192), hematuria and proteinuria. Treatment with prednisone 60mg/d and azathioprin 50mg/d was initiated. During in-patient regime for the treatment, she presented with hyperglycemia, glucosuria, HBA1c levels of 9,1%. Fasting triglyceride levels were low. Insulin treatment was initiated, and then she went on develop hypoglycemia with adrenergic and neuroglycopenic symptoms. Despite discontinuing insulin treatment, she experienced continued episodes of transient hypoglycemia and hyperglycemia. Initial workup showed a fasting blood glucose of 51mg/dL (NR: 70-100mg/dL), an insulin levels of 11,6 µU/mL (NR<25,0 µU/mL), C-peptide of 1,8 ng/mL (NR:0,4-3,6 ng/mL). Screening test for oral hypoglycemic agents was negative. An oral glucose tolerance test was performed. Four hours after the administration of 75 g dextrose, her glucose levels dropped to 35 mg/dL, insulin was 178,5 mcg/ml, C-peptide was 9,6 ng/mL. Mixed-meal diagnostic test showed hypoglycemia at fasting and after three, four and five hours of test. Abdominal and thoracic MRI were normal. Serum anti-insulin antibodies were absent; however, serum anti-insulin receptor antibodies were positive (RIA) making the diagnosis of type B insulin resistance. Hyperandrogenism was observed with elevated testosterone levels. Pelvic ultrasound showed cystic enlargement of the ovaries. After two months of immunosuppressive treatment, there was apparent restitution of glucose homeostasis. **Conclusion:** Autoimmune forms of hypoglycemia are uncommon. However type B insulin resistance must be considered in any patient with hypoglycemia, mainly if it alternates with hyperglycemia in patients with a coexistent autoimmune disorder.

(1) Lupsa BC et al., *Medicine* 2009; 88: 141-153.

(2) Arioglu E et al., *Medicine* 2002; 81: 87-100.

Nothing to Disclose: PMM, DI, MAAP

P3-567

Clinical Predicates of Survival in Emphysematous Pyelonephritis (EPN).

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Background: EPN is a rare and potentially lethal complication of ascending urinary tract infections with (most commonly) *E. coli* or *K. pneumoniae*. It is associated with 80% mortality when diagnosed late or treated inappropriately. However, early diagnosis is hampered by non-specific presenting symptoms (malaise, fever, and abdominal pain, rather than flank pain, which occurs in only 25% of cases), and even though immediate nephrectomy is life-saving, it runs counter to the natural instinct of the internist to preserve the kidney at any cost (1). We present a case that highlights the importance of a high index of suspicion in the appropriate clinical context, leading to survival with appropriate management.

Clinical Case: A 61 year old white female with hyperlipidemia and hypertension, presented to an outside hospital with 2 weeks of malaise, fever, abdominal pain, nausea and vomiting, as well as polyuria, polydipsia, and 50 lb weight loss over the preceding year. A urinary tract infection was diagnosed (confirmed when *E. coli* were cultured in urine) and treated with ceftriaxone. Despite this, her condition deteriorated, culminating in hemodynamic collapse requiring pressor support, and she was transferred to our center in septic shock, with fever (102.5F), hypotension (BP 80/54), renal failure (creatinine 6.6 mg/dl) and hyperglycemia (glucose 472 mg/dl, HbA_{1c} 12.8%). CT scan showed extensive parenchymal gas and edema in the right kidney without perinephric fluid or dilatation of the calyceal system. She underwent emergency right nephrectomy and, after an uneventful postoperative course marked by recovery of adequate renal function (creatinine 2.2mg/dl), was discharged on a multiple daily insulin regimen.

Teaching Lessons: This case exemplifies the twin clinical predicates leading to survival in EPN, namely, a high diagnostic suspicion in the appropriate clinical context and aggressive surgical intervention. The typical clinical scenario of a female (6 out of 7 patients with EPN) with diabetes (80% of cases) and an upper UTI (fever and abdominal pain) must trigger imaging studies to confirm the presence of gas in the kidney (1,2). Immediate nephrectomy, except in the most unusual of circumstances such as a single kidney or bilateral disease, is imperative for survival, because it has a reported success rate of 90%, compared to ~ 50% for medical management, with or without percutaneous drainage (2).

(1) Joshi, N., Caputo, G., Weitekamp, M., Karchmer, A.W. Infections in patients with diabetes mellitus. NEJM, 1999; 341: 1906-12.

(2) Huang, J., Tseng, C., Emphysematous pyelonephritis: clinicoradiological classification, management, prognosis, and pathogenesis. Arch Intern Med, 2000; 160: 797-805.

Nothing to Disclose: AEC, BJD, RHR

P3-568

Study of Thyroid Peroxidases (TPO) Gene Polymorphism in Hypothyroidism.

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Thyroid Peroxidase (TPO) enzyme is a membrane-bound glycoprotein that catalyzes incorporation of iodine into tyrosine residues and plays the critical enzymatic step in thyroxine biosynthesis. Mutations in the thyroid peroxidase (TPO) gene (particularly non-synonymous cSNPs) can lead to severe defects in thyroid hormone production. Most of different mutations of the TPO gene have been reported in exons 8, 9, 10, 11, and 14, which are present in the catalytic site. It is hypothesized that various ethnic groups in the Indian population have acquired distinct mutations in the TPO gene.

Aims & objectives:

1. What proportion of the diagnosed Indian hypothyroid patients screened is associated with mutations in the TPO gene?
2. What types of TPO gene mutations are prevalent in the screened population?
3. Is there a possible association of mutated TPO in the genesis of auto-immune thyroiditis?

Materials & methods:

154 consecutive hypothyroid cases and 150 controls have been studied for this purpose. Thyroid function test and Anti TPO antibody is done for evaluation of cases and control population. Control population is euthyroid & TPO antibody negative. Mutation in TPO gene is studied by molecular techniques: PCR and capillary DNA sequencing.

Results:

Among our study cases 56.5% is primary hypothyroid with goiter, 19.5% is primary hypothyroid without goiter, 14.3% congenital hypothyroid and 5.8% subclinical hypothyroid. Mean age of cases 30.1 ± 14.1 and controls 32.9 ± 9.5 yrs. 11% of hypothyroid patients have catalytic site TPO gene mutation. Among the catalytic site mutation most involved exon is 8 (3.2%), others are 11 (1.9%), 10(2.6%), 13(1.9%). Mutation is more common in congenital hypothyroid (22.7%) compared to acquired hypothyroidism (9.3%). TPO antibody positivity of total study case population is 29.9%. Mutation in TPO antibody +ve group is 24% and TPOAb -ve group is 4.8% which is statistically significant ($P < 0.001$). Highest number of mutation in TPO antibody +ve cases is seen in chromosome 8 (23.5%).

Conclusion:

Our study shows good number of our hypothyroid population is having TPO gene mutation, of which exon 8 mutation is the highest. TPO mutation is found to be significantly associated with TPO antibody positivity. We are planning for functional in vitro analysis of TPO mutations for detection of functional affection by this mutation.

Nothing to Disclose: AS, SC, MD, KP

P3-569

Three Novel PAX8 Mutations in Three Unrelated Patients with Congenital Hypothyroidism.

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We identified three novel PAX8 mutations (S54R, R133Q, E234K) in three unrelated patients with congenital hypothyroidism. Two of them (S54R and R133Q) are located within the area of the PAX8 gene which encodes the PAX8-DNA binding domain. E234K lies outside of the DNA binding domain as well as outside of the activation domain. We functionally characterized all three mutations using various *in vitro* assays. S54R showed no activity on the TG- and the TPO-promoters *in vitro*. It showed only little response when co-expressed with TTF1. Also R133Q had no activation of the TG- and TPO-promoters but co-expression of TTF1 showed only an increase of 50 % compared to the WT. In contrast E234K, which is located outside the DNA-binding domain, exhibits the same transactivation ability as the WT control. All three mutant proteins are correctly localized in the nucleus. The absence of promoter activation of S54R is due to a loss of the ability to bind to DNA, thus transcription of downstream targets such as TG and TPO is inhibited. The other two mutant PAX8 proteins do not show any alteration on their DNA binding ability. The molecular mechanism by which R133Q has an impaired function might be a dominant negative effect. Interestingly, *in silico* analysis of the E234K mutation results in an additional exonic splice enhancer. Thus, this mutation might have impact on the correct splicing of the PAX8 gene which could result in an altered transcript with impaired function if it was expressed at all.

Nothing to Disclose: PH, HG, CF, MM, HGD, SR, JP

P3-570

Thyronamines in Humans: Development of a Validated Isotope Dilution Liquid Chromatography/Tandem Mass Spectrometry Method for Their Measurement.

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Background: Thyronamines are decarboxylated and deiodinated metabolites of the iodothyronines. They activate the trace amine-associated receptor 1 (TAAR1) - a G protein-coupled receptor activated by monoamines and amphetamine-related psychostimulants. Systemic administration of synthetic thyronamines to rodents have been shown to induce acute bradycardia, hypothermia, decreased metabolic rate, and hyperglycemia.

Objective: To develop a method to measure thyronamines at biological levels in humans

Methods: An API-5000 isotope dilution tandem mass spectrometer equipped with TurbolonSpray source and Shimadzu HPLC system was employed. 200 µL of human plasma/serum was deproteinized by adding 200 µL of acetonitrile containing internal standards. After centrifugation, 250 µL of supernatant was diluted with 250 µL distilled de-ionized water and a 250 µL aliquot was injected onto an Agilent Zorbax SB-C18 (2.1 x 30 mm, 1.8-micron) chromatographic column, where it underwent cleaning with 2% (v/v) methanol in 0.01% formic acid at a flow rate of 0.25 mL/min. After a 5 min wash, the switching valve was activated and the analytes eluted with a water/methanol gradient at a flow rate of 0.25 mL/min. Quantification by MRM analysis was performed in the positive mode.

Standards and Controls: Stock solutions and internal standards were prepared to obtain a concentration of 1 mg/mL for each. 40% ammonium hydroxide (v/v) in methanol was used as a solvent except that methanol was used for dissolving 3,3'-diiodo-L-thyronine (3,3'-T2) and its internal standard 3,3'-T2-13C6. The stock solutions were diluted with methanol to obtain spiking solutions. The solutions were stored at -20°C and were stable for at least 6 months. Calibration curve standards in the range of 2.5-1000 pg/mL for 3-Iodothyronamine (3-T1AM), 2.5-1000 pg/mL for 3,3'-T2, 0.025-10 ng/mL for T3 and 0.5-200 ng/mL for T4 were prepared by adding spiking solutions to 3% human γ-globulin (< 2% of final volume). In-house quality control solutions were prepared at three concentrations to evaluate within-day and between-day precision as well as the accuracy of the method. A diluted solution containing 0.2 ng/mL 3-T1AM- d4, 0.2 ng/mL 3,3'-T2-13C6, 1.5 ng/mL T3-13C6, and 10 ng/mL T4-d5 in acetonitrile was used as working internal standard solution. This LC/MS/MS-ESI method, demonstrates excellent specificity, accuracy (93-103% for all analytes) and precision (CVs of 4.1-6.2% for all analytes) and is simple and fast (<9 min).

Sources of Research Support: In part by the National Institutes of Child Health and Development NICHD Obstetric-Fetal Pharmacology Research Unit Network (OPRU) Grant 5U10HD0478925 (OPS), the Office of Research on Women's Health (OPS) and by the GCRC at Georgetown University Medical Center.

Nothing to Disclose: OPS, JG, SJS

P3-571

A Practical Approach for Development of Age-, Gender- and Race-Specific TSH Reference Limits.

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Four recent reports show that age- and race-specific reference limits for TSH need be employed to avoid significant misclassification of patients with thyroid dysfunction (1-4). Developing such limits is an imposing task that requires TSH measurements in large numbers of people in these sub-populations who are free of thyroid disease. This is likely to be beyond the capability of most laboratories.

We determined TSH median, 2.5th and 97.5th centiles as a function of age, sex, ethnicity and antithyroid antibodies (AB) in Whites, Blacks and Mexican Americans (MA) using data from NHANES III. We compared TSH limits of the defined thyroid disease-free population (DF) (n=15,277) to a reference population (REF) (n=13,344) formed by exclusion of subjects with antithyroid antibodies as well as 144 subjects with TSH > 10 mIU/L or less than 0.1 mIU/L, who very likely had thyroid dysfunction. We then quantitatively determined the effect of age, race, gender, body weight and urinary iodine concentration on TSH reference limits by building quantile regression models in the NHANES III AB- population that estimated the influence of each predictor on the chosen quantile.

The presence of AB did not significantly affect the 2.5 centile and median TSH concentration in any group or the 97.5 centile in Blacks. However, the 97.5 centile of the DF Whites and MA was, on average, 1.0 mIU/L greater than that of the REF group, indicating a small effect of AB+ subjects. After exclusion of AB+ patients, we built multivariate quantile regression models. Age significantly increased the 2.5th, 50th, and 97.5th centile of TSH. Blacks had significantly lower 2.5th, 50th, and 97.5th centile than Whites, while all TSH centiles in MA were comparable to Whites. Women had significantly lower 2.5th and 50th centiles than males, but the 97.5th centile was not affected by gender. Urinary iodine and body weight did not significantly affect any quantile of TSH. From these models, we developed equations to predict the 2.5th and 97.5th centiles of TSH using weighted effects of age, race and gender. When programmed into a laboratory computer with known patient demographics, it provides TSH limits for individual patients.

Our study provides a method to determine TSH limits in individual patients of different ages, gender and ethnicities whose AB status is uncertain. It will enable clinicians to determine whether a patient's TSH is within the reference limits for their specific subpopulation.

(1) Surks MI, Hollowell JG. *J Clin Endocrinol Metab.* 2007;92(12):4575-82.

(2) Boucai L, Surks MI. *Clin Endocrinol (Oxf).* 2009;70(5):788-93.

(3) Atzmon G, Barzilai N, Hollowell JG, Surks MI, Gabriely I. *J Clin Endocrinol Metab.* 2009;94(4):1251-4.

(4) Atzmon G, Barzilai N, Surks MI, Gabriely I. *J Clin Endocrinol Metab.* 2009;94(12):4768-75.

Nothing to Disclose: LB, JGH, MIS

P3-572

Relationship between Thyrotropin and Body Mass Index in Euthyroid Subjects.

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Background: Several studies have reported that thyrotropin (TSH) levels are slightly increased in obese subjects in comparison to normal weight humans. This may be due to a higher prevalence of hypothyroidism in obesity. On the contrary, other studies have provided no evidence for an association between thyroid status and body mass index (BMI).

Aim: Our aim has been to evaluate the relationship between TSH and BMI in a group of subjects with obesity, overweight and normal weight with serum TSH levels within the reference range, excluding subjects with hypothyroidism.

Subjects and methods: We evaluated a cohort of 778 euthyroid subjects (488 women, mean age 58.5±15.9 yr) with TSH values between 0.4 and 5.0 mU/l (median [IQR], 1.41[1.03-2.12] mU/l). There were 321 subjects with obesity (BMI≥30 kg/m²), 319 with overweight (25 to 29.9 kg/m²) and 138 with normal weight (18.5 to 24.9 kg/m²). There were no differences in sex distribution among groups. Subjects with overweight were significantly older (60.5±15.9 yr) than those with obesity (56.2±15.7 yr).

Results: Serum TSH levels significantly increased with weight category in studied subjects (normal weight, median [interquartile range], 1.24[0.94-1.72]; overweight, 1.31 [1.00-1.93]; obesity, 1.66 [1.15-2.44] mU/l; P<0.001). Free T4 was not statistically different in subjects within the three weight categories. A significant correlation was found between TSH and BMI (r=0.217, P<0.001). Subjects with serum TSH levels in the upper tertile (1.84 to 5.0 mU/l) exhibited a BMI value (30.8[26.9-34.9] kg/m²) significantly higher (P<0.001) than that found in subjects with TSH levels in the middle (1.13 to 1.83 mU/l, 28.8[25.8-32.3] kg/m²) or in the lower tertile (0.4 to 1.12 mU/l, 27.4[25.4-30.6] kg/m²). There were no differences in BMI in patients classified according to tertiles of free T4. However, when studying a subgroup of patients with negative thyroid autoimmunity (n=375), we could not observe any significant difference in TSH levels between obese (n=106, 1.24[0.90-1.81] mU/l) and non-obese subjects (n=269, 1.23 [0.90-1.80] mU/l). No correlation was found between TSH and BMI in this subgroup of subjects with negative thyroid autoimmunity (r=-0.035, NS).

Conclusion: These results suggest that the relationship between TSH and BMI observed in euthyroid subjects is lost when subjects with negative autoimmunity are selected.

Nothing to Disclose: JJJ, PI

P3-573

The Relationship between Thyroid Function and Fatigue in the General Population: The Nijmegen Biomedical Study.

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Fatigue is a frequently reported symptom of both hyper- and hypothyroidism. Although thyroid dysfunction is relatively common, only few studies have investigated the relationship between thyroid function and fatigue in the population. The aim of our study was to investigate whether thyroid function is associated with fatigue in the general population. The subjects of this study are participants of the Nijmegen Biomedical Study (NBS), a large population-based survey performed in Nijmegen. Age- and sex-stratified randomly selected adults received a questionnaire on lifestyle, medical history and symptoms, including questions about the presence and severity of fatigue and the Shortened Fatigue Questionnaire. 9371 (41.7%) recipients responded and for 6434 individuals TSH and FT4 levels were available. After excluding subjects with reported thyroid disease and/or thyroid surgery and/or the use of thyroid hormone and/or thyrostatic drugs and/or the use of medication interfering with thyroid function, data of 5778 participants were analysed.

Of the 5778 subjects, 1988 reported to be tired (34.4%). Subjects with abnormal thyroid function reported the symptom fatigue more frequently than euthyroid subjects (39.6% versus 34.0%, RR 1.2, CI 1.0-1.3). After adjustment for gender and age, the RR was 1.1 (CI 1.00-1.2). The attributable risk was 14.6%, the population attributable risk was 1%.

| | N | n Fatigue (%) | RR (95%CI) |
|----------------------------|------|---------------|---------------|
| Overt thyrotoxicosis | 9 | 5 (55.6%) | 1.6 (0.9-2.9) |
| Subclinical thyrotoxicosis | 192 | 71 (37.0%) | 1.1 (0.9-1.3) |
| Euthyroid | 5333 | 1812 (34.0%) | 1 (ref) |
| Subclinical hypothyroidism | 208 | 89 (42.8%) | 1.3 (1.1-1.5) |
| Overt hypothyroidism | 21 | 6 (28.6%) | 0.8 (0.4-1.7) |

The table shows the relationship between fatigue and thyroid function. Even within normal range, higher FT4 levels and lower TSH levels were related with a higher fatigue score, estimated with the Shortened Fatigue Questionnaire (data not shown)

In conclusion, in the general population thyroid dysfunction is associated with a higher prevalence of fatigue, even within normal range of TSH and FT4. Thyroid dysfunction however explains only one percent of cases with fatigue in the population.

Nothing to Disclose: AvdV, AR, FS, AH, MdH

P3-574

Thyroid Function and CTLA4 Polimorphisms in Chronic Infected Hepatitis C Patients Treated or Not with Interferon alpha and Ribavirine.

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Introduction: Association between autoimmune thyroid diseases (AITD) and hepatitis C(HCV) infection is controversial, but thyroid disturbance is well known during IFN treatment.

Objective: To determine frequency and characterize thyroid diseases in HCV patients, prior and during interferon treatment (IFN), beside correlation among genotypes of CTLA4 polymorphisms.

Materials and methods: 112 chronic HCV infected patients were prospectively evaluated and 183 normal subjects. 23% of patients received IFN treatment. Function, autoimmunity and ultrasound(US) analysis were performed. CTLA4 polymorphisms C/T-318, A/G49, CT60 and AT(n)3'UTR were evaluated. **Results:** Total T3 and T4 were above normal values in HCV group(T3T IC95%112-246 vs 40-180ng/dL; T4T 7.8-15.2 vs 4.5-12mg/dL). Free T4(FT4) levels were lower than normal controls($p < 0.001$) despite no differences in TSH. Thyroxine binding globulin levels were higher than normal values(IC95%17-47 vs 14-31 mg/L) and correlated to T3T and T4T($p < 0.001$) in HCV group. AITD frequency in HCV was similar to control subjects(10.7 vs 13.5%, $p = 0.67$). Thyroid US revealed high frequency of increased vascularization in HCV(49%). AITD were associated with parenchima heterogeneity($p = 0.029$) and hipoechogenicity($p = 0.008$). IFN induced autoimmune(19%) and non-autoimmune(16%) thyroid disturbances, corresponding to 3 cases of antithyroidal antibodies surge, 2 autoimmune hypothyroidism, 2 non-autoimmune subclinical hypothyroidism and 2 destructive thyroiditis. Female sex represented risk factor to IFN induced AITD and lower T3T and T4T levels risk factors to non-AITD. During treatment, T3T reduced since 3rd month($p = 0.038$) concomitant to ALT decrease($p = 0.055$) in patients without thyroid disturbance. T4T had significant reduction in 3($p = 0.039$) and 12 months($p = 0.008$). There was no significant difference in FT4 or TSH levels.

Repetitions AT(8) in 3'UTR region of CTLA4 were more frequent in HCV($p = 0.027$). Viral genotypes 1 and 3 were related to GG/AG genotypes of A/G49 (OR0.31, $p = 0.008$; OR3.28, $p = 0.01$) and CC genotype of C/T-318 polymorphism (OR0.18, $p = 0.026$; OR9.66, $p = 0.01$). **Conclusions:** AITD was not more frequent in our HCV cohort. T3T and T4T were higher in HCV group due to TBG excess. Apart from autoimmune and non-autoimmune thyroiditis, IFN causes T3T and T4T decrease without FT4 and TSH change even in those without thyroid disturbance. CTLA4 polymorphisms were related to viral genotype, but not to thyroid disturbance in treated or not HCV patients.

Sources of Research Support: Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP)06-06080-2.

Nothing to Disclose: DLSD, EUL, MCM-C, MCC, HZ, RKB, MK, SM

P3-575

Antiruthenium Antibody Interference.

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Introduction: There are many causes of interference in immunoassays causing erratic patient results. A method specific interference due to antiruthenium antibodies in the Roche fT4 and fT3 assay has been described previously. Recently, antiruthenium antibody interference in the Roche TSH assay was found in our laboratory as well. This raised the question whether other assays on this platform would also give incorrect results in patients with antiruthenium antibodies.

Methods: In the past years, in 7 patients interference due to antiruthenium antibodies was supposed because of discrepancies between TSH, fT4 and fT3 concentrations. Samples of these seven patients were analysed in Roche Diagnostics Laboratory. It was demonstrated that the found discrepancies were indeed explained by interfering antiruthenium antibodies. Subsequently these patients were asked to donate 37 ml blood once for further evaluation. The plasma and serum was used to assay 22 analytes (AFP, CA 15.3, CA 19.9, CA 125, CEA, CKMB, cortisol, estradiol, fT4, fT3, ferritin, folate, FSH, HCG, LH, fPSA, tPSA, prolactin, progesterone, troponine-T, TSH and vitamin B12) on the Modular E and on a ruthenium-independent platform. The results were compared with each other, taking into account the known differences between the distinct methods.

Results: Four patients participated in the study. As expected, significant interference was found in the TSH, fT4 and fT3 assay. The other assays however, both competitive as immunometric assays, did not show any interference.

Conclusions: Although antiruthenium antibodies can interfere theoretically in all assays on the Elecsys and / or Modular E platform, interference was only found in the TSH, fT4 and fT3 assays.

Nothing to Disclose: MMB, JPMCG, EE

P3-576

The Effect of Hemodialysis and Anticoagulant Used for Blood Sample Collection, on Thyroid Function Parameters in Patients with Chronic Renal Failure.

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Background: Chronic renal failure and hemodialysis (HD) are associated with alterations in the concentration of circulating thyroid hormones (TH). These alterations are in turn associated with systemic acidosis, concentrations of organic acids, time on dialysis, and some markers of endothelial damage and inflammation. Heparin treatment in vivo can lead to an unusual in vitro artefact that gives spuriously high estimates of circulating free T4 (FT4).

Aim: The aim of this study was to compare the parameters of thyroid function in patients undergoing HD before and after their HD session and using two different anticoagulants (EDTA and heparin) for blood sample collection.

Material and methods: 28 patients with chronic renal failure undergoing HD have been selected for the study (15 females and 13 males). Before HD every patient had their TSH, FT4, FT3, T4, the total binding capacity of serum for T4 (T-uptake), anti-peroxidase antibodies (TPO), prealbumines determined and free thyroxine index (FTI) was calculated. Thyroid function parameters have been determined from blood samples drawn using EDTA and heparin as anticoagulant. After HD all of the above parameters have been determined from blood samples drawn using heparin as anticoagulant only.

Results: In 23 patients euthyroidism was confirmed, 1 was diagnosed with hypothyroidism and 5 with hyperthyroidism. In 1 of the patients the anti-TPO antibodies were present. Before HD all of the tested parameters, except for the value of prealbumin, proved to be higher when determined from the EDTA blood samples comparing to the heparin samples. We have also found that the serum FT4, T4, T-Uptake and FTI were higher in those samples drawn after HD. All of the results were statistically significant at p-values of 0.02 or less.

Conclusions: The aforementioned results confirm the influence of heparin in vitro and in vivo on the determination of the parameters of thyroid function. The metabolic disturbances typical for chronic renal failure may enhance the in vivo effect of heparin on determination of thyroid function parameters and hence make the assessment of thyrometabolic status of these patients more difficult. The thyroid function parameters in patients with chronic renal failure should be estimated before HD using EDTA as an anticoagulant.

Nothing to Disclose: AL, BB, WM, ZE-T, KS, PW

P3-577

Thyroid Function in Women with Oligo- or Amenorrhea.

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BACKGROUND: Women presenting with oligo- or amenorrhea are classified based on diagnostic criteria recommended by the World Health Organization (WHO) in WHO1 (hypogonadotropic), WHO2 (normogonadotropic), WHO3 (hypergonadotropic) categories. Recent studies have shown disturbances of serum TSH levels in women with WHO class 2 with polycystic ovary syndrome. In this study we describe the serum TSH levels in different WHO classes.

OBJECTIVE: The aim of this study was to describe the prevalence of thyroid dysfunction reflected by serum TSH levels below (<0.4 mU/L) or above (>4.3 mU/L) the normal range in women with oligo- or amenorrhea.

MATERIALS AND METHODS: Serum TSH levels were obtained in 2734 women who visited our out-patient clinic with oligo- or amenorrhea. Patients were classified into WHO1, 2 or 3 categories. TSH levels >4.3 mU/L or <0.4 mU/L were compared between the three categories. A second comparison was made between WHO2 patients with and without PCOS.

RESULTS: Serum TSH levels >4.3 mU/L were observed in 5.6% (4/71) of WHO1 subjects, in 3.2 % (72/2218) of WHO2 subjects, and in 4.9% (22/445) of WHO3 subjects. Serum TSH levels were <0.4 mU/L in 12.7% (9/71) of WHO1 subjects, in 2.3% (52/2218) of WHO2 subjects, and in 3,6% (16/445) of WHO3 subjects. No significant difference was seen between the WHO classes in the prevalence of serum TSH levels >4.3 mU/L ($p < 0.14$). A significant difference was seen between the WHO classes in the prevalence of serum TSH <0.4 mU/L ($p < 0.00$). In the WHO2 group, 3.0% (56/1844) of the PCOS women and 4.5% (11/244) of the non-PCOS women had a TSH higher than 4.3 mU/L.

CONCLUSION: In this study we describe the serum TSH levels in women with oligo- or amenorrhea. The prevalence in the WHO classes appears to be consistent with the prevalence in general population. A significant difference was seen between the WHO classes in the prevalence of serum TSH <0.4 mU/L ($p < 0.00$). Further research is needed, including measurement of FT4 levels and TPO antibodies, to know the implications of the TSH levels in these classes.

Nothing to Disclose: MLB, YVL, TJV, JSEL

P3-578

Multinodular Goiter with Obstructive Symptoms. Should We Look for Cancer?.

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Purpose:

The incidence of thyroid cancer in multinodular goiter is comparable to that which exists in solitary thyroid nodules(1). However, the risk of malignancy in thyroid nodules occurring within a multinodular goiter (MNG) presenting with obstruction symptoms has not been studied.

Methods and Results:

Clinical and pathological data of 1200 consecutive patients who underwent thyroidectomy at a single institution from 2002 to 2009 was collected prospectively. From this database, a retrospective review of 41 patients with MNG presenting with obstruction symptoms was performed. From the 41 patients, 95% (n=39) were female, mean age was 49 years, 27% (n=11) were Caucasian, 34% (n=14) Hispanic and 37% (n=15%) African American. None of the patient reported a history of radiation exposure nor family history of thyroid cancer. The average diameter of the dominant nodule measured by ultrasound was 2.73 cm. A total of 24 patients underwent ultrasound guided fine-needle aspiration; adequate material was obtained in 92% of cases; 9% (n=2) of them were follicular lesion of undetermined significance, 5% (n=1) suspicious for malignancy and 86 % (n=19) were benign. Surgical intervention was performed in all 41 cases. Thyroid cancer was found in seven patients including 3 cases of papillary cancer, 3 cases of follicular variant of papillary cancer, and 1 case of follicular cancer.

Conclusions:

The overall malignancy rate in thyroid nodules among the patients with MNG presenting with obstruction symptoms was 17%. This incidence of malignancy is higher than ones reported for solitary nodules and MNG with no obstruction symptoms (5%). Due to this high incidence, surgical intervention may be considered an option in all patients with MNG presenting with obstruction symptoms to not only pursue symptom relief, but also to evaluate for potential malignancy.

Tollin SR, Mery GM, Jelveh N, Fallon EF, Mikhail M, Blumenfeld W, Perlmutter S . The use of fine-needle aspiration biopsy under ultrasound guidance to assess the risk of malignancy in patients with a multinodular goiter. *Thyroid* 2000 Mar;10(3):235-41.

Nothing to Disclose: PB, AS, CS

P3-579

Short Term Follow-Up of Thyroid Function and Auxological Changes in Patients Who Received Hematopoietic Stem Cell Transplantation during Childhood.

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Background: Thyroid dysfunction has been known as a common long-term complication following hematopoietic stem cell transplantation (HSCT). Limited data are available regarding thyroid dysfunction as a short-term complication during childhood. We evaluated changes in thyroid function and auxological parameters in patients who underwent HSCT during childhood.

Methods: We enrolled 44 patients (28 boys and 16 girls) who checked thyroid function and auxological parameters among patients underwent HSCT, at the Catholic HSCT center, The Catholic University of Korea between December 2005 and September 2006. The mean age was 9.5 ± 4.4 (range 1.2-18.8) years. Thyroid function was assessed by serial measurement of basal TSH, free T4 and T3 levels before HSCT and at 3 month, 6 month, 9 month, 12 months after HSCT.

RESULTS: Seventeen patients (38.6%) had thyroid dysfunction over 12 months after HSCT. Thyroid dysfunction included subclinical hypothyroidism (n=13, 30.0%), euthyroid sick syndrome (n=4, 9.1%), overt hypothyroidism (n=2, 4.5%) and overt hyperthyroidism (n=1, 2.3%). Serum T3 levels significantly decreased at 3 months after HSCT (1.52 ± 0.24 vs. 1.20 ± 0.27 ng/mL, $P=0.000$) and almost recovered at 12 months. Meanwhile, serum free T4 and TSH levels did not show significant change during follow-up.

Weight-SDS decreased at 3 months after HSCT (0.70 ± 1.40 vs. 0.32 ± 1.11 , $P=0.02$) and normalized within 6 months.

Height-SDS decreased at 12 months after HSCT (0.45 ± 1.43 vs. -0.08 ± 1.13 , $P=0.01$) and BMI-SDS increased at 9 months after HSCT (0.43 ± 1.22 vs. 0.85 ± 0.91 , $P=0.01$). There were no auxological differences between the patients who had thyroid dysfunction and those with normal thyroid function.

Conclusions: We observed thyroid dysfunction in 38.6% of patients and auxological changes over 12 months following HSCT. These results suggest that thyroid function and auxological parameters should be assessed regularly even before 12 months after HSCT in childhood.

Nothing to Disclose: WKC, SHP, SHH, MHJ, BKS

P3-580

Thyroid Function, Lipid Profile, Insulin Resistance, Homocysteine and High Sensitivity C-Reactive Protein in Patients with Autoimmune Thyroid Disease.

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Background: Thyroid dysfunction is associated with detrimental cardiovascular effects. **Objective:** To evaluate the association between thyroid function, lipid concentrations, C-reactive protein (CRP), homocysteine and insulin resistance in patients with treated autoimmune thyroid disease. **Patients and methods:** We assessed thyroid function tests, anti-thyroglobulin (anti-Tg), anti-TPO, TRAb, BMI, total cholesterol (TC), HDL-cholesterol, LDL-cholesterol, triglycerides (TG), apolipoprotein B (ApoB), ApoA1, lipoprotein(a) [Lp(a)], homocysteine, CRP, folic acid, B12 vitamin, and insulin resistance markers comprising the HOMA-IR, HOMA-B, the Quantitative Insulin Sensitivity Check Index (QUICKI), HISI (Hepatic Insulin Sensitivity Index), WBISI (Whole-Body Insulin Sensitivity Index), IGI (Insulinogenic Index) in 73 patients with Graves' disease (GD) and in 73 patients with Hashimoto's thyroiditis (HT). The patients with GD were treated with propylthiouracil until they normalized the levels of TSH, FT3 and FT4. The patients with HT were treated with levothyroxine, in order to normalize the FT3, FT4 and TSH levels. A 75-g OGTT was performed in the morning, and blood samples were obtained every 30min for 120min for measurements of plasma glucose, insulin, and C-peptide. Statistical analysis was performed with Student's t-test and Person's correlation test. Results are expressed as mean±SD or percentage. A p<0.05 was considered significant. **Results:** We found that patients with HT had significantly higher levels of C-peptide (3.20±1.44 vs 2.30±0.70ng/mL;p<0.001), CT (207.42±39.39 vs 189.53±37.15mg/dl;p<0.01), LDL (128.35±32.29 vs 114.26±27.83mg/dl;p<0.01) TG (133.69±98.23 vs 92.60±47.79mg/dl;p<0.01), ApoB (98.81±22.57 vs 85.39±23.07mg/dl;p=0.03), and Lp(a) (32.52±38.88 vs 17.11±19.92mg/dl;p=0.02). In patients with GD we found significant correlations between FT3 and glucose (R=0.52;p<0.001), TSH and TG (R=0.36;p<0.001), TSH and ApoB (R=0.39;p<0.01), anti-Tg and QUICKI (R=0.34;p=0.04). In patients with HT we found significant correlations between TSH and TG (R=0.26;p=0.03), anti-Tg and homocysteine (R=0.37;p<0.01), FT3 and folic acid (R=-0.30;p=0.03), FT4 and QUICKI (R=0.32;p=0.03), anti-Tg and WBISI (R=0.47;p=0.01), HISI (R=0.31;p=0.03) and HOMA-B (R=-0.32;p=0.03). **Conclusion:** In patients with treated GD and HT, the interrelationships between thyroid function, lipid profile, insulin resistance, homocysteine and CRP may determine an increased cardiovascular risk.

Nothing to Disclose: CN, RR, CG, MB, MA, MP, EC, IP, CP, JPR, DC, LD, JLM

P3-581

Diastolic Dysfunction in Women with Subclinical Hypothyroidism.

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Introduction: Diastolic dysfunction (DD) is very common and appears to cause a lot of morbidity from heart failure. Some studies report an increased frequency of DD in patients with Subclinical Hypothyroidism (SH). The aim of the present study was to evaluate the resting diastolic cardiac function by echocardiography (Isovolumic relaxation time, Transmitral inflow and E:A ratio) in SH.

Methods: A cross sectional study was performed in 60 adults women (group A and group B) 30 (group A) with SH and 30 (group B) euthyroid patients. Both groups had similar demographic characteristics as blood pressure, BMI, smoking, menopause. None of the patients were receiving SH group management with LT4. **Isovolumic relaxation time** (IVRT, IRT: The time from aortic valve closure to mitral valve opening, a normal IRT is about 70 ± 12 ms. With abnormal relaxation, the value is usually in excess of 110ms). **Transmitral inflow** (E/A; E wave deceleration. Deceleration of inflow of the E wave is measured by Deceleration Time DT). **E:A ratio** (With impaired relaxation, the E component will be reduced, resulting in a lower E:A ratio) were performed in all patients.

Results: 19 patients with SH showed mild dysfunction (IVRT 160 ± 12 vs 75 ± 8 ms) with A mitral inflow pattern of abnormal relaxation [E<A with a prolonged DT] in comparison with women with normal thyroid function ($p=0.043$).

Conclusion: The results suggest an increase in the frequency of mild diastolic dysfunction at rest in women patients with SH. Is necessary to evaluate the diastolic function under LT4 treatment in patients with SH.

Nothing to Disclose: HVU

P3-582

Study of the Frequency of Thyroid Autoantibodies in Thai Patients with Autoimmune Thyroid Diseases.

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Background: The prevalence of thyroid autoantibodies in autoimmune thyroid disease (AITD) varies, depending on age, gender, ethnicity, amount of iodine intake, and the method of the test¹. Since inadequate iodine intake has been reported in central Thais², this may affect the sensitivity of these antibodies to diagnose AITD. We therefore conducted a cross-sectional study to examine the frequencies of detectable thyroid autoantibody comparing between different categories of AITD in Thais.

Methods: Serum was collected from subjects with Graves' disease in hyperthyroid state and subjects with Hashimoto's thyroiditis. We excluded subjects with pregnancy, on treatment of immunosuppressive drugs, renal or liver dysfunction. Serum from healthy blood donors was obtained as controls. Serum free thyroxine (FT4), thyrotropin (TSH), anti-thyroperoxidase antibody (TPOAb), anti-tyroglobulin antibody (TgAb), and anti-TSH receptor antibody (TRAb) concentrations were measured in all subjects. The sensitivity, specificity of each antibody to diagnose AITD was calculated and receiver operating characteristics (ROC) curve analysis was performed.

Results: Four Hundred and forty three serum samples were tested (Graves' disease=127, Hashimoto's thyroiditis=102, control= 214 samples). The positive results were defined using reference cut-off levels from the kits. The incidence of positive TPOAb, TgAb, and TRAb were 9.34%, 13.55%, and 3.74%, respectively in healthy controls. Subjects with Graves' disease had positive TPOAb, TgAb, and TRAb of 72%, 51.18%, and 85.83%, respectively. TRAb provided the best test to diagnose Graves' disease with the sensitivity of 85.83% and the specificity of 96.26% (AUC=0.96±0.01, p<0.05). Positive TRAb test alone is more sensitive than either TPOAb or TgAb test (the sensitivity = 72.44%) in Graves' disease. Subjects with Hashimoto's thyroiditis had positive TPOAb, TgAb, and TRAb of 79.41%, 85.29%, and 14.70%, respectively. In contrast with previous report from the Western population³, TgAb provided the most sensitive for Hashimoto's thyroiditis with the sensitivity of 85.29% and specificity of 86.45% (AUC=0.91±0.02, p<0.05). The sensitivity increased to 93.14% when we combined with the TPOAb test.

Conclusions: TRAb is the most sensitive test for diagnosis of Graves' disease. With the highest frequency in Hashimoto's thyroiditis, the authors suggest using TgAb for diagnosis of Hashimoto's thyroiditis instead of using only TPOAb in Thais.

1. Saravanan P et al., Endocrinol Metab Clin North Am 2001;30:315
2. Rajatanavin R., Public Health Nutr 2007;10:1602
3. Nordyke RA et al., Arch Intern Med 1993;153:862

Sources of Research Support: Reagents and an analyzer for this study were provided by Roche Diagnostics.

Nothing to Disclose: CS, L-OC, SV, WJ

P3-583

Age and the TSH Response to Hypothyroxinemia and Recombinant Thyrotropin.

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Background

Thyrotropin (TSH) production is generally thought to decrease with increasing age. However, recent studies suggest that serum TSH distributions shift to higher concentrations with aging. We tested the hypothesis that serum TSH concentration is affected by age in both hypothyroidism and after recombinant human TSH administration.

Methods

We retrospectively studied three populations of 112 patients, each divided into four age subgroups (< 35 yrs, 35-49 yrs, 50-64 yrs, > 64 yrs). The populations were: (1) patients newly diagnosed with primary hypothyroidism; (2) thyroid cancer patients undergoing levothyroxine withdrawal; and (3) thyroid cancer patients receiving rhTSH. The latter two groups were being prepared for diagnostic or therapeutic radioiodine.

Results

In spontaneously hypothyroid patients, the mean TSH concentration decreased non-significantly in each ascending age group (69 mIU/L, 49 mIU/L, 43 mIU/L, 29 mIU/L) with free thyroxine (FT4) concentrations that remained similar in each group. In patients with iatrogenic hypothyroidism the mean TSH concentration decreased significantly in each ascending age group (156 mIU/L, 115 mIU/L, 74 mIU/L, 46 mIU/L, $p < 0.001$), despite comparable FT4 concentrations. In patients receiving rhTSH the day of the first injection was designated day 1. The TSH concentration on day 3 and day 5 both increased significantly with ascending age groups. Day 3 values were 96 mIU/L, 107 mIU/L, 142 mIU/L, 196 mIU/L ($p < 0.0001$). Day 5 values were 9 mIU/L, 11 mIU/L, 20 mIU/L, and 29 mIU/L ($p < 0.0001$). Multivariate analyses showed that the relationship between the log-transformed TSH and FT4 was significantly and inversely affected by age in spontaneous hypothyroidism ($p = 0.0005$) and iatrogenic hypothyroidism ($p < 0.0001$). Conversely there was a significant positive correlation between day 3 and day 5 TSH levels and age ($p < 0.0001$) in patients receiving rhTSH.

Conclusion

Age modifies the pituitary response to comparably reduced FT4 concentrations, resulting in less pronounced serum TSH elevation in older hypothyroid patients. Following administration of rhTSH, there is greater elevation of serum TSH levels in older patients. These observations, which suggest altered regulation, distribution, metabolism, or clearance of TSH, are relevant to the management of patients with both hypothyroidism and thyroid cancer. For example, age affects the ability to achieve particular TSH values after both levothyroxine withdrawal and rhTSH protocols.

Nothing to Disclose: RO, SM, HN-M, KB, JJ

P3-584

Soy Phytoestrogens Precipitate Overt Hypothyroidism in Patients with Subclinical Hypothyroidism: A Randomized Double Blind Crossover Study.

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Context: Subclinical hypothyroidism has been associated with increased cardiovascular disease that may be mediated through increased insulin resistance. Soy phytoestrogens have been reported to improve insulin resistance and lipids, effects that may be offset by causing deteriorating thyroid function.

Objective: To determine the effect of soy phytoestrogens on thyroid and cardiovascular risk indices in patients with subclinical hypothyroidism.

Design, Setting and Patients: A randomized, double-blind cross over study involving 60 patients with subclinical hypothyroidism was undertaken to determine the effects of soy phytoestrogens on thyroid and cardiovascular risk factors.

Intervention: Patients were randomly assigned to either a low dose phytoestrogen (30g of soy protein with 2mg phytoestrogens) or a high dose phytoestrogen (30g of soy protein with 16mg phytoestrogens) treatment regime for 8 weeks and then crossed over after an 8 week wash out period.

Main Outcome Measures: The primary outcome of the study was progression to overt hypothyroidism with blood pressure and HOMA-IR being secondary outcome measures.

Results: Six patients in the study progressed into overt hypothyroidism during the 6 month period with a standardised rate ratio of 3.6(95%CI-1.9, 6.2), though no deterioration in fT3, fT4 and TSH were seen in the remainder of the study population. Systolic blood pressure [140.7 ± 2.4 vs. 133.6 ± 2.8 mmHg $p < 0.01$] and diastolic blood pressure [76.7 ± 1.8 vs. 72.1 ± 1.4 mmHg $p < 0.02$] decreased after the 16 mg phytoestrogens, whilst systolic pressure alone [141.4 ± 2.0 vs. 136.8 ± 1.8 mmHg ($p < 0.03$)] decreased with 2 mg phytoestrogens. Insulin resistance (mean \pm SD) [HOMA-IR 3.5 ± 0.09 vs. 2.6 ± 0.08 $p < 0.02$] and hsCRP decreased [4.9 ± 0.04 vs. 3.9 ± 0.03 , $p < 0.01$] with 16mg phytoestrogens alone. The lipid profile remained unchanged.

Conclusion There is an increased risk of thyroid failure with dietary soy phytoestrogens in subclinical hypothyroidism. However, high (16mg) soy phytoestrogen levels, equivalent to that seen in a vegetarian diet, significantly reduce the insulin resistance, hsCRP and blood pressure in these patients.

Sources of Research Support: The phytoestrogen standards were produced as part of a Food Standards Agency (contract T05001) and were donated for use in this project by Dr Nigel P Botting, Department of Chemistry, University of St Andrews, UK. This study was supported by the Food Standards Agency, United Kingdom (contract T05029). Duality of Interest: Natalie Thatcher is currently employed by the Food Standards Agency, United Kingdom. All of the other authors do not have any duality of interest to report. Other than this none of the authors have anything to disclose. Views expressed are those of the authors not those of the FSA.

Disclosures: NJT: Employee, Food Standards Agency, United Kingdom.

Nothing to Disclose: TS, AMM, ASR, ESK, TC, SLA

P3-585

Incident Thyroid Dysfunction in the Elderly: The Cardiovascular Health Study.

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Background: Thyroid dysfunction is common in elderly individuals, but there are few data from cohort studies examining its incidence. We examined the incidence of thyroid function testing abnormalities over a four-year period in an older population who were euthyroid at baseline.

Methods: Serum TSH, free T4, and total T3 concentrations were measured in serum from 1992-93 (baseline) in 3,593 US community-dwelling people aged 65 or over who were enrolled in the Cardiovascular Health Study and not taking thyroid medications. Serum TSH levels were also measured in specimens from 1996-97 (Year 4), with free T4 if TSH was abnormal and T3 if TSH < 0.1 mU/L. We identified 3,057 participants who were euthyroid (TSH 0.45-4.5 mU/L) at baseline and analyzed their thyroid testing status at Year 4.

Results: Of those who were euthyroid at baseline (n=3057), 56% were female, 19% were non-white, and mean age was 74.6 years. Over the four-year follow-up, 12.9% died and an additional 19.6% did not have available blood for thyroid function testing and were not taking thyroid medications at Year 4. Of those remaining (n=2064), 0.05% developed overt hyperthyroidism, 0.8% subclinical hyperthyroidism, 0.1% overt hypothyroidism and 5.7% subclinical hypothyroidism, with 92.1% remaining euthyroid and 1.3% initiating thyroid medication. The median baseline TSH levels for the 17 subjects who developed subclinical hyperthyroidism and the 117 subjects who developed subclinical hypothyroidism by Year 4 were 0.73 and 3.53 mU/L, respectively. The median change in TSH for those who remained euthyroid (n=1900) was -0.01 mU/L from a median baseline TSH of 1.93 mU/L. For those with baseline TSH 0.45-2.50 mU/L (n=1342), the median TSH was stable from 1.58 to 1.62 mU/L; for TSH 2.51-4.50 mU/L (n=558), the median TSH decreased from 3.10 to 2.86 mU/L. Changing the euthyroid range to 0.45-7.5 mU/L (n=2192) did not alter the median four-year change ($\Delta=0$). Additional data will be presented incorporating Year 2 thyroid function testing and baseline antiTPO antibody status.

Conclusions: A large proportion of elderly people with normal thyroid testing remain euthyroid with no change in the distribution of TSH over four years, even if the upper limit is extended to 7.5 mU/L. Those who were euthyroid at baseline and subsequently developed subclinical hyperthyroidism or subclinical hypothyroidism tended to have lower or higher TSH levels at baseline, respectively, compared to those who remained euthyroid.

Sources of Research Support: NIA Grant R01-AG032317 awarded to ARC; NHLBI contracts N01-HC-85079 through N01-HC-85086, N01-HC-35129, and N01 HC-15103.

Nothing to Disclose: ADS, ARC, AMA, LPF

P3-586

Absorption of Levothyroxine When Co-Administered with Various Calcium Formulations.

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Calcium carbonate is a commonly used dietary supplement and has been shown to interfere with levothyroxine (T4) absorption. However, calcium citrate, which is also used for supplementation purposes, has not been studied previously. Calcium acetate, which is used to treat hyperphosphatemia in renal failure, has been reported to show little or no interference with T4 absorption in a retrospective pharmacoepidemiologic study (1). We performed single-dose pharmacokinetic studies in which we compared T4 absorption when each of these calcium preparations was co-administered with T4 versus the absorption seen when T4 is given alone.

Seven healthy adults completed this study. Each subject was tested on four separate occasions, three weeks or more apart, each after an overnight fast. Subjects were first given 1.0 mg T4 alone (control). At the next three visits, subjects received 1.0 mg T4 and simultaneously ingested either calcium carbonate, calcium acetate or calcium citrate in doses containing 500 mg elemental calcium. Blood samples were collected over the course of six hours for T4 and TSH measurements. The area under the T4 response curve above baseline (AUC) was calculated for each individual test using trapezoidal integration. Mean T4 AUC responses were first analyzed using one-way analysis of variance for repeated measures, followed by Dunnett's multiple comparisons test to compare the T4 responses of T4 plus each of the calcium preparations with T4 alone (control).

The average AUC above baseline for T4 alone was 1763 ± 80 (SEM) $\mu\text{g}\cdot\text{min}/\text{dL}$; for T4 plus calcium carbonate 1416 ± 165 $\mu\text{g}\cdot\text{min}/\text{dL}$; for T4 plus calcium citrate 1417 ± 170 $\mu\text{g}\cdot\text{min}/\text{dL}$; and for T4 plus calcium acetate 1316 ± 151 $\mu\text{g}\cdot\text{min}/\text{dL}$. The preliminary analysis of variance showed that there were significant differences in the mean T4 AUC between the 4 tests ($p = 0.014$). Co-administration of each of the three calcium preparations significantly ($p < 0.05$) reduced T4 absorption compared to T4 given alone.

Conclusion: Simultaneous ingestion of levothyroxine with calcium carbonate, calcium citrate or calcium acetate decreases the expected rise in serum T4 concentrations over the ensuing six hours. Contrary to a prior report, our data suggest that calcium acetate interferes with T4 absorption in a manner similar to that seen with calcium carbonate or calcium citrate. Hypothyroid patients should be cautioned to take their levothyroxine well-separated from all of these calcium formulations.

(1) Diskin CJ et al., International Urology and Nephrology 2007; 39: 599-602

Sources of Research Support: General Clinical Research Center, Stony Brook University Hospital, M01RR10710.

Nothing to Disclose: IZ, HEC

P3-587 Thyroid Dysfunction in Patient's Treated with Sunitinib - A Retrospective Analysis.

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Sunitinib, a multi-targeted receptor tyrosine kinase inhibitor, inhibits VEGF-mediated tumour angiogenesis. Following NICE approval, it is increasingly used in the United Kingdom for the treatment of metastatic renal cell carcinoma. Hypothyroidism in sunitinib-treated individuals was first described in 2005. The aetiology remains uncertain and possibly reflects a destructive thyroiditis. Incidence rates of hypothyroidism from case series vary between 36% and 85%. We report a retrospective analysis of thyroid-function in patients treated with Sunitinib for metastatic renal cell carcinoma at a UK tertiary hospital.

Twenty-six patients (18 men, 8 women; median age 62.5 years (range 32-80)) were started on Sunitinib between September 2007 and April 2009. All patients received Sunitinib for more than three cycles (25-50mg/day; 4-weeks-on and 2-weeks-off per cycle). Sixteen patients (61%) had baseline thyroid function tests (Mean TSH 2.44(SD+2.45) mIU/l, NR 0.27-4.20mIU/l; Mean FT4 16.23(SD+2.62) pmol/l, NR 12-22 pmol/l). Pre-treatment TSH was elevated in two patients (4.61 mIU/l and 10.7 mIU/l) increasing to 47.4 mIU/l and 42.1 mIU/l respectively, after one cycle. At the first follow-up thyroid assessment, which was done between the 1st and 8th cycle (median 3 cycles), the mean TSH was 5.0(SD+8.09) mIU/l (Δ TSH 2.56, 95%CI 1.76-6.78). Free-T4 was normal with a mean value 15.86(SD+1.80) pmol/l. One patient had a thyrotoxic phase before progressing to hypothyroidism. Median time-point for the 2nd follow-up was after 4 cycles (range 3-12) and the third follow-up occurred after 6 cycles (range 3-13). Thirteen patients (50%) had a high TSH (Median 11.4 mIU/l(Range 5.48-80) during follow-up and 8 (30%) were started on levothyroxine due to a progressive increase in TSH.

In conclusion, hypothyroidism is a common complication of Sunitinib therapy and may adversely affect tolerance. Local protocols for thyroid surveillance and thyroxine replacement will enhance recognition and enable early treatment of this unusual clinical entity.

Nothing to Disclose: KM, RG, DJ, SR, SC, BM, PC, JP

P3-588

Prevalence of Hypothyroidism in Children up to 5 Years of Age in Moderate to Severe Iodine Deficient Area of Northern India.

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Recently we reported prevalence of moderate to severe iodine deficiency disorder (IDD) indicating the failure of universal salt iodization program (1). Hypothyroidism in children can cause prolonged jaundice, feeding problems, hypotonia, delayed bone maturation. Importantly, permanent neurologic damage results if treatment is delayed. Aim of our study was to assess the prevalence of hypothyroidism in children up to the age of 5 years in moderate to severe iodine deficient area of northern India.

The work was carried out in 6 blocks of Gonda district of Uttar Pradesh in northern India which are moderate to severely affected by IDD. All children up to age of 5 years underwent evaluation of goiter size by palpation and graded as per the WHO classification. Blood as well as urine samples along with house hold salt samples were collected after informed consent of the parents. Serum TSH and T₄ were measured by IRMA and RIA methods respectively. Urinary iodine concentration was measured using a micro titer plate based kit. Salt iodine was estimated by iodometric titration. 512 children with mean (\pm SD) age of 3.1 \pm 1.1 yr (range, 0.9–5 yr) showed a goiter rate of 32.4%. The Mean \pm SD T₄ was 103.0 \pm 22.04 mmol/L and median TSH was 3.1 \pm 3.5 μ U/ml. Sub-clinical hypothyroidism and overt hypothyroidism is confirmed in approximately 5.5% and 2.5% of the children respectively. And 38.3% children having normal urinary iodine concentration (UIC) with median for study population being 84 μ g/L. 24.1% of children had UIC <50 μ g/L. Only 16% of study children receive adequate iodine (~ 15 ppm) through salt and median salt iodine is 6.1 ppm. The

clustering of majority of samples with low salt iodine levels and urinary iodine excretion is a noticeable ($R^2=0.11$ $p=0.000$). These results can be largely attributed to the fact that more than 25% of subjects still receive negligible iodine from salt consumption and 50% receive less than adequate amount of iodine from salt source. Overall goiter prevalence rate in district has significant decreased over last 2 decades but above the internationally accepted norm of <10%. The median levels of outcome indicators like UIC indicate unsatisfactory implementation of universal salt iodization program. In view of high prevalence of hypothyroidism study indicates need to put more emphasis on pregnant women, lactating mothers and children up to 5 years and more important in Indian communities as the only source of iodine up to 1 year is mother milk.

Yadav S et al., Public Health Nutr. 2009 Jun 11:1-6.

Sources of Research Support: Intramural grant of Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow to Dr. Subhash Yadav, (No PGI/DIR/RC/412/2007).

Nothing to Disclose: SY, NK, MG

P3-589

Effect of Therapy with Thalidomide Derivatives on Thyroid Function in Multiple Myeloma.

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Thalidomide and its derivative lenalidomide are novel agents approved for the treatment of multiple myeloma. Case reports of hypothyroidism associated with thalidomide and lenalidomide treatment have been reported. There has also been a case report of associated transient thyroiditis. **Objective:** The goal of this study was to evaluate thyroid function and the incidence of hypothyroidism and thyroiditis before and after therapy with thalidomide and lenalidomide in patients with multiple myeloma. **Methods:** We reviewed medical records of 412 consecutive patients seen at the Mayo Clinic who received thalidomide (n=184) or lenalidomide (n=228) as first line therapy for multiple myeloma. Pre and post-treatment thyroid status, physical exam, and thyroid function tests were recorded. Any changes of thyroid function status or abnormal thyroid function were also recorded. Change in TSH levels was assessed using the Wilcoxon Signed Rank test. **Results:** 142 patients (62%) treated with lenalidomide and 52 patients (28%) treated with thalidomide, had baseline TSH values. 107 patients (47%) treated with lenalidomide and 73 (40%) treated with thalidomide had TSH values during or following treatment. There were 55 patients (24%) treated with lenalidomide and 8 patients treated with thalidomide on whom both a baseline and a follow up TSH value post-therapy were available for analysis. Overall, there was no statistically significant change in the TSH values from baseline to the follow-up measurement (P= 0.24). The incidence of hypothyroidism or thyroiditis was rare. Two patients (0.9%) developed silent thyroiditis and one other patient (0.4%) developed hypothyroidism during the course of treatment with lenalidomide. Forty-five patients (11%) had preexisting hypothyroidism on replacement therapy, and three of these patients (7%) required minor (25 mcg) dose adjustments (one up and two down). **Conclusion:** Thyroid dysfunction (hypothyroidism or thyroiditis) occurs in approximately 0.7% of patients following therapy with lenalidomide or thalidomide. Based on this study we conclude that this occurrence is a relatively rare event since TSH values do not change significantly during the course of therapy with these drugs in patients receiving these agents, and only 3 patients (7%) with existing hypothyroidism required adjustments in thyroxine dosage.

Nothing to Disclose: AQH, SVR, VF

P3-590

Thyroid-Related Adverse Effects of Interferon- β and Glatiramer Treatment of Multiple Sclerosis.

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Multiple sclerosis is a demyelinating disease of the central nervous system of an autoimmune nature. Previous studies have shown an association between thyroid dysfunction and MS (1,2) with the prevalence estimated to range between 2-11% (3). Patients with relapsing-remitting multiple sclerosis (MS) are usually treated with interferon- β or glatiramer acetate. Both immunomodulating agents have been found to be similar in efficacy. Thyroid dysfunction has been reported in 1-13% of MS patients who are treated with interferon β (2,3) whereas no such data exist for glatiramer. Our objective was to determine and compare the effects of interferon- β and glatiramer acetate on thyroid function in patients with multiple sclerosis.

Patients with relapsing-remitting MS, treated with either interferon- β or glatiramer acetate were evaluated using data from the Cleveland Clinic electronic medical record. TSH values were normal prior to the start of treatment and at least one thyroid function test was obtained within one year after treatment. Patients who were on neither medication were also evaluated. The frequency of patients who developed hyperthyroidism (TSH <0.4), hypothyroidism (TSH >5.5) or both was calculated.

Of the 221 patients (35 male and 186 female) with MS, 77 were treated with glatiramer acetate and 144 with interferon- β . The frequency of hyperthyroidism during treatment was 11% vs. 13% (P=0.68) in the interferon- β vs. glatiramer-treated patients, respectively, whereas the frequency of patients who developed hypothyroidism was 4% vs. 5% (P=0.22), respectively. Among the 540 patients who were not treated with either medication, 7% developed hyperthyroidism and 6% developed hypothyroidism. These values are not significantly different from the treatment groups (P=0.11 and P=0.71, respectively).

There have been conflicting data regarding the incidence of thyroid dysfunction in MS patients treated with interferon- β . Our study shows a similar incidence in the development of thyroid dysfunction in MS patients treated with either interferon- β or glatiramer acetate which is not different from patients who are on neither medication. We conclude that the high incidence of thyroid dysfunction in MS patients treated with interferon- β and glatiramer could be the result of inherent autoimmunity associated with MS.

(1) Rotondi M et al., J Endocrinol Invest. 1998; 21: 748-752

(2) Caraccio N et al., JCEM 2005; 90(7): 4133-4137

(3) Durelli L et al., JCEM 2001; 86(8): 3525-3532

Disclosures: RZ: Speaker, Daiichi Sankyo Inc., Merck & Co., Pfizer, Inc., Novo Nordisk, GlaxoSmithKline, Bristol-Myers Squibb.

Nothing to Disclose: VC, HB

P3-591

Discriminating Ability of Various Signs and Symptoms of Hypothyroidism To Predict Biochemical Thyroid Status in Hypothyroid Patients on Levothyroxine Replacement.

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Hypothyroidism is a relatively common disorder encountered in clinical practice. Patients with hypothyroidism on replacement therapy with levothyroxine (LT4) often need adjustment of their therapy based on their clinical features and biochemical thyroid profile including TSH and Free T4. The primary objective of this study was to assess the discriminating ability of various signs and symptoms associated with hypothyroidism to predict the biochemical thyroid status. This was a retrospective study reviewing charts of the patients with a diagnosis of hypothyroidism on replacement therapy with LT4 being followed in our Endocrinology clinics. Clinical and biochemical data was collected from their last visit for analysis. Presence and absence of each of the following symptoms was assessed in every patient from their electronic medical records: Fatigue, dry skin, weight gain, hoarseness, constipation, cold intolerance, generalized muscle weakness, hair loss, soft tissue swelling, memory problems, somnolence and subjective depression. Similarly the presence and absence of following signs was assessed: dryness of skin, goiter, swelling, and delayed tendon reflexes. To be included in the study the presence or absence of each of the above mentioned signs and symptoms had to be documented or else the charts were excluded from the final analysis. Over 200 charts were reviewed; only 78 were included in the final analysis, rest being excluded due to a diagnosis of thyroid cancer or incomplete documentation. Mean patient age was 55 years. Fatigue was reported by 70% of patients, weight gain by 50%, subjective depression by 30% and cold intolerance by 20%. The prevalence of rest of symptoms and signs was low among our patient cohort. A cut-off TSH value of 2.8 was used to categorize patients into adequate and inadequate biochemical thyroid control. There was no significant difference in the prevalence of any of the specified signs and symptoms of hypothyroidism among patients assigned to inadequate or adequate biochemical control. Discriminating ability of various signs and symptoms of hypothyroidism to predict biochemical thyroid status in hypothyroid patients on LT4 replacement was poor in our study cohort. Most of the hypothyroid symptoms and signs are non-specific and might not be useful in making therapeutic adjustments of LT4 therapy. Our study was limited by its small sample size and studies with larger numbers are needed to conclusively address this issue.

Nothing to Disclose: SG, TG, AY

P3-592

Myxedema Coma: A 10-Year Retrospective Study in a Tertiary Hospital.

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Objective

Myxedema coma is an extreme manifestation of hypothyroidism, uncommon but potentially a lethal condition. This retrospective study was undertaken to study the epidemiology, clinical features, biochemical abnormalities and the response to treatment in myxedema coma.

Methods

All patients presenting with severe hypothyroidism and myxedema coma between 2000-2009 were included. Myxedema coma was defined as biochemical hypothyroidism in conjunction with hypothermia, hypoventilation with CO₂ narcosis or decreased level of consciousness. Data was collected from the medical records in a retrospective manner.

Results

Nineteen patients presented with severe hypothyroidism during this period. About 79% (15/19) of cases were females and 63% (12/19) were elderly (>60yrs of age). Denovo hypothyroidism (52%) occurred equally as known hypothyroidism (48%). Denovo hypothyroidism was diagnosed on admission in 63% of cases. Significant clinical features included altered mental state (73.7%), cardiac failure (26.3%), hypothermia (32%), bradycardia (21%), ileus (21%) and non-specific giddiness (5.3%). About 30% of them had pneumonia as a precipitating event. Median free T₄ (RI: 21-30 pM) was 4 pM (range :0-6 pM) and median TSH (RI: 0.34-5.64mIU/L) was 56.6 mIU/L (range : 4.42-100). Other biochemistry abnormalities included renal impairment (63%), increased creatine kinase (53%) and hyponatremia (42%). Thirteen patients were treated with oral thyroxine (50-100 ug) and 6 patients were treated with low dose intravenous thyroxine (100-300ug) for the first 2-3 days followed by oral thyroxine. No correlation was seen between mortality and the treatment option of oral or intravenous thyroxine. The mortality rate was 30% amongst the 32% patients admitted to the intensive care unit. Median APACHE II score was 23 (range: 7-43).

Discussion

Although decompensation of a hypothyroid patient into coma is extremely rare in our local setting, the mortality rate can still be 30% amongst very ill patients. It is more common in elderly women and altered level of consciousness is the most common presenting feature. Although the outcome was not influenced by the choice of treatment in this study, there may have been a treatment bias in this retrospective analysis. Future prospective and randomized studies will help to clarify this better.

(1) Rodríguez I et al.,RV.J Endocrinol. 2004;180(2):347-50.

(2) Yamamoto et al.,Thyroid. 1999;9(12):1167-74. Review.

Nothing to Disclose: WHH, RD, JLK, SN

P3-593

Impact of Hypothyroidism on the Outcome of Severe Sepsis.

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Aim: To determine the mortality rate, intensive care unit and hospital length of stay and duration on the mechanical ventilation in patients with known primary hypothyroidism who were admitted with severe sepsis to the ICU and to compare their outcome to those without hypothyroidism.

Method: This retrospective study was performed on the patients who were admitted with severe sepsis to an ICU between 6/2006 and 12/2008. Patients were included if TSH was measured within 72 hours of admission and prior to the initiation of any steroid or pressors.

Result: In 25 patients with known hypothyroidism, 40% were men with a mean age of 66 years. In 82 patients without prior history of hypothyroid, 65% were men with a mean age of 62 years. Clinical characteristics and outcome of the patients were summarized in table1.

table1

| | Mean TSH level (mU/L) (95% CI: 4.9-15.3) | Mean APACHE II score (p 0.17) | Mean Glasgow Coma Scale (p 0.11) | ICU stay in days (p 0.27) | Hospital stay in days (p 0.18) | Duration on the ventilation in days | Non-survivors (p 0.182) |
|---|--|-------------------------------|----------------------------------|---------------------------|--------------------------------|-------------------------------------|-------------------------|
| Patients with known hypothyroid (N=25) | 13.7 ±4.5 | 17.8±1.2 | 11.8±0.7 | 11.1±2.4 | 20.4±4.0 | 12.4 | 36% |
| Patients without known hypothyroid (N=82) | 3.6 ±0.5 | 23.9±1.1 | 9.1±0.5 | 8.6±1.0 | 14.5±1.4 | 8.4 | 51% |

Discussion: The impact of hypothyroidism on the outcome of severe sepsis is unknown. In our study, the mortality rate was 36% in patients with known hypothyroid and 51.2% in those without. The difference was not statistically significant though there was a significant difference in mean TSH level between 2 groups. Those without hypothyroid were sicker with higher APACHE II scores and lower GCS scores. However, ICU and hospital stay and duration on the ventilation were much longer in those with hypothyroid, suggesting that they may take longer to recover from severe sepsis when compared to those without hypothyroidism.

Conclusion: The mortality rate in patients with known hypothyroidism who were admitted with severe sepsis to the ICU was 36%; and there was no statistically significant difference in mortality rate between those with hypothyroidism and those without. Patients with known hypothyroidism had longer ICU and hospital stay and longer duration on the mechanical ventilation when compared to those without. Further studies with larger number of cases are needed to confirm the findings.

Nothing to Disclose: SN, FG, ZR, SA, VB

P3-594

Congenital Hypothyroidism and Necrotizing Enterocolitis in a Term Infant: Coincidence or Cofactor?.

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Background: Few prior reports describe concurrent necrotizing enterocolitis (NEC) and congenital hypothyroidism (CH) in full term infants. These reports described infants with additional risk factors predisposing to the development of NEC. We describe a full term infant lacking obvious risk factors who had concurrent CH and NEC.

Clinical case: This female infant was the 3100 gm product of an uncomplicated 39 week gestation to a 34 yo primagravida mother. Delivery was by Caesarian section due to maternal hypotension and failure to progress. Apgars were 8 and 9. The infant passed meconium within 24 hours of age. Mother and infant were discharged together on day of life (DOL) 3. The infant was breastfed, noted to be jaundiced on DOL 6 (T. bilirubin: 11.3 mg/dl), and treated with blanket phototherapy for 2 days. On DOL8, PA state newborn screen was reported to be positive for congenital hypothyroidism. The infant was referred for evaluation. Physical examination revealed a well appearing female infant without a goiter. Diagnostic evaluation showed the following values: TSH 326.389 uIU/ml; T4 0.6 mcg/dl; free T4 0.07 ng/dl, and T3 0.62 ng/ml. Thyroid sonogram demonstrated a normally positioned and sized gland. A Tc-99m Pertechnate scan showed no uptake. Thyroid hormone replacement was initiated.

Over the next 12 hours, the infant had repeated bouts of forceful emesis, refused to nurse, became inconsolable, and had a rigid abdomen. Abdominal X-ray revealed pneumatosis intestinalis consistent with the diagnosis of NEC. She was admitted on DOL 10 for treatment of NEC. Intestinal biopsy was negative for Hirschsprung's. LT4 was continued intravenously. Her hospital course was complicated by feeding difficulties and several operations to relieve intestinal strictures.

Conclusion: NEC is relatively uncommon in term infants, accounting for only 10% of cases, and appears to have different presentation and clinical course. Predisposing factors include congenital heart disease, sepsis, Hirschsprung's, and IUGR (1). Apart from a brief period of maternal intrapartum hypotension, none of these other risk factors were present in this infant. It is unclear whether the CH contributed to the NEC in this infant or whether it was merely a coincidental event.

1. Lambert DK, et al. Necrotizing enterocolitis in term neonates: data from a multihospital health-care system. *J Perinatology* 2007;27:437-43

Nothing to Disclose: DF, SFW

P3-595

Intraepidermal Nerve Fiber Density Reduction as a Marker of Preclinical Asymptomatic Small-Fiber Sensory Neuropathy in Hypothyroid Patients.

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Aim: to evaluate, by using skin biopsy technique, the intraepidermal nerve fiber (IENF) density in a group of untreated patients with hypothyroidism, either overt or subclinical, who did not complain of neurologic symptoms.

Methods: we evaluated 18 neurologically asymptomatic patients newly diagnosed with hypothyroidism, overt and subclinical. Fifteen healthy age-matched controls were also studied. A nerve conduction study was performed. Skin biopsy was carried out from the skin of upper thigh and distal leg. Nerve fibers density was measured using an immunofluorescence technique. The density of innervation was calculated by counting only fibers crossing the basement membrane.

Results: electroneurographic parameters were similar in patients and controls. When compared with healthy controls, patients with overt or subclinical hypothyroidism showed a significantly lower IENF density. As assessed by the proximal/distal fiber density ratio, the hypothyroid neuropathy was length-dependent. When individually considered, an abnormally reduced IENF was observed in 60% of patients with overt hypothyroidism at the distal leg and in 20% at the proximal site. In patients with subclinical hypothyroidism, an abnormal IENF density was found at the distal leg in 25% and at the proximal thigh in 12.5% of cases.

Conclusions: Our study provides the first direct demonstration of reduced IENF density in patients with overt and subclinical hypothyroidism. In all patients the IENF density reduction was length-dependent. These findings suggest that a considerable number of untreated hypothyroid patients may have a preclinical asymptomatic small fiber sensory neuropathy and represent a further argument favouring the treatment of subclinical thyroid failure.

Nothing to Disclose: FM, MB, AO, MR, AG, SA, AC, LC

P3-596

Radioiodine Therapy for Graves' Disease Is Associated with Increased Rate of the Metabolic Syndrome.

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Introduction: Although the treatment of Graves' thyrotoxicosis with radioactive iodine is generally perceived as clinically efficient, cost effective and safe, most radioiodine-treated patients become hypothyroid rapidly after radioiodine treatment and need life-long thyroxine replacement. Further, observational studies show that radioiodine therapy is linked to increased mortality due to cardio- and cerebrovascular disease. Here we evaluated the cardiometabolic outcome of radioiodine-treated (RI) vs. medically treated (Med) subjects with Grave's disease as assessed after ≥ 3 years of follow up.

Patients/Methods: All subjects were actively recruited to undergo complete physical examination and blood testing which included glucose, HgbA1C, CRP, liver, kidney and thyroid function. Non-diabetic patients also underwent 75 gram oral glucose tolerance test. Arterial stiffness was evaluated using applanation tonometry and pulse wave analysis by different standard devices which assess distinct measures of arterial stiffness: pulse wave velocity (PWV), augmentation index, and large/small artery compliance (C1 and C2). Additionally, all subjects were referred for ambulatory blood pressure monitoring.

Results: Sixty five RI-treated and 33 Med-treated patients with Graves' disease were included in the study. Mean age (53 ± 12 vs. 48 ± 13.6 yrs, $p=NS$), gender (M-28 vs. 21%, $p=NS$), duration of the disease (5.2 vs 5.3 yrs), and thyroid function tests (TSH- 2 ± 1.4 vs. 1.6 ± 1.1 mU/L) were similar in the RI and Med groups, respectively. Post-RI-therapy patients had higher BMI, waist circumference, systolic blood pressure, PWV, hs-CRP levels (4.14 ± 5.3 vs. 1.62 ± 1.44 ; $p=0.032$), higher prevalence of the metabolic syndrome and lower C2 than Med-treated patients. After adjustment for age and gender, RI- treated patients had higher BMI, hs-CRP levels and higher prevalence of the metabolic syndrome (52.3 vs. 15.6% ; $p=0.003$)

Conclusions: Radioiodine treated patients with Grave's disease gain more weight and have higher rate of the metabolic syndrome compared with medically treated subjects with this condition. The difference is seen despite normal and indistinguishable thyroid hormone levels. We suggest that in the choice between these two major therapeutic modalities, physicians and patients should be aware of this emerging difference in outcome. Additionally, RI-treated patients should be carefully followed in an attempt to reduce the risk of weight gain and the metabolic syndrome.

Nothing to Disclose: EI, EO, MY, KT, YG, GS, YT, JS, IA, MI-S, RL, NS

P3-597

131 Iodine Therapy Is Effective in the Management of Diffuse and Nodular Non-Toxic Goiter.

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Objective: To describe the clinical outcomes of patients with diffuse and nodular non-toxic goiter treated with radioactive iodine.

Methodology: Medical records of patients from an endocrine specialty clinic with diffuse and nodular non-toxic goiter who received radioactive iodine treatment were reviewed. Demographic characteristics were recorded. Thyroid volume, size and number of thyroid nodules before and after treatment were compared.

Results: A total of 82 medical records, 79 females and 3 males with mean age of 43.49 ± 15.46 years were reviewed. Eleven patients had diffuse non-toxic goiter while 71 patients had nodular non-toxic goiter. Mean TSH pretreatment was 0.929 uIU/mL. Mean dose of radioactive iodine was 14.4mci(range 6-35mci). Follow-up period ranged from 1 - 50 months with a mean of 5.53 months. Mean thyroid volume in the right pretreatment was 12.09cm² vs. 8.34cm² post-treatment, with percent reduction of 32%. Mean thyroid volume in the left was 11.31cm² vs. 7.88cm² post-treatment with a percent reduction of 30.3%. The average nodule size pre-treatment and post treatment was 1.92cm² vs. 1.18cm², with a percent reduction of 38%. There were 114 nodules pretreatment vs. 67 nodules post-treatment with complete disappearance in 17 patients. The reduction in size and number of nodules resulted in clinical improvement. Post-treatment, levothyroxine replacement dose ranged from 25-100 mcg/day. No recurrence of goiter and nodules were noted so far.

Discussion: With the issue of osteoporosis as a complication of long-term suppression with levothyroxine, radioactive iodine therapy is an effective and safe alternative in the management of diffuse and nodular non-toxic goiter.

Nothing to Disclose: AYS-A, LBM-A

P3-598

Response to Therapy for Amiodarone-Induced Thyrotoxicosis in Patients with Congenital Heart Disease.

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¹Mayo Clin Rochester, MN.

Background - The incidence of amiodarone-induced thyrotoxicosis (AIT) is higher in patients with congenital heart disease than in those with acquired cardiac disease yet the response to treatment and the natural history of the disease in this group have not been described.

Methods - All patients treated with amiodarone between 1986 and 2007 were identified from the database of the Adult Congenital Heart Disease Clinic at Mayo Clinic in Rochester, MN. From those, we identified and studied all cases of thyrotoxicosis that occurred after a minimum of 3 months on amiodarone in patients without prior history of hyperthyroidism. Treatment selection, duration, complications and responses as well as complications of the disease itself were abstracted by a physician from Mayo electronic medical records or reports from local treating providers.

Results - A total of 23 cases of AIT were identified in a cohort of 169 patients at risk; in 2 of these outcome data were not available. We found that 11/21 patients received medical treatment, 5 had thyroidectomy and 7 were observed. Two patients moved from medical therapy to surgery due to failure of medical management. Medical therapy included a combined steroid and antithyroid drug regimen in 5 cases, potassium perchlorate and antithyroid drugs in 4 cases, antithyroid drugs alone in one case and potassium perchlorate alone in one case. Radioiodine uptake was performed in 7 patients, and was >5% in one who was unsuccessfully treated with radioiodine. In all 21 cases the thyrotoxicosis ultimately resolved. AIT complications (CHF, hospitalizations, arrhythmias, edema, and weight loss) occurred in 14/21 cases (67%). The average duration of the AIT episodes was 273 days. Hypothyroidism developed in 5/15 patients (33%) who did not undergo surgery (3 post medical therapy and 2 after observation). Amiodarone was continued in 11/15 patients who didn't have thyroidectomy and one patient (9%) developed recurrent AIT that required surgery.

Summary - The treatment selection for AIT varied widely, yet the disease uniformly resolved arguing for a generally self-limited course. The complication rate was high and the duration of episodes was significantly longer than that seen in other causes of thyroiditis. In our cohort, thyroidectomy was performed without significant complications despite the high cardio-vascular comorbidity of the group. Hypothyroidism is a common outcome after AIT resolution, while recurrence of thyrotoxicosis is rare.

Nothing to Disclose: MNS, MS, MDB, RSB

P3-599

Clinical Manifestations and Outcomes of Encephalopathy Associated with Autoimmune Thyroid Disease in Patients with Graves' Disease.

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The encephalopathy associated with autoimmune thyroid disease (EAATD) is characterized by neurological and/or psychiatric symptoms, high titer of anti-thyroid antibodies, increased cerebrospinal fluid protein concentration, non-specific electroencephalogram abnormalities, and responsiveness to the corticosteroid treatment in patients with an autoimmune thyroid disease. Almost all patients with EAATD are affected by Hashimoto's thyroiditis (HT), but fourteen EAATD patients with Graves' disease (GD) have been also reported. With the goal of analyzing the clinical features and the management issues of GD patients with EAATD, we have recorded the clinical, biological, radiological, and electrophysiological findings and the data on the therapeutic management of all GD patients with EAATD reported so far as well as the clinical outcomes in those of them followed-up in the long term. All subjects with EAATD and GD were women but two. The majority of GD patients with EAATD presented with mild hyperthyroidism at EAATD onset or shortly before it. Evidence of active anti-thyroid autoimmunity was reported in all cases. Most of the patients dramatically responded to the corticosteroid treatment. The long term clinical outcome was overall benign but EAATD can relapse, especially at the time of corticosteroid dose tapering or withdrawal. When comparing the clinical features of the GD patients with those of HT patients described in the literature, the EAATD presents as a single nosological entity characterized by clinical, biological, radiological, and electrophysiological findings and management issues that are unaffected by the nature of the underlying autoimmune thyroid disease. In conclusion, either GD or HT can equally represent the background condition for the development of EAATD, which should be considered in the differential diagnosis of all patients with encephalopathy of unknown origin and an autoimmune thyroid disease, regardless of the nature of the autoimmune thyroid disease itself.

Nothing to Disclose: GT, YC, RS, MD, KK, GG, BIL, CH, GM

P3-600

71 Episodes of Thyrotoxic Periodic Paralysis: An Update and Focus on Recurrence.

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Background: Thyrotoxic Periodic Paralysis (TPP), hyperthyroidism presenting with weakness or paralysis and hypokalemia, is underrecognized as a cause for lower extremity weakness and can recur despite medical attention. Here we present an update of our previous report on TPP with a focus on factors that are associated with recurrent attacks requiring medical attention.[1]

Methods: We performed a retrospective study of 71 episodes in 53 patients with TPP between June 1985 and July 2009 from diagnosis of hyperthyroidism to euthyroid status. Chart and electronic medical records were analyzed for age at presentation, gender, ethnicity, dates of medical encounters, etiology of hyperthyroidism, thyroid function tests, electrolytes and potassium administered at initial presentation, hyperkalemia during hospitalization, location of weakness and other symptoms or precipitants, initial and maximum thionamides (antithyroid drugs or ATD) and beta-blocker (BB) dose. Those with repeated episodes requiring hospitalizations were defined as having recurrent TPP and were analyzed as subgroup.

Results: 94% were male. Mean age was 33±8 years old. 53% were Asian while 40% Latino. 89% had Graves. In recurrent TPP group, median duration between first presentation and recurrence was 7 days (range 1 to 425 days). There was no significant difference in gender (all were male), age, ethnicity, degree of body involvement or associated symptoms of pain when compared to the non-recurrent group. Recurrent patients had higher free thyroxine levels, lower potassium and phosphorus levels, and less rebound hyperkalemia, but these differences did not reach statistical significance. Patients not receiving ATD and BB at initial presentation had increased risk for recurrence of TPP (OR 8.8, p=0.04).

Conclusions: TPP continues to be underrecognized. TPP patients who do not receive thionamides and beta blockade are at significantly increased risk for recurrence. Increased attention must be placed on identifying patients presenting with TPP and initiating prompt therapy to reduce recurrence.

Manoukian MA et al., Clinical and Metabolic Features of Thyrotoxic Periodic Paralysis in 24 Episodes. Arch Intern Med 1999; 159:601-606

Nothing to Disclose: CC, AMT, HL, VL, LMC

P3-601

¹³¹I mCi/Gram of Thyroid Size Correlates with RAI Outcomes.

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Background:

Currently there is no consensus regarding optimal radioiodine dose. In our institution Graves' patients are treated with doses of ¹³¹I iodine therapy not based on radioiodine uptake. Instead, various doses of ¹³¹I are used depending on clinical judgment. Initial data showed a correlation between the administered ¹³¹I dose per gram of clinically estimated thyroid size (mCi/g) and response rate. In this study, we wanted to validate this finding.

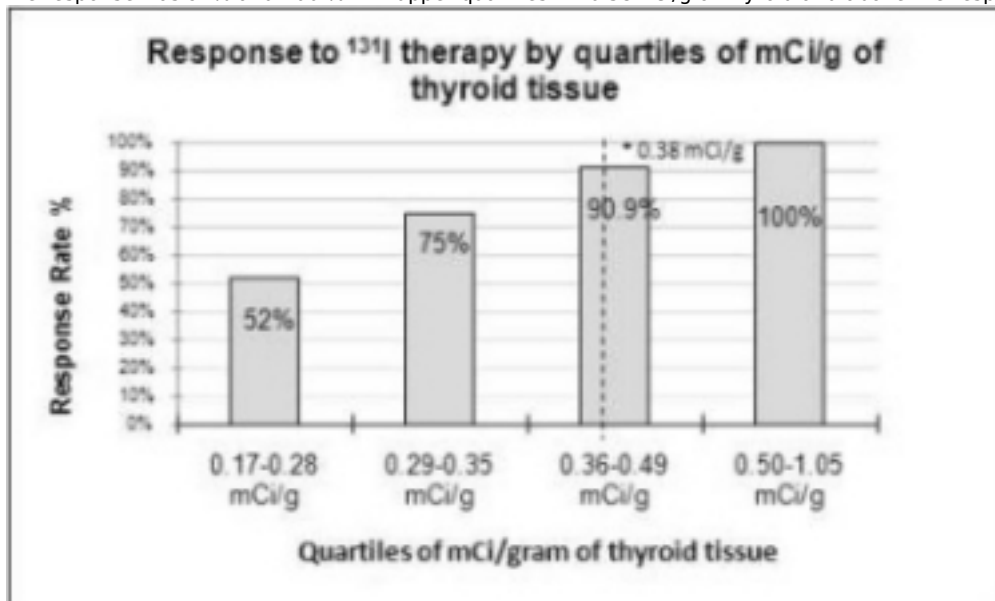
Methods

Retrospective chart analysis of patients treated with ¹³¹I therapy and had clinical or laboratory criteria for Graves' disease was done. Only patients who were treated with methimazole prior to ¹³¹I therapy but not after were included. The ¹³¹I therapy was given if patients had elevated thyroid hormone levels or increase uptake after discontinuation of methimazole for at least one week. Responders were patients that achieved euthyroid/hypothyroid state within 12 months post ¹³¹I therapy.

Results: Ninety patients (65% females, mean age 40 years) were analyzed. The mean thyroid size was 52 g and 16.6 % had a size bigger than 80 g. The mean FT4 at the time of the diagnosis was 5.5 ng/dl and before therapy was 4.26 ng/dl.

The mean ¹³¹I therapy dose was 19.1 mCi (range 10.1 - 29.9 mCi). The mean ¹³¹I mCi/g was 0.41. The total ¹³¹I dose correlated with thyroid size (p=0.001).

The overall response rate was 78.9%. The strongest independent predictor of response using multivariate regression analysis was mCi/g of thyroid (p=0.001). While ¹³¹I dose (p=0.02), FT4 at diagnosis (p=0.06) and age (p=0.03) were negatively correlated. Using quartiles of mCi/g (graph) there was a significant dose response relationship (p=0.001). The response was 91% and 100 % in 2 upper quartiles. At 0.38mCi/g of thyroid and above the response rate was 100%.



Conclusion:

In this study, the mCi/g of thyroid was a strong predictor of response. These results may support the use of clinical estimate of ¹³¹I doses based on thyroid size and optimal mCi/gram criterion. Giving a dose of 0.38 ¹³¹I mCi/g of thyroid may allow very high success rates. Further prospective studies are needed to confirm such an approach.

Nothing to Disclose: BMP, GS, SB, DT, LF

P3-602

Untreated Hyperthyroidism and Post-Operative Outcomes in Nonthyroidal Surgery.

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Background:

Thyroid disease is highly prevalent in the general population, so a large number of patients undergoing non-thyroidal surgeries are likely to have concomitant thyroid disease. There are no clear guidelines for management of hyperthyroidism in the post-operative setting for non-thyroidal surgeries. We retrospectively looked at the post-operative outcomes of untreated hyperthyroidism upto 3 months after non-thyroidal surgery.

Methods and Results: 72 adult patients [(M : 13, F: 59), mean age 65 y (M: 67y, F 64 y)] with TSH < 0.3 uU/mL (0.4-5.5 uU/mL) with a normal or elevated free T4 up to 6 weeks prior surgery were included. Exclusion criteria were treatment for hyperthyroidism, use of thyroid hormones supplements, non-availability of TSH prior to surgery, and thyroid surgery as current procedure. Post surgical outcomes included angina, myocardial infarction, arrhythmia, respiratory failure, sepsis and death.

There was no mortality in this group. 14% patients (N=11) had complications. 6% (n= 5; 1 M - underwent ophthalmologic procedure; 4 F- underwent ophthalmological procedure, cardiothoracic surgery, plastic surgery, general surgery) patients had angina and 5% (N=4) had arrhythmias (all females, 2- underwent cardiothoracic surgery, 1-orthopedic procedure, 1-urological procedure). None of the patients had myocardial infarction or sepsis. 2 patients were diagnosed with respiratory insufficiency (both females who had undergone cardiovascular procedure).

Conclusion:

Mortality was not increased in patients with untreated hyperthyroidism undergoing non-thyroidal surgery. Type of surgery did not affect the post-surgical outcomes. Complications seen were comparable to that in the general population. (Complications in general population are between 11-20% for cardiothoracic surgery).

Nothing to Disclose: NTN, KBD, RR, BAH

P3-603

The Prevalence of Post Radioactive Iodine Hypothyroidism in Patients Treated for Hyperthyroidism in the West of Ireland.

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Background: Hypothyroidism is a predictable sequelae following treatment of hyperthyroidism with radioactive iodine (RAI). The prevalence of post-RAI hypothyroidism in the West of Ireland is unknown.

Aim:

1. To determine the rate of hypothyroidism at one year following RAI therapy.
2. To identify predisposing determinants for developing hypothyroidism.

Methods: Patient data including demographic, biochemical and clinical variables were obtained using patient records and a computer based laboratory system. Patients who were hypothyroid one-year post RAI were identified. These included patients who were biochemically hypothyroid at one year and patients who were euthyroid at one year but receiving treatment for hypothyroidism. Subjects identified as being hypothyroid were further investigated to identify determinants predisposing them to hypothyroidism. Factors investigated included underlying disease, age, sex, presence of thyroid antibodies. In this study all patients received a fixed dose of RAI.

Result: Initially 100 patients were included in the study. However in order to increase statistical power an additional eleven subjects were included to allow two controls for every patient identified as hypothyroid at one year. 111 subjects, 89 females who had received RAI between 2006 and 2008 were studied. Thyroid status at twelve months was available for the 111 selected. The incidence of hypothyroidism in the original 100 was 37% with a 95% confidence interval. Descriptive analysis was used to look at the breakdown of original disease among the 37 patients hypothyroid at one year. 65% of patients had Graves' disease, 3.8% had a solitary thyroid nodule, and 28% had multinodular goitre. Statistical analysis in the form of a t-test and binary regression logistic model was used to investigate age and gender as predisposing factors. Youth was found to be a statistically significant predisposing factor with a p-value on T-test of 0.0119 and a p-value on Binary logistic regression model of 0.0135 and a mean age difference of 9 years. Male gender was found to approach statistical significance as a determinant for hypothyroidism when analysed with a p-value of 0.15.

Conclusion: The rate of hypothyroidism at twelve months post RAI therapy for hyperthyroidism in patients attending a thyroid clinic in the West of Ireland was 37%. This study found that the tendency to develop hypothyroidism post RAI is strongly influenced by age, with younger patients being at a greater risk

Nothing to Disclose: RTC, MB, BD

P3-604

Enhancement of Aldosterone-Induced Mineralocorticoid Receptor Activation by the THP-1 Macrophage Secretory Products.

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[Aim] Elevation of plasma aldosterone level or mineralocorticoid receptor (MR) activation is involved in resistant hypertension, vascular inflammation and cardiac remodeling. Macrophages play a crucial role in the inflammation of cardiovascular tissues. We therefore hypothesized that macrophage might activate MR in the inflammation of blood vessels, investigated whether macrophage secretory products directly stimulate MR transcriptional activity. [Methods] We used a human monocytic leukemia cell line, THP-1, which differentiates into macrophages when treated with phorbol 12-myristate 13-acetate. Utilizing coculture of THP-1 and COS-7 cells in which MR and MR-responsive reporter plasmids were transfected, we investigated the effect of transfer of the monocyte-macrophage conditioned media from single culture of THP-1 cells into COS-7 cells on the MR transcriptional activity. We also investigated the effect of coculture of THP-1 conditioned media on endogenous MR target gene expression in stably MR-expression 293-MR cells. Subsequently, we investigated the effect of THP-1 conditioned media on the levels of expression of MR mRNA and protein by quantitative real time RT-PCR and western blotting. The same experiments utilizing glucocorticoid (GR) and androgen receptor (AR) plasmids were performed. [Results] Transient transfection in COS-7 cells showed that 10^{-10} M aldosterone increased MR transactivation by 12-fold. The MR transcriptional activity was minimally affected with coculture of THP-1-monocytes, whereas significantly enhanced by 4-fold with coculture of THP-1-macrophages. The THP-1 conditioned media transfer experiments confirmed that macrophage conditioned media enhanced MR transactivation by 5.2-fold, whereas repressed GR and AR transactivation by 0.7- and 0.4-fold, respectively, indicating that enhancing effects of macrophage-conditioned media on MR activity is selective. Interestingly, treatment with macrophage conditioned media did not change mRNA, but increased protein levels of MR by 3.5-fold. [Conclusion] The THP-1 macrophage secretory products enhanced aldosterone-induced MR transactivation via increased MR protein, but not mRNA levels. The THP-1 conditioned media conversely repressed hormone-activated GR as well as AR transactivation. The alternative MR activation pathways by unidentified humoral factor(s) from THP-1 macrophage conditioned media may play a crucial role in cardiovascular inflammation and fibrosis by infiltrated macrophages.

Nothing to Disclose: TH, HS, IK, YM, AM-T, YM, RJ, HI

P3-605

Activation of Epidermal Growth Factor Receptor Enhances Mineralocorticoid Receptor Action Via Deubiquitylation of the Receptor: Non-Genomic and Genomic Pathway Crosstalk.

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[Aim] Epidermal growth factor (EGF) receptor is a downstream target of mineralocorticoid receptor (MR) and the EGF receptor-tyrosine kinase-ERK signaling may be involved in aldosterone-induced cardiovascular fibrosis. On the other hand, tyrosine kinase and ERK are shown to be involved in non-genomic pathways of MR. We therefore investigated whether tyrosine kinase-ERK signaling as a non-genomic MR pathway affects the genomic MR action.

[Methods] The reporter assay using COS-7 cells was performed to investigate MR-mediated transcriptional activity. The expression level of MR and Sgk were examined with real time RT-PCR and Western blot analysis using HEK-293 cells. The ubiquitylation of MR was investigated with coimmunoprecipitation.

[Results] EGF potentiated aldosterone-mediated transactivation of a MR-responsive reporter as well as endogenous Sgk mRNA levels by 2-fold. The enhanced MR activation by EGF was inhibited by EGF receptor antagonists such as PD153035 and AG1478, as well as an ERK inhibitor, PD98059. As expected, depletion of endogenous EGF receptor expression by introducing small interfering RNA for EGF receptor completely abrogated the enhanced MR response. Interestingly, EGF treatment significantly increased protein, but not mRNA levels of MR which was accompanied by deubiquitylation of MR. The MR mutant K367A/K715E, in which two ubiquitylation lysine residues were mutated and were partially resistant to proteasome-mediated degradation, showed enhanced aldosterone-induced MR transcriptional activity by 3-fold with increased levels of MR protein. The enhanced MR activation by EGF treatment was canceled for the K367A/K715E MR mutant. In parallel with MR transcriptional activities, effects of EGF treatment on increased MR protein levels were also abrogated by PD 153035, AG1478, and EGF receptor siRNA treatments. These data support that EGFR-ERK signaling enhanced MR transcriptional activity via deubiquitylation of MR. [Conclusion] Activation of EGF receptor enhanced aldosterone-induced MR transactivation by inhibiting MR degradation. The MR protein was modified by the tyrosine kinase-ERK signaling-mediated phosphorylation and deubiquitylation, thus resulting in increased MR protein levels. The novel mechanisms by a non-genomic pathway crosstalk with genomic action may shed a new light on alternative activation of MR.

Nothing to Disclose: YM, HS, IK, KY, AM-T, RJ, TH, YM, HI

P3-606

Anti-apoA-1 IgG Is a New Cardiovascular Prognostic Marker Interacting with Non Genomic Aldosterone Signaling in Ventricular Cardiomyocytes.

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Background. Anti-apolipoprotein A-1 IgG (anti-apoA-1) have been reported in autoimmune diseases associated with high cardiovascular risk and myocardial infarction (MI), but their clinical significance remains undetermined. We have recently shown that MI patients positive for these auto-antibodies have a higher resting heart rate and a higher incidence of adverse events during the one-year follow-up (Vuilleumier et al., 2010, Eur Heart J). We also demonstrated *in vitro* that anti-apoA-1 exert a positive chronotropic action on spontaneously beating rat cardiomyocytes primed with aldosterone. The aim of the present study was to identify aldosterone-induced mechanisms making myocytes prone to respond to anti-apoA-1. **Methods.** The rate of spontaneous contractions was determined *in vitro* in freshly isolated neonate rat ventricular myocytes. **Results.** Plasma from MI patients containing anti-apoA-1 induced a 4-fold acceleration of cell contractions. Spiking monoclonal anti-apoA-1 also induced a dose-dependent chronotropic effect, fully reversed by apoA-1. Control plasma or the addition of unrelated IgG had no effect on myocytes. The chronotropic response to anti-apoA-1 was maximal within 20 min and observed only in cells previously exposed to aldosterone. This “permissive” action of aldosterone was not mimicked by other steroids and reached a maximum at 10 nM. Inhibition of PKA, calcineurin, p42-44 or p38 MAPK pathways did not affect aldosterone action that was nevertheless dependent on PI3K activation, suggesting a non genomic action through the mineralocorticoid receptor (MR), a fact confirmed by the rapid kinetics of aldosterone effect (10 min). Eplerenone, but not spironolactone, antagonized aldosterone action. Upon oxidative conditions, corticosterone mimicked the non genomic action of aldosterone through MR. In contrast, upon normal redox state, the glucocorticoid functioned as an efficient mineralocorticoid antagonist, preventing the chronotropic response to aldosterone. **Conclusions.** In post MI patients, anti-apoA-1 is an independent predictor of cardiovascular complications, significantly affecting RHR. The chronotropic action of this antibody is reproduced *in vitro* in isolated rat ventricular cardiomyocytes in a fashion dependent on a non genomic signaling pathway activated by aldosterone. Sensitization of cardiomyocytes to anti-apoA-1 by aldosterone can be prevented by glucocorticoids in the absence of oxidative stress or with the MR antagonist eplerenone.

Nothing to Disclose: MFR, NV, SP, MP, AM, RJ, FM, PR-L

P3-607

Phenotypical Characterization of GILZ-Deficient Mice.

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The glucocorticoid-induced leucine zipper (GILZ) is a X-linked transcription factor and was originally described as a dexamethasone-induced transcript in murine thymocytes. It is a member of the transforming growth factor β 1-stimulated clone 22 domain (TSC22D) family. GILZ is known as TSC22D3 and described as 4 isoforms. GILZ is widely expressed and an important role in immunity, adipogenesis, renal sodium handling has been proposed. Using the Cre-Lox recombination technique, we generated mice constitutively lacking the vital region of the GILZ gene, thus abolishing the function of this gene in mice. The expression of GILZ mRNA transcripts and protein is completely abolished in all the tissues tested from knockout mice. Knockout mice are viable, but the males are sterile and present a severe testis hypoplasia. Although different processes in inflammation and immune system have been tested, only minor defects were observed. Changes in fat metabolism and adipogenesis are observed with age. Following sodium and water deprivation experiments, water and salt homeostasis is conserved. Sterility of knockout males is associated with a severe testis dysplasia. Generally, seminiferous tubules are smaller and the number of Sertoli and germ cells is reduced while apoptosis is increased as evidenced by TUNEL staining. The interstitial Leydig cell population is augmented, and higher plasma FSH and testosterone levels are found. In summary, these new GILZ-deficient mice allow to proof in vivo its implication in various glucocorticoid-dependent and -independent biological processes.

Nothing to Disclose: PES, EGR, JCS, SR, JW, RS, OG, VP, AW, TR, FP, DP, JT, BCR, EH

P3-608

Roles of BMP System in Endothelin- and Aldosterone-Induced Mitosis of Pulmonary Arterial Smooth Muscle Cells Isolated from Pulmonary Arterial Hypertension.

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Primary arterial hypertension (PAH) is a life-threatening disease that has prevalence of 1 to 2 occurrences per one million individuals. This disease is characterized by excessive proliferation of vascular endothelium and smooth muscle cells, causing thickening the walls of pulmonary arterioles and the formation of plexiform lesions that occlude the vascular lumen. Identification of heterozygous germline mutations in BMPR2 gene in familiar and sporadic PAH has proven to be a crucial breakthrough in elucidating the pathogenesis of PAH. However, it remains difficult to explain the entire mechanism of pulmonary arterial smooth muscle cells (PASMCs) mitogenesis in PAH lungs. Disequilibrium in vasoactive mediators in the lungs, including activated thromboxane, endothelin-1 (ET1), RAA system, and decreased prostacyclin and NO leads to vasoconstriction and proliferation of PASMCs. In the present study, we investigated the functional link of the BMP system and other vasoactive factors associated with PAH (including ET, angiotensin II and aldosterone) in the mitotic actions of PASMCs isolated from idiopathic and secondary PAH lungs. ET1 and aldosterone stimulated PASMC proliferation of idiopathic PAH more efficaciously than that of secondary PAH, whereas angiotensin II and ET3 failed to activate mitosis in either cell type of PASMC. The effects of ET1 and aldosterone were blocked by an ET type-A/B receptor (ETA/BR) antagonist bosentan and a selective mineralocorticoid receptor (MR) blocker eplerenone, respectively. Among the BMP ligands examined, BMP-2 and -7 but not BMP-4 and -6 significantly increased cell mitosis in both cell types of PASMCs. Notably, ET1- and aldosterone-induced mitosis as well as MAPK phosphorylation were significantly increased in the presence of BMP-2 and -7 in PASMC isolated from idiopathic PAH, although the additive effects were not observed in PASMC isolated from secondary PAH. Inhibition of ERK signaling suppressed basal, ET1- and aldosterone-induced PASMC mitosis more potently than that of SAPK/JNK inhibition. Given that BMP-2 and -7 upregulated ETA/BR and MR expression and that BMP-2 decreased 11 β HSD2 levels in PASMC isolated from idiopathic PAH, BMPR-Smad signaling may play a key role in amplifying the ETA/BR- and/or MR-ERK signaling in PASMCs of PAH lung. Collectively, the functional link between BMP and ET and/or MR system may be involved in the progress of PASMC mitosis, leading to the development of clinical PAH.

Nothing to Disclose: FO, RY, KN, HO, MT, TM, KI, HM

P3-609

The Target Genes of Aldosterone in Vascular Smooth Muscle Cells.

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Although recent in vivo findings suggest direct deleterious effect of aldosterone on cardiovascular tissues, the precise molecular mechanism is not completely understood. Since mineralocorticoid receptor (MR) is an aldosterone receptor and a transcription factor as well, in this study, we examined the effect of aldosterone on the transcriptional activities of a variety of genes expressed in vascular smooth muscle cells (VSMC). Using the rat VSMC(A10) cells and the promoter-luciferase reporter constructs in vitro, we found that aldosterone(10-100 nM) potently stimulated the promoter activities of ion channel genes(ENaC, NKCC1, NHE, Na/K ATPase subunits, NCX1/2), among which that on ENaC was most prominent. Aldosterone also stimulated the transcription of inducible transcription factors such as Fos/AP1, those of calcium-activated transcriptional factor(NFAT, SRF, NF-κB)-dependent genes, inflammation/coagulation-related genes(CRP, IL6, MCP1, PAI-1, ICAM1), and RAS-related genes(p22phox, p47phox, p67phox, gp91phox, AT1, ACE). Based on those in vitro findings, we assume that that aldosterone/MR facilitates the expression of ion channel in the expression of inflammation, coagulation, and RAS-related genes, most of which may be responsible for the vascular inflammation, fibrosis, and the atherosclerosis.

Nothing to Disclose: MT, YI, MN, TT, MO, YT, SN, LFZ, MK, TT, KH, YT

P3-610

A Role of Bone Morphogenetic Proteins in Aldosterone-Induced Glomerular Injury.

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In recent years, aldosterone has been recognized as the risk factor of multiple organ damages including cardiovascular system, brain and kidney. Aldosterone directly induces renal glomerular damages by stimulating mesangial cell proliferation and increasing extracellular matrix through activation of MAPK pathway via mineralocorticoid receptor (MR). According to the recent studies, bone morphogenetic proteins (BMPs), in particular BMP-7, play an inhibitory role in various renal diseases including diabetic and obstructive nephropathy. We earlier reported the antagonistic effects of BMPs on renal mesangial cell proliferation induced by aldosterone in vitro. In that study, BMP-4 and -7 antagonized aldosterone-induced mesangial cell proliferation through inhibiting ERK1/ERK2 activation and MR expression. In the present study, we further investigated the in vivo roles of BMPs in aldosterone-induced renal glomerular injury. BALB/c mice aged 6 weeks were treated with aldosterone injection (5 µg/day i.p.) and/or oral administration with 2% saline for 9 weeks. Systemic blood pressure, body weight and kidney weight were not significantly changed by aldosterone and/or saline treatment. However, renal histological examination revealed that increases in glomerular cellularity and glomerular diameter were apparently observed in the groups treated with aldosterone injection and 2%-saline administration even for 3 weeks. BMP ligands (BMP-4 and -7), BMP type-I receptors (ALK-2, -3 and -4), BMP type-II receptors (BMPRII and ActRII) and MR mRNA levels were detected in mouse kidney tissue by RT-PCR. Immunohistochemical studies revealed the expression of BMP-4, -7 and BMP type-I and -II receptors in the glomerular mesangial region. MR mRNA expression in renal cortex was transiently increased for 3-week treatment with 2%-saline, whereas MR expression was rather decreased by 9-week treatment with aldosterone. Furthermore, the expression of BMP-4 and -7 mRNA was enhanced in the renal cortex treated with aldosterone plus 2%-saline after 3-week and 9-week periods, respectively. Thus, these findings suggest the possibility that renal BMP system can be activated by aldosterone and/or 2%-saline treatment, which may play a key role in antagonizing mesangial cell proliferation induced by aldosterone and/or high-salt diet in vivo.

Nothing to Disclose: JS, FO, KI, TM, MT, NT, EN, HO, TO, HM

P3-611

Mineralocorticoid Receptor Blockade Attenuates Hypertension and Myocardial Oxidative Stress without Reducing Left Ventricular Hypertrophy.

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Effects of elevated aldosterone levels on the heart involve an interaction with the renin-angiotensin system (RAS) to promote myocardial oxidative stress mediated by NADPH oxidase generated superoxide synthesis. Thus, in RAS-dependent hypertension mineralocorticoid receptor blockade could be beneficial in the myocardium. We previously reported that a sub-depressor dose of spironolactone had favorable effects on oxidative stress and perivascular fibrosis in the myocardium of hypertensive Ren2 rats, a model of tissue RAS activation and hyperaldostolemia. We extend that study by testing the hypothesis that a higher dose of spironolactone likely to lower blood pressure is similarly beneficial in the Ren2 myocardium. Ren2 rats and their Sprague-Dawley (SD) littermates received 5 mg·kg⁻¹·day⁻¹ spironolactone or placebo for 21 days starting at 5 weeks of age. Spironolactone treated Ren2 rats had reduced systolic blood pressure compared to Ren2 controls (163±7 vs 190±3; P<0.05); nonetheless, blood pressure was still elevated above SD controls (140±6 vs 163±7; P>0.05). Compared to control Ren2 rats the LV of spironolactone-treated Ren2 rats exhibited reductions in markers of oxidative stress, including NADPH oxidase activity (33.2±0.8 vs 29.2±2.1; P<0.05), superoxide levels (5140±1045 vs 3778±700; P=0.061), and 3-nitrotyrosine (40±3 vs 7±1; P<0.05). Markers of LV hypertrophy (LVH), including LV (plus septum) weight, myocyte cross sectional area, and LV septum thickness were not reduced following spironolactone treatment (P>0.05). The increase in mitochondrial number associated with LVH in the Ren2 rat was also unchanged by spironolactone (P>0.05). Spironolactone reduced periarterial fibrosis in the Ren2 (P<0.05). In conclusion, a dose of spironolactone sufficient to achieve a partial reduction in blood pressure, reduced perivascular fibrosis, and markers of LV oxidative stress, but was not sufficient to reduce markers of LVH. Given that LVH is a significant risk factor for heart failure, further study is needed to determine whether increasing the duration or dose of spironolactone therapy, using more specific MR blockers, or using spironolactone in combination with other antihypertensive therapies are needed to reduce LVH in Ang II-dependent hypertensive models.

Sources of Research Support: NIH and VA Merit to JRS and CDA-2 for AWC and Forest Research Institute.

Nothing to Disclose: VGD, ATW-C, JH, MRH, LM, LP, JRS

P3-612

Protein Kinase D Stabilizes Aldosterone-Induced ERK1/2 MAP Kinase Activation in Renal M1 Cortical Collecting Duct Cells To Promote Cell Proliferation.

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Aldosterone rapidly stimulates ERK1/2 MAP kinase and this activation is stabilised via protein kinase D1 in M1 cells. Here we describe a novel effect of aldosterone on renal cell growth modulated by the ERK1/2 – PKD1 pathway. Aldosterone (10nM) promoted the growth of M1 cells over 48hr, an effect that was PKD1, PKC δ and ERK1/2-dependent. This increase in cell growth was a result of stimulated proliferation and not due to suppression of apoptosis or necrosis. Aldosterone induced the rapid activation of ERK1/2 with peaks of activation at 2min and 10-30min, followed by sustained activation lasting >120min. Aldosterone promoted the association of PKD1 with ERK1/2 within 2min. M1 cells suppressed in PKD1 expression using siRNA exhibited only the early transient peaks in ERK1/2 activation without the sustained phase. Furthermore, aldosterone induced the subcellular redistribution of ERK1/2 to nuclei at 2min and to cytoplasmic sites proximal to the nuclei at 30min as measured using immuno-fluorescence confocal microscopy. The redistribution of ERK1/2 was inhibited in PKD1 knockdown cells. The stabilization of ERK1/2 activation and sub-cellular redistribution by PKD1 are required for aldosterone-induced cell proliferation.

Sources of Research Support: The Wellcome Trust; Higher Education Authority of Ireland Programme for Research in Third Level Institutions; Science Foundation Ireland Research Frontiers Programme.

Nothing to Disclose: RD, VM, BJH, WT

P3-613

Post-Transcriptional Control of Renal Mineralocorticoid Receptor Expression by the mRNA Binding Protein Tis11b and MicroRNAs under Hypertonic Stress.

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Aldosterone exerts its effects via the Mineralocorticoid Receptor (MR), a transcription factor highly expressed in the distal nephron. There is much interest in elucidating the molecular mechanisms governing MR expression that is altered in several pathophysiological disorders (mineralocorticoid resistance, renal disease). Recently, we generated a novel highly differentiated cortical collecting duct cell line (KC3AC1), which enabled us to show that hypertonicity drastically reduced MR expression through post-transcriptional mechanisms (Viengchareun et al, Mol Endocrinol, 2009, 23:1948). We first showed that hypertonic stress enhanced the expression of NFAT5/TonEBP, an osmoregulatory transcription factor, which binds tonicity response elements (TonE). Interestingly, we identified four TonEs in the promoter of Tis11b gene encoding a mRNA-destabilizing protein, and demonstrated that hypertonicity induced a parallel increase in Tis11b expression at both mRNA and protein level. We hypothesized that Tis11b accounted for the hypertonic-induced decrease in MR expression via its binding to six AU-rich elements (AURE) located in the 3'-untranslated region (3'-UTR) of MR mRNA. Transient transfection assays using a series of Tis11b promoter mutants, together with gene expression studies with siRNA strategies and RNA-ChIP, should enable us to decipher these post-transcriptional regulatory mechanisms.

In parallel, we hypothesized that microRNAs (miRNAs), might also be involved in the control of renal MR expression. Such small non coding RNA regulatory molecules can bind the 3'-UTR of targeted mRNA and activate RNA interference pathway to alter gene expression by either cleaving or repressing the translation of targeted mRNA. Our working hypothesis is supported by the identification of several miRNA target sites in the 3'-UTR of the MR gene by using two prediction programs (Targetscan and miRanda). A high throughput approach using MicroRNA TaqMan Low Density Arrays was used to follow the expression of 768 miRNAs in KC3AC1 cells under various experimental conditions. Validation of several miRNAs candidates, induced or repressed under hypertonic stress, is currently performed by quantitative PCR.

Understanding how Tis11b and/or miRNAs affect MR mRNA stability and protein level may open new perspectives to modulate mineralocorticoid signaling in pathophysiological situations, most notably in renal failure, hypertension, or mineralocorticoid resistance.

Sources of Research Support: INSERM and University Paris-Sud 11.

Nothing to Disclose: SV, VK, GM, FB, JB, NC, ML

P3-614

Assessment of Glucocorticoid Receptor Function in Peripheral Blood Mononuclear Cells of Critically Ill Children.

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Introduction: Due to their anti-inflammatory actions, glucocorticoids have been utilized since the 1950s as adjunctive therapy for septic shock. However, both beneficial and deleterious outcomes have been reported regarding its use among critically ill patients. Furthermore, evidence-based data regarding the safety and efficacy of cortisol therapy in the critically ill child have been particularly limited. Current methods of evaluating hypothalamic-pituitary-adrenal (HPA) axis have significant limitations such as hypoproteinemia and inconsistencies in laboratory assays. Cellular responses to glucocorticoids are mediated primarily by the glucocorticoid receptor (GR), which functions as a ligand-dependent transcription factor. With our goal to establish a more physiologic way of assessing HPA function, we aimed to analyze and compare measures of cellular GR function with currently available measures of adrenal function among critically ill children.

Methods: Pediatric patients (1-18 yrs) with the following critical illness were recruited from Children's Hospital of Pittsburgh: 1) Sepsis; 2) Septic shock; and 3) Traumatic Brain Injury (TBI). Healthy children with primary hypothyroidism (but normal thyroid studies) served as controls. Serum total and free cortisol, cortisol binding globulin (CBG), urinary free cortisol (UFC), and salivary cortisol were measured. PBMCs were isolated from 4 ml samples of blood. Total, cytoplasmic and nuclear protein lysates were prepared, followed by infrared immunoblotting for GR and modulators or downstream targets of the receptor.

Results: Total cortisol levels were comparable among the critically ill patients and controls. Serum free cortisol, salivary and UFC levels were higher in patients with TBI and septic shock compared to controls ($p < 0.05$). CBG was significantly lower in patients with septic shock and TBI compared to controls ($p < 0.05$). Western blot analyses showed significantly lower levels of total and cytoplasmic GR in critically ill children compared to controls.

Conclusion: The lack of correlation between circulating hormone levels and GR expression suggests that among critically ill patients, responses to glucocorticoid therapy may not be accurately predicted based solely upon their endogenous cortisol levels. Further studies are essential to elucidate putative treatment regimens that could either restore GR expression in critically ill patients or enhance the activity of the limited pool of available receptors.

Sources of Research Support: Pfizer, Inc. Investigator-Initiated Proposal; National Institutes of Health Grant M01-RR-00084 (Pediatric Clinical and Translational Research Center (PCTRC); Children's Hospital of Pittsburgh of UPMC); National Center for Research Resources (NCRR) Grant UL1 RR024153 (Basic to Clinical Collaborative Research Pilot Program (BaCCoR), Clinical & Translational Science Institute, University of Pittsburgh).

Nothing to Disclose: CSC-V, JI, SFW, IW, SV, RM, RS, NZ, DBD

P3-615

A Possible Role for Protein Kinase D in the Angiotensin II-Induced Aldosterone Priming Response in Adrenal Glomerulosa Cells.

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Priming refers to a process in which pretreatment of bovine adrenal glomerulosa cells with angiotensin II (AngII) sensitizes them to respond with greater aldosterone secretion to succeeding stimuli. Thus, following exposure to and removal of AngII, subsequent addition of a second AngII stimulus or of an agent that increases calcium influx results in an enhanced aldosterone secretory rate relative to cells that have not received a prior AngII treatment. We have recently shown that the serine/threonine kinase, protein kinase D (PKD) mediates, at least in part, acute aldosterone secretion in response to AngII. To determine if PKD activation persists following AngII washout and could thus be involved in priming, we pretreated cells with AngII for 30 minutes, removed the hormone and then monitored PKD activation status, as measured by autophosphorylation at serine 916. Phosphoserine 916 immunoreactivity remained significantly elevated for at least 30 minutes after AngII removal, suggesting that PKD remains active during the 30-minute washout period. Our previous results indicate that AngII-stimulated phospholipase D (PLD), which hydrolyzes phosphatidylcholine to produce phosphatidic acid, is involved in priming. Phosphatidic acid can be dephosphorylated by lipid phosphate phosphatases to yield diacylglycerol, a known activator of PKD, and persistent diacylglycerol is thought to mediate priming. To determine whether PLD activity underlies AngII-induced PKD activation, we treated cells with AngII in the presence and absence of 1-butanol, an organic alcohol that inhibits PLD-mediated lipid signal generation. 1-Butanol inhibited AngII-elicited PKD activation, as seen by a reduction in serine 916 autophosphorylation, suggesting a link between PLD and PKD in the AngII-induced aldosterone secretory response and possibly priming. Finally, we investigated the ability of a recently described PLD inhibitor, 5-fluoro-2-indolyl des-chlorohalopemide (FIPI), to reduce AngII-stimulated PLD activity. We found that FIPI significantly inhibited AngII-induced PLD activity, returning activity to a level not significantly different than the control value. These results suggest the possible utility of FIPI as a tool for determining the role of PLD in AngII-elicited PKD activation, aldosterone secretion and priming in bovine adrenal glomerulosa cells.

Sources of Research Support: National Institutes of Health #HL70046; American Heart Association Grant-in-Aid #0350166N.

Nothing to Disclose: WBB, BAS, MVG

P3-616

AMP-Activated Protein Kinase Increases the Expression of StAR in Adrenocortical Tissues.

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AMP-activated protein kinase (AMPK) plays an important role in the function of many tissues and often is involved in the effects of cytokines upon these tissues. Because cytokines affect the secretion of steroids from the adrenal cortex, we determined if AMPK activation modified the expression of adrenal steroidogenic acute regulatory protein (StAR), an important protein involved in the regulation of steroidogenesis. Two model systems were utilized in this study. The first model was the adrenocortical tumor cell line H295R. H295R cells were exposed to 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), an activator of AMPK, and the expression of phosphorylated AMPK determined by Western blot. AMPK was expressed in H295R cell and AICAR increased the AMPK phosphorylation (active form of AMPK). H295R cells were then transfected with the promoter for StAR linked to luciferase and the luciferase activity determined following exposure of the H295R cells to AICAR (0.5-2 mM). AICAR increased StAR promoter activity at 120 and 240 min in a concentration-dependent manner. In the second model, zona fasciculata tissue was isolated from bovine adrenal glands and the effects of AICAR exposure determined. AICAR (0.1 to 3 mM) exposure (1h) increased the expression of StAR mRNA (RT-PCR) and StAR protein (Western blot) in a concentration-dependent manner at 1 h. These effects were time dependent in that 1 mM AICAR increased StAR mRNA and protein within 30 min and the effects increased in magnitude through 3 h. In summary, adrenal tissue express AMPK and activation of this enzyme increases StAR promoter activity, mRNA expression, and protein expression in adrenal tissue. Therefore, this enzyme may have a role in regulating adrenal steroidogenesis.

Nothing to Disclose: AWD, RMA, TBW, BDB, JCS, KAD, TLO, AMJ

P3-617

Aldosterone-Induced Sodium Reabsorption in Pregnant Rat Kidney Distal Nephron: Expression and Activity of Epithelial Sodium Channel.

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Aldosterone is a potent stimulator of sodium reabsorption by the kidney distal nephron. It acts through its binding to the mineralocorticoid receptor to maintain the epithelial sodium channel (ENaC) at the apical membrane of the collecting duct's principal cells which promotes sodium influx. Late gestation is characterized by increased serum aldosterone, sodium retention and blood volume. Our hypothesis is that the increased sodium retention observed in late gestation is due, at least in part, to enhanced aldosterone-induced sodium reabsorption by ENaC in the distal nephron. The aim of this study is to compare the renal mRNA, protein and activity of ENaC along the last week of gestation in rats. Sprague-Dawley pregnant rats were sacrificed on days 14, 17, 19 and 22 of gestation. Blood was collected and kidneys were removed. Plasma sodium, potassium and hematocrit were evaluated. Plasma renin activity (PRA) and serum aldosterone were measured by radioimmunoassay. ENaC subunits mRNA and protein expression were measured respectively by real-time PCR and Western blot in cortex and outer medulla. ENaC activity was evaluated by whole-cell patch-clamp in fresh isolated split-open cortical collecting duct. We observed that late-pregnant rats (day 22) had lower plasma potassium while sodium remained stable. Late-pregnant rats had also a higher PRA when compared to days 14 and 17 rats, and they also had a higher serum aldosterone when compared to days 14, 17 and 19. The mRNA levels of α , β , and γ ENaC in renal cortex and outer medulla were similar between the different stages of pregnancy. Protein expression of α ENaC was higher in late-pregnant rats compared to days 14, 17 and 19 in the outer medulla, but was not different in the cortex. The β and γ ENaC protein levels were similar between the four groups. ENaC activity was also comparable between the groups. Despite the increased aldosterone levels during late gestation, the level and activity of ENaC, one major target of aldosterone action in the distal nephron, were only slightly affected. In this specific physiological context, instead of acting on ENaC, the pregnancy-induced increase of aldosterone could closely act with angiotensin II to enhance Na/Cl cotransporter activity in the distal tubule to reabsorb sodium. This possibility remains to be elucidated.

Nothing to Disclose: VH, GF, MP, JS-L, LGP, MB

P3-618

Ascorbic and Retinoic Acid Increase the Yield of Dopaminergic Neurons Derived from Chromaffin Progenitors Isolated from Adrenal Medulla.

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Chromaffin cells and sympathetic neurons derive from a common sympathoadrenal progenitor cell. Unlike sympathetic neurons, chromaffin cells are able to proliferate throughout life. In this study, we characterized and explored self renewal and differentiation capability of bovine chromaffine progenitor/stem cells. Similar to neuronal progenitors that can be maintained as neurospheres, isolated chromaffin cells formed chromospheres (CS) that are able to proliferate in a low-attachment environment. They expressed several progenitor markers such as nestin, Hes5, Hes 1. Our data establish enrichment of progenitor cells within CS. Presence of progenitor/stem cells was confirmed by presence of side population with low brightness due to exclusion of Hoechst dye from stem cells as previously reported. On average, up to 1,5% of isolated chromaffin cells displayed low brightness characteristic for side population consisting of progenitor/stem cells.

The capability of progenitor cells for self-renewal and to form CS depended on the density of seeding and age. An increased sphere initiation capacity up to 2 weeks of culturing was observed, suggesting enrichment of progenitor/stem cells. Under differentiation conditions, chromaffin precursor cells were capable to derive neurons positive for tyrosine hydroxylase, dopamine β hydroxylase, dopa decarboxylase and serotonin. Shift towards neuronal differentiation was accompanied with remarkable downregulation of neural progenitor markers such as Hes 1, Hes 5, Nestin and Notch-2. Moreover, frequency of generated dopaminergic neurons positive was significantly elevated after treatment with retinoic acid (RA) and ascorbic acid (AA). Unlike stimulated neurons derived by standard procedure, stimulation of neurons derived after RA and AA treatment revealed increased dopamine production with a tendency to lower nor- and epinephrine synthesis. In addition, typical neuronal excitability and existence of voltage-dependant channels was found after patch-clamping. Herewith, functional maturity of generated neurons was confirmed.

In summary, our data establish the presence of progenitors/stem cells with time limited self renewal in CS culture but pronounced ability to differentiate into dopaminergic neurons. These data support use of somatic progenitors/stem cells for therapeutic purposes. Particularly, chromaffin precursors might be promising source in the treatment of neurodegenerative diseases such as Parkinson's disease.

Nothing to Disclose: VV, JS, SL, NQ, K-FC, AH, GE, MA, ET, SRB, ME-B

P3-619

Is Overexpression of the Hypoxia-Inducible Factor-1alpha Natural Antisense Transcript a Marker of the Malignant Potential of Pheochromocytoma?.

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BACKGROUND 25-30% of pheochromocytomas (PHEO) are caused by germline mutations of the susceptibility genes RET, von Hippel-Lindau (VHL), neurofibromatosis 1 (NF1) and succinate dehydrogenase subunit B, C and D (SDHB/C/D). The tumorigenesis of VHL- and SDH-related PHEO is characterized by activation of hypoxic and angiogenic pathways, with high expression of hypoxia inducible factor 1-alpha (HIF-1 α) protein and its target gene vascular endothelial growth factor (VEGF). In other cancers, tumor aggressiveness and unfavorable prognosis are associated with high levels of HIF-1 α and VEGF. In vitro experiments have suggested that HIF-1 α expression is inhibited by its natural antisense transcript aHIF. The role of aHIF in the pathophysiology of PHEO is unknown. We hypothesize that there is a genotype-specific overexpression of aHIF in PHEO, which is associated with an increased metastatic potential.

AIM To investigate whether genotype-specific expression of aHIF is a biomarker of the metastatic potential of PHEO

METHODS Frozen tissue samples of primary tumors from 87 patients with PHEO were investigated. After histological confirmation of the representativeness of the samples, RNA was extracted followed by cDNA synthesis. Quantitative PCR was performed to assess the expression of aHIF and VEGF, and of other genes that are known to be up- or down-regulated in tumors of hypoxia-related tumorigenesis. Gene-expression was correlated with metastasis-free survival.

RESULTS Of 87 patients, 10 developed metastatic disease after a mean \pm SD of 5.50 \pm 8.15 years since the diagnosis. The other 77 remained free of metastases during 7.04 \pm 5.07 years of follow-up. Both aHIF and VEGF were overexpressed in PHEOs belonging to the 'hypoxia-cluster', i.e. VHL (n=3), SDHB (n=3), SDHD (n=1), as compared to tumors of the 'non-hypoxia cluster', i.e. RET (n=11) and NF1 (n=7). Both aHIF and VEGF were also overexpressed in primary tumors that later metastasized (n=10, including 2 SDHB) as compared to 'benign' tumors (n=77). Patients with high expression of aHIF and VEGF had a lower metastasis-free survival than those with low expression.

CONCLUSION aHIF and VEGF are overexpressed in PHEOs with a genotypic signature of hypoxia-related tumorigenesis. Moreover, overexpression of these genes represents a biomarker of the metastatic potential of PHEO.

Nothing to Disclose: PNS, SBJOO, JWML, CGJS, PW, FHVN, RRDK, ARMMH, HJLMT

P3-620

Reactive Oxygen Species and HIF 2 α Stabilization in Aggressive Mouse Pheochromocytoma Cells.

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Tumor cells often shift from efficient ATP synthesis via oxidative phosphorylation (oxphos) to increased levels of glycolysis. Increased glycolysis can be generated by suppression of oxphos. Oxphos suppression is caused by hypoxia in tumor areas with poor blood and oxygen supply as well as pseudohypoxia promoted by mutation of key regulatory genes or increased reactive oxygen species (ROS) levels.

Development of pheochromocytomas (PHEOs) and paragangliomas (PGLs), particularly those with underlying VHL, SDHB or SDHD germline mutations has been linked to aerobic glycolysis/pseudohypoxia. Despite this commonality tumor aggressiveness is very distinct: metastases rarely occur in VHL derived PHEO/PGL, while SDHB derived PHEO/PGL often metastasize.

Here we aimed to evaluate the involvement of energy metabolism and pseudohypoxia related pathways in aggressive PHEO/PGL.

As a first step, differential protein expression of cultured mouse PHEO cells (MPC) and their more aggressive filial mouse tumor tissue (MTT) cells was studied by 2D-gel analysis and mass spectrometric protein identification. Several energy metabolism and ROS related genes were identified as differentially expressed (FC>2, p<0.05). The involved pathways were further explored, revealing that in MTT cells lactate dehydrogenase (LDH) A is increased, while LDHB is decreased, supporting a glycolytic phenotype. Additionally glucose starvation resulted in reduced proliferation of MTT, but not MPC. However, respirometry revealed no difference in oxygen consumption of complex I and II, although increased ROS production at complex I was detected (p<0.01) in MTT cells. In addition, MTT expressed HIF 2 α , but not 1 α under normoxic conditions. Elevated ROS have been discussed as trigger for pseudohypoxia in SDH related tumors.

Here we show that aggressive mouse PHEO cells show a supposedly HIF 2 α provoked glycolytic phenotype. Stabilization of HIF 2 α may be due to increased ROS, caused by a leaky oxphos complex I. Besides promoting hypoxia, elevated ROS were shown to induce DNA damage and may act as second messengers in tumorigenesis, potentially leading to highly aggressive tumor cells. ROS may induce tumor aggressiveness in MTT cells as well as human SDHB related PHEO/PGL. Thus, further study of the mouse cell culture model may enhance the understanding of aggressive PHEO/PGL and lead to the identification of new diagnostic and prognostic markers and potential therapeutic targets.

Sources of Research Support: Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health.

Nothing to Disclose: SMJF, NK, XSJ, OS, PT, FDP, PB, LM, ALY, SP, NP, HL, KP

P3-621

The Role of Adrenal Cortex in Formation and Differentiation of the Adrenal Medulla in Mice.

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The goal of this study is to investigate whether abnormal development of the adrenal cortex affects differentiation of the medulla in mice. Steroidogenic factor 1 (Sf1) and beta-catenin are two genes critical for adrenal cortex development. Loss of Sf1 led to adrenal dysgenesis at birth and insufficiency of beta-catenin resulted in decreased proliferation in the fetal adrenal and increased apoptosis in the adult adrenal. Other than controlling medulla's development, the adrenal cortex also plays roles in medulla function. Catecholamine synthesis, the major function of adrenal medulla, is under the control of glucocorticoids produced by adrenal cortex. To further investigate how adrenal cortex controls medulla development and function, we generated four different genetic models with various defects in adrenal cortex development. By using the Sf1/Cre mouse line that conditionally inactivates/activates genes in the adrenal cortex, we produced mice that developed cortex dysgenesis (Sf1/Cre-mediated beta-catenin knockout), cortex hypoplasia (Sf1/Cre-mediated Sonic hedgehog or Shh knockout), mice with progressive degeneration of fetal adrenal cortex (Sf1/Cre-mediated Dicer1 knockout), and mice with adrenal tumor (Sf1/Cre-mediated beta-catenin activation). We found that adrenal medulla formed regardless the defects in adrenal cortex development. The expression of Phenylethanolamine N-methyltransferase (PNMT), a key enzyme of catecholamine synthesis, remained unaffected in all models, suggesting that the numbers of cortical cells do not affect the migration or the differentiation of neural crest cells at fetal stages. However, the beta-catenin knockout mice as well as the beta-catenin activation mice both developed misplaced medulla outside of adrenal proper, indicating that beta-catenin pathway in the adrenal cortical cells plays an indirect role in controlling cell organization of the adrenal medulla.

Nothing to Disclose: C-CJH, C-FL, HY

P3-622

The Effect of DHEA on Proliferation and Differentiation of Chromaffin Progenitor Cells.

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Dehydroepiandrosterone (DHEA) and its sulfated form DHEAS are the most abundant steroid hormones in the human body. They are secreted by the inner adrenocortical zone, zona reticularis, which is in direct contact with the adrenal medulla. These intermingled tissues exhibit complex paracrine interactions which regulate their endocrine function and their differentiation. Our previous research has shown that DHEA and DHEAS influence the chromaffin cells proliferation and differentiation caused by growth factors. We therefore addressed the question on a potential influence of DHEA and DHEAS on the chromaffin progenitor cells proliferation and differentiation.

Proliferation-competent sphere-forming cells with progenitor properties have been successfully isolated from the bovine adrenal medulla. Compared to primary chromaffin cells, gene specific for differentiated chromaffin cells, such as the epinephrine synthesizing enzyme PNMT, was dramatically downregulated while the expression of progenitor markers nestin, Musashi1, Sox1 and Sox9 were upregulated. The sphere-forming cells had self-renewing capacity and differentiated into neuronal and endocrine cell lineages with the inductions of respective growth factors. The proliferation and differentiation of chromaffin progenitor cells was influenced by DHEA. The data from MTS assay indicated that the proliferation of chromaffin progenitor cells is hampered by high concentrations of DHEA (100 μ M). In contrast to dexamethasone, which induced the expression of PNMT, neither DHEA nor DHEAS had an effect on the expression of this enzyme. However, revealed by real-time PCR analysis, DHEA increased β -III-tubulin mRNA expression as a marker for the differentiated neuron.

In summary, our data indicate that DHEA influences the proliferation and differentiation of chromaffin progenitor cells; in vitro it promotes the neuronal differentiation of these cells. The high DHEA concentrations present in the fetal and adult adrenal gland may therefore play an important role in the proliferation and differentiation of the adrenomedullary cells.

Nothing to Disclose: KFC, VV, WBH, SRB, ME-B

P3-623

Analysis of the D104N Polymorphism of the *COL18A1* in Patients with Benign and Malignant Pheochromocytoma.

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Background: *COL18A1* is a component of basement membranes. Proteolytic cleavage within its C-terminal domain releases a fragment, endostatin, which has been reported to have antiangiogenic effects. The presence of an endogenous inhibitor of angiogenesis encoded by a gene on chromosome 21 has been suggested upon the observation that individuals with trisomy-21 (Down's syndrome) rarely develop solid tumors. A single nucleotide polymorphism was identified in the endostatin domain of *COL18A1* (D104N) that leads to a less active protein. It was observed that individuals with N104 allele in homozygous state have an increased chance of developing prostate and breast cancer. Alterations in this angiogenesis inhibitor might be associated to the development of solid and vascularized tumors. Pheochromocytomas are vascularized catecholamine-secreting tumors that arise from the chromaffin cells of the sympathoadrenal system with a malignant behavior in 10% of patients. **Objective** To evaluate whether the D104N polymorphism may play a role in the behavior of pheochromocytomas. **Subjects** 14 patients with pheochromocytoma diagnosis were selected (12 benigns and 2 metastatic pheochromocytoma) and 150 controls were also studied. **Methods** DNA were extracted from blood and adrenal medulla tumor tissue, respectively. The D104N polymorphism was genotypes by PCR-RFLP using the *MseI* enzyme and the final product was submitted to electrophoresis in a 2% agarose gel. **Results** Among the 150 individuals of the control group, 124 (83%) had the D104D genotype, 23 (15%) had the D104N genotype, while 3 (2%) had N104 genotype. The D104N polymorphism was not found in any patient with benign or malignant pheochromocytomas. **Conclusions** Although the pheochromocytoma are solid and vascularized the D104N or N104N polymorphism located in the antiangiogenic endostatin *COL18A1* we did not identify it in benign as well malignant pheochromocytomas in our cohort.

Sources of Research Support: FAPESP- 07/515048.

Nothing to Disclose: BMPM, TCR, RAT, MAAP, APSSdS, BBM, MCBVF

P3-624

Adrenal Vein Sampling for Catecholamines: A Normal Value Study.

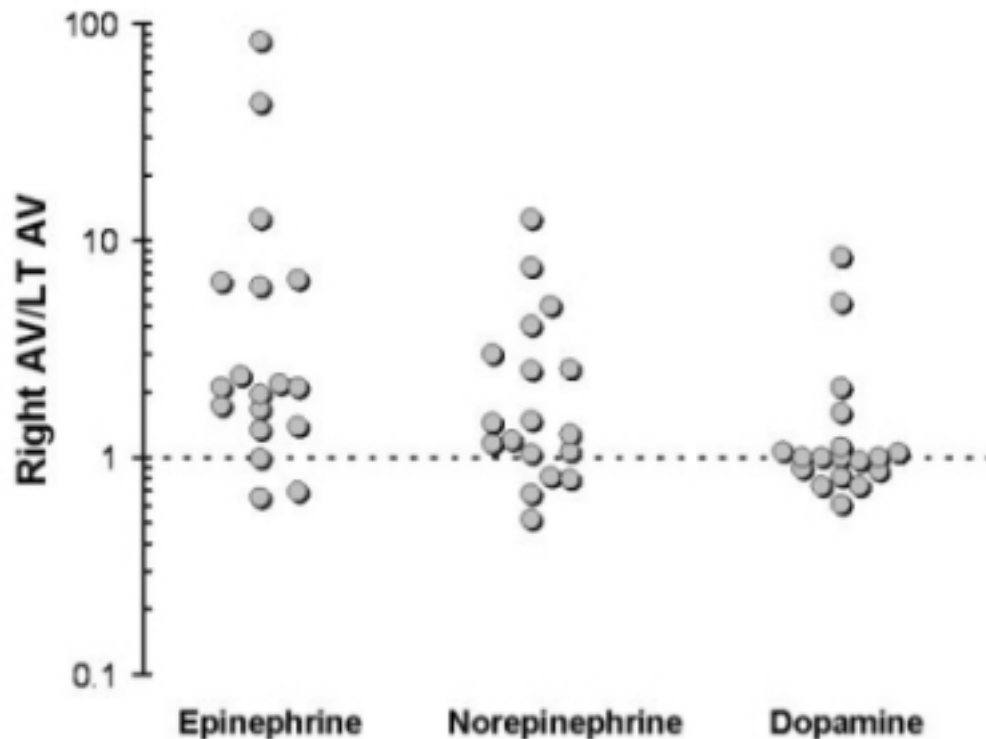
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Pheochromocytomas are rare, but potentially fatal, neoplasms. The diagnosis and localization of pheochromocytoma can be challenging and recently there has been some debate regarding the role for adrenal venous sampling (AVS). The utility of AVS in this setting is hampered by a lack of normative value data for adrenal vein catecholamine concentrations and the reliability of lateralization ratios. We sought to address these concerns by analyzing AVS catecholamine concentrations from patients who did not have pheochromocytoma

Methods: Eighteen patients underwent successful AVS for evaluation of cortisol-producing adrenal masses. All had normal 24 hour urinary excretion of fractionated catecholamines and metanephrines, thus excluding the diagnosis of pheochromocytoma.

Results: There was a wide range of catecholamine concentrations in both the right (epinephrine 389 - 118326 pg/mL; norepinephrine 156 - 11193 pg/mL) and left (epinephrine 113 - 9327 pg/mL; norepinephrine 229 - 2216 pg/mL) adrenal veins. The right adrenal vein-to-left adrenal vein epinephrine gradient was as high as 83:1 (median, 2.1:1; $P < .02$);).



Although less striking, similar findings were also seen for norepinephrine (Figure 1).

Conclusions: This report provides a reference range for adrenal vein catecholamine concentrations in nonpheochromocytoma patients and illustrates the wide variation in epinephrine and norepinephrine concentrations. Epinephrine and norepinephrine concentrations are statistically significantly higher in the right versus the left adrenal vein; in the case of epinephrine, up to an 83-fold difference was found between the right and left adrenal veins. These data highlight why AVS should not be used in the investigation of adrenal pheochromocytoma.

Nothing to Disclose: EMF, AWS, GBT, CSG, DRF, MLR, WFY

P3-625

Safety and Efficacy of Outpatient Preoperative Alpha Blockade of Pheochromocytoma.

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Background:

There is currently no standard of care for the preoperative medical management (preopmedmx) of patients with pheochromocytomas (PHEO). In Europe patients are usually treated as inpatients for close monitoring during preoperative alpha blockade. In the US, with increasing pressure to minimize healthcare costs and length of hospital stay (LOS), patients are routinely managed preoperatively in the outpatient setting.

Methods:

We conducted a retrospective chart review of 65 patients who were managed by a single physician and in nearly all cases a single surgeon. Our objective was to evaluate the safety and efficacy of outpatient preopmedmx of PHEO. All patients received phenoxybenzamine, metyrosine, and a beta blocker (BB) as needed for tachycardia. Patients monitored blood pressure (BP) and heart rate (HR) at home and communicated readings regularly with physician.

Results:

43 female and 22 male patients were treated between 2003 and 2009. Mean age was 51 ± 12 years. This included 1 Asian, 51 white and 13 black patients with 51 adrenal and 14 extra-adrenal pheochromocytomas. Patients were prescribed phenoxybenzamine for 29 ± 16 days and metyrosine for 13 ± 2 days. 30/65 patients received a BB preoperatively. 56 patients were admitted on the day of surgery. 2 were admitted 1-2 days early due to pre-operative complications: 1 with transient, severe hypertension and 1 with symptomatic hypotension. Both were admitted for closer monitoring until surgery. 7 were admitted the night before for logistical reasons. Mean post-operative LOS was 4.3 ± 2.1 days. Results of hospital course BP analysis are depicted below. 21 of 65 patients received postoperative pressors, but only 3 of those 21 required more than 24 hours of postoperative pressor support. No patients experienced cardiovascular complications.

Mean BP Throughout Hospital Course

| | Mean Preoperative Blood Pressure (mm Hg) | | Mean Intraoperative Blood Pressures (mm Hg) | Mean Postoperative Blood Pressures (mm Hg) |
|------------|--|----------------|---|--|
| <i>SBP</i> | 132 ± 22 | <i>SBP Min</i> | 86 ± 12 | 99 ± 19 |
| | | <i>SBP Max</i> | 167 ± 26 | 135 ± 22 |
| <i>DBP</i> | 77 ± 12 | <i>DBP Min</i> | 50 ± 7 | 55 ± 11 |
| | | <i>DBP Max</i> | 97 ± 17 | 77 ± 12 |
| <i>HR</i> | 79 ± 13 | <i>HR Min</i> | 57 ± 11 | 79 ± 14 |
| | | <i>HR Max</i> | 97 ± 19 | 100 ± 17 |

Conclusion:

This study demonstrates that outpatient preopmedmx for PHEO is safe and effective. Only 2 patients were admitted early with preoperative complications, only 3 of 65 patients required pressors for more than 24 hours postoperatively, and the average post-operative LOS was only 4.3 ± 2.1 days.

Nothing to Disclose: KMR, DLF, DLC

P3-626

Survival Rates in Patients with Metastatic Pheochromocytomas and Paragangliomas.

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Background: Ten to 15% of sympathetic PHEO/PGLs are malignant, most of which present with distant metastases at diagnosis.

Methods: We reviewed 371 cases (269 PHEO and 102 PGL) treated at MDACC from 1953 through 2009. The aim of the study was to determine the impact of metastatic disease on survival in malignant PHEO/PGLs. In all cases diagnosis was confirmed pathologically. Tumor location and metastases 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. Kaplan-Meier curves were used to compare survival distributions across metastatic subgroups. A log-rank test was used to make statistical comparisons.

Results: The mean age of diagnosis was 43 years (9-79 years) with median follow-up of 8.2 years (0.-523 months). Thirty five percent (131/371) of patients had metastases: 25% PHEO (68/269) and 60% PGL (63/102) (chi-square test $p < 0.001$). Forty nine percent had metachronous and 51% had synchronous metastases. The metastatic sites were the skeleton (60%), lymph nodes (44%), liver (43%) and lungs (31%). Brain metastases were present in 2% of the patients. The median overall survival (MOS) for synchronous metastatic disease was 53 months vs. 191 months for metachronous disease (log-rank test $p < 0.001$). When comparing patients with vs. without liver metastases, the MOS was 55 months vs. 166 months, respectively (log-rank test $p = 0.008$). When comparing patients with vs. without lung metastases, the MOS was 60 months vs. 120 months respectively (log-rank test $p < 0.05$). When comparing patients with vs. without bone metastases the MOS was 81 months vs. 115 months respectively (log-rank test $p < 0.05$). When comparing patients with vs. without lymph node metastases the MOS was 79 vs 113 months respectively (log-rank $p = 0.721$).

Conclusions: The overall survival rate of metachronous PHEO/PGL metastatic disease is 3.6 fold greater than in synchronous metastatic disease. Metastases in the lungs and liver are associated with an early decrease in overall survival while skeletal metastases are associated with a late decrease of survival. Lymph node metastases are not associated with a decreased overall survival.

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

P3-627

The Prevalence and Associated Factors of Testicular Adrenal Rest Tumor in Patients with Congenital Adrenal Hyperplasia Caused by 21-Hydroxylase Deficiency.

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Purpose: Congenital adrenal hyperplasia (CAH) is a lifelong disorder, issues on quality of life, sexual function and reproductive capacity should be considered in early adulthood. Testicular adrenal rest tumor (TART) in these patients has been known as a common and important cause of the infertility. Therefore, in the present study we aim to describe the result of the testicular sonography and to analyze factors associated with TART in patients with CAH.

Patients and Methods: Testicular sonography was performed in 50 male patients (salt-wasting, 38; simple-virilizing, 12) with 21-hydroxylase deficiency, who were between 10.6 and 27.1 years old. The prepubertal patients were excluded. We analyzed the serum 17-hydroxyprogesterone (17-OHP) level from the time of sonography back to the past. The number of the serum 17-OHP level of over 10 ng/mL was divided by the total number examined during the follow-up period and it was defined as undertreatment percentage. The time of sonography was regarded as 0 year and mean serum 17-OHP concentration with an interval of 2 years was named as 0 to 2 year average concentration (0_2YAC), 2_4YAC and 4_6YAC in order.

Results: TART was detected by sonography in 33 (66%) out of 50 patients. The median of maximal cross-sectional area of TART was 0.71 cm² (range 0.01-9.41 cm²) and the median of volume was 0.170 cm³ (range 0.001- 16.558 cm³). Patients with TART showed lower corrected final height and higher 2_4YAC than without TART, whereas other factors showed no difference. The maximal cross-sectional area of TART was positively correlated with undertreatment percentage ($r=0.448$, $P=0.009$), 2_4YAC ($r=0.413$, $P=0.017$) and 4_6YAC ($r=0.538$, $P=0.001$).

Conclusion: TART was found in 66 percent of patients and the size of TART was related with the serum 17-OHP which is an indirect index of disease control. Especially the serum 17-OHP level between two and six years before the time of sonography showed positive association with the size of TART, therefore, strict disease control in childhood and adolescence is important and regular testicular sonographic screening is recommended. Furthermore, evaluation of the fertility is considered in adult CAH patients with TART.

Nothing to Disclose: MJK, SHL, JHK, HHL, YAL, CHS, SWY

P3-628

Prevention of Virilization in Pregnancies at Risk of Congenital Adrenal Hyperplasia with Dexamethasone: A Systematic Review and Metaanalysis.

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Background: Little is known about the potential side effects of treatment with dexamethasone in pregnancies at risk of virilization due to CAH.

Objective: To conduct a systematic review and meta-analysis of randomized and observational studies that evaluated the effects of dexamethasone administration during pregnancy.

Data Sources: We searched MEDLINE, EMBASE, and Cochrane CENTRAL through August 2008. Review of reference lists and contact with CAH experts further identified candidate studies.

Study Selection: Reviewers working independently and in duplicate determined trial eligibility. Eligible studies reported the effects on fetal and maternal outcomes of dexamethasone administered during pregnancy compared to a control group that did not receive any treatment.

Data Extraction: Reviewers working independently and in duplicate determined the methodological quality of studies and collected data on patient characteristics, interventions, and outcomes.

Results: Of 1083 candidate studies, 8 were eligible. The methodological quality of these studies was low. Dexamethasone treatment initiated early during pregnancy reduced the degree of fetus virilization measured by Prader score in comparison with previous non-treated pregnancies of the same families (weighted mean difference = -2.33, 95%CI= -3.38, -1.27). No deleterious effects of dexamethasone on stillbirths, spontaneous abortions or fetal malformations were found. There was increased edema and striae in the mothers treated with dexamethasone. Neuropsychological developmental outcomes in children exposed to dexamethasone were similar to those unexposed. Quality of evidence is low due to imprecision and methodological limitations of included studies.

Conclusions: Low quality evidence suggests that dexamethasone reduces fetus virilization, has no deleterious effects on pregnancy outcomes but is accompanied by excess of minor symptoms attributable to hypercortisolism in the mothers.

Nothing to Disclose: KM, MMF-B, HMM, GS, JFL, MBE, NOAE, KBE, NA, JFG-O, PJE, VMM

P3-629

Normal Cortisol Response to High Dose Synacthen and Insulin Tolerance Test in Children and Adults with Prader-Willi Syndrome.

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INTRODUCTION: Prader-Willi syndrome (PWS) is associated with hypogonadism and partial growth hormone insufficiency, which are thought to be partly explained by hypothalamic dysfunction. In addition partial insufficiency of the hypothalamic-pituitary-adrenal axis has recently been suggested, as an insufficient response to an overnight single-dose metyrapone test in 15 out of 25 (60%) randomly selected children with PWS was found.

OBJECTIVE: The aim of the present study was to further explore the above mentioned potentially dangerous condition in PWS by means of usual clinical tests for adrenal insufficiency.

METHODS: During a one-year period all PWS patients attending our out-patient clinic for rare diseases were consecutively enrolled. All were clinically and genetically verified as having PWS. Twenty-nine women and 22 men, median age 22 yr (range 0.5-44 yr) and median BMI 22.6 kg/m² (range 13.6-42.7 kg/m²), were examined with a standard high dose synacthen test (HST). Two women and 6 men, median age 26 yr (range 16-36 yr) and median BMI 30.1 kg/m² (range 22.7-53.0 kg/m²), were examined with a standard insulin tolerance test (ITT). Two out of these 8 persons were also tested using the HST. In case of admittance to our department with acute illness a spot cortisol was measured.

Four adults had diabetes (two Type 1). One child and 2 adults had hypothyreosis. Thirty-seven were treated with growth hormone and 16 were treated with sex steroids. None were treated with systemic hydrocortisone.

RESULTS: Median cortisol in the HST at t^{0min} was 179 nmol/L (range 58-1020 nmol/L), and at t^{30min} was 698 nmol/L (range 474-1578 nmol/L). Three had a cortisol level less than 550 nmol/L at t^{30min}, and one of them even less than 500 nmol/L. In the latter ITT showed a normal peak cortisol of 583 nmol/L.

Using the ITT the median cortisol at t^{0min} was 188 nmol/L (range 175-281 nmol/L), and the median peak cortisol was 668 nmol/L (range 502-822 nmol/L).

During the study period three children (age 0.42, 1 yr, and 4 yr) with febrile illness or asthma were admitted to the acute ward at our hospital. The spot cortisol were 1372, 775 and 1080 nmol/L, respectively.

CONCLUSION: In this cohort of 59 children and adults with PWS we were not able to confirm the finding that patients with PWS are at an increased risk of having central adrenal insufficiency. Clinically significant insufficiency of the hypothalamic-pituitary-adrenal axis in PWS is rare.

Disclosures: SF: Clinical Researcher, Novo Nordisk. RS-C: Clinical Researcher, Novo Nordisk. JSC: Investigator, Novo Nordisk; Speaker Bureau Member, Novo Nordisk; Advisory Group Member, Novo Nordisk.

Nothing to Disclose: JRO, CH

P3-630

Clinical Aspects and Adrenal Functions of Ten Japanese Patients with Childhood X-Linked Adrenoleukodystrophy.

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X-linked adrenoleukodystrophy (X-ALD) is a genetic disease associated with demyelination of central nervous system, adrenal insufficiency, and accumulation of very long chain fatty acids (VLCFAs). It is a clinically heterogeneous disorder ranging from severe neuropsychological dysfunction to no symptom. Addison's disease may be the first symptom of X-ALD. However, Addison's disease without neurological symptoms was not found in 286 ALD patients registered in a nationwide survey in Japan between 1990 and 1999 (1). The aim of the present study was to assess the clinical aspects and adrenal functions of Japanese ALD patients.

Patients and methods:The medical records of ten Japanese boys with ALD between 1990 and 2009 in our hospital were evaluated. The diagnosis of ALD was made based on clinical manifestation, accumulation of VLCFAs, and genetic analysis.

Results: The present ages were ranged from 5 to 13 years old. The ages at diagnosis were ranged from 2 to 11 years old. Neuropsychological abnormalities were first symptoms in seven patients: gait disturbance (2), visual impairment (3), convulsions (1), school failure, intellectual and behavioral changes (1). One boy was incidentally found by abnormality in brain MRI. Two brothers with no neurological abnormalities were found by genetic analysis, and the elder had moderate skin pigmentation and general fatigue. Their clinical classifications were divided into four types as childhood cerebral form (7), olivo-ponto-cerebellar form (1), Addison's disease only (1), and presymptomatic form (1). Adrenal function was rated as abnormal in five cases (50 %): baseline plasma ACTH level >100 pg/mL (146-1558 pg/mL) and also suboptimal cortisol response to ACTH stimulation test (7.2-18.1 microgr/dL). Hydrocortisone replacement therapy has been prescribed in seven patients: soon after the diagnosis (5), half a year after the diagnosis (1), since the stem cell transplantation (1). The dosage was gradually increased according to the laboratory date of serum morning cortisol, ACTH and urinary free cortisol excretion. The present daily amount was various on each case. Four patients received allogeneic stem cell transplantation (ages 2-8).

Conclusion: We experienced with a high prevalence of unrecognized adrenocortical insufficiency in ten Japanese ALD children. Early identification of impaired adrenal function and early detection of presymptomatic patients are important for hormone replacement therapy and better prognosis.

Takemoto Y et al., J Hum Genet 2002; 47: 590

Nothing to Disclose: YM, MT, YH, YH, YK, HY, NS, KO

P3-631

Clinical and Subclinical ACTH-Independent Macronodular Adrenal Hyperplasia (AIMAH) Affecting Members of a Large Brazilian Kindred.

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Background: AIMAH is a rare cause of Cushing's syndrome (CS) and in the majority of cases appears to be sporadic. The prevalence of familial forms of AIMAH is unknown and a systematic familial screening has not been conducted. **Objective:** To perform a systematic screening for CS in a large Brazilian family known to harbor two siblings with CS due to AIMAH, in order to delineate epidemiological, clinical, laboratorial and radiological features. **Methods:** After identifying the two index cases with AIMAH and CS, a total of 44 family members were initially selected for investigation after informed consent. Clinical and laboratory evaluation was performed in all individuals and the following systematic screening tests were used to assess the presence of CS: 1-mg vo dexamethasone suppression test (DST) (cortisol cutoff <1.8µg/dL) and midnight salivary cortisol (normal value ≤ 13µg/dL). To assure that DST was efficiently performed, plasma dexamethasone concentrations were also measured (expected value between 180-550 ng/dL). The abnormal tests results were repeated. Adrenal morphology was assessed using computed tomography (CT) scan in 27 individuals. **Results:** Most potentially affected individuals presented signs and symptoms consisted with subclinical CS. In all cases analyzed the plasma dexamethasone was in the expected levels. Eleven individuals presented abnormal DST and in 8 of them the midnight salivary cortisol levels were in the normal range. Adrenal CT-scan showed abnormalities morphological 7 members. Thus far, we could identify others 7 family members with classical features of AIMAH (5 females and 2 males ranged from 32 to 74 years) involving three different generations. **Conclusion:** The follow-up and systematic screening of this family involving several generations for AIMAH may allow a better understanding of clinical development and also to diagnose unsuspected cases of subclinical CS. DST test was more sensitive for detection of subclinical CS associated with AIMAH than midnight salivary cortisol. An asymmetric development of adrenal nodules observed in some individuals could have led to the erroneous diagnosis of unilateral pathology. In addition, these kindred AIMAH seems to have an autosomal dominant pattern of transmission as previously reported in other small series, future linkage analyses of this family might identify a germline genetic defect related to familial AIMAH.

(1)Lacroix A, Best Pract Res Clin Endocrinol Metab 2009; 23:245

(2)Masserini B et al., Eur J Endocrinol 2008; 160:87

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Nothing to Disclose: GAA, AML, IB, MSR, GCG, BBM, AL, MCBVF

P3-632

ACTH-Dependent Ectopic Cushing's Syndrome: The University of Texas M.D. Anderson Cancer Center Experience.

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Introduction: Ectopic ACTH secretion is associated with various benign and malignant tumors. It can lead to characteristic Cushing syndrome (CS) or go unrecognized in patients who have symptoms such as weight loss due to their primary malignancy.

Methods: A retrospective review of ACTH-dependent ectopic CS was undertaken at a comprehensive cancer center from 1952-2009. Clinical features and outcomes were summarized.

Results: 300 patients with CS were identified through an institutional tumor registry of whom 39 had ACTH-dependent ectopic CS defined as: CS with tumors known to produce ACTH, CS with positive ACTH staining in non-pituitary tumors, CS with lack of gradient on inferior petrosal sinus sampling (IPSS). Median age at diagnosis was 63 years (range 24-74) with 18 men and 21 women. Median body mass index at diagnosis was 37.4 (range 17.4-54.9) with 14 patients reporting weight gain and 10 patients having weight loss at initial presentation. New onset/worsening hypertension was documented in 31 patients, new onset/worsening hyperglycemia in 24 patients, and hypokalemia in 11 patients. IPSS was done in 5 patients and suggested an ectopic ACTH source. Octreotide scan identified the source of ACTH in 12/18 patients. The source of ACTH was found at the time of diagnosis in 25 patients and delayed in 14 patients. Tumors associated with this syndrome included, 9 bronchial carcinoids, 8 small cell lung carcinomas, 6 medullary thyroid carcinomas, 4 pancreatic neuroendocrine tumors, 2 prostatic neuroendocrine tumors, 1 thymic carcinoid, 1 small bowel carcinoid, 1 ovarian endometrioid adenocarcinoma, and 7 with unknown source of ACTH. As definitive therapy, 9 patients underwent bilateral adrenalectomy and 18 had their primary tumor removed. Venous thromboembolism was documented in 3 patients. At last follow-up, 15 patients were alive while 24 were dead from variable causes, mainly attributed to their underlying malignancy.

Conclusions: ACTH-dependent ectopic CS accompanies a wide range of benign and malignant tumors. Patients may not have obvious CS as their clinical presentation may be masked by symptoms of the underlying tumor. The diagnosis can be challenging, with the source of ACTH production difficult to identify. The true prevalence of this syndrome is likely underestimated in this retrospective review. We propose conducting a prospective study to estimate the true prevalence of this syndrome and its clinical implications in these patients.

Nothing to Disclose: SE, SGW, RVS, NLB, CJ, AKY, MIH, MEC, RFG, MAH

P3-633

Cardiovascular Risk Factors in Patients with Cushing Syndrome: An Evaluation of the Endothelial Stressor Biomarkers.

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It has been reported that Patients with Cushing syndrome have increased cardiovascular risk even those already cured. Moreover the cardiovascular risk after long term cured has been related with a state of low-grade inflammation due to the presence of central fat mass. The aim was to evaluate different endothelial stressor biomarkers in these patients before and after being cured compared with healthy population. There were selected 20 hypercortisolic women with Cushing syndrome, 20 normocortisolic women with cured Cushing syndrome, of which 12 were from the previous treatment group and 8 from the cured Cushing's syndrome group who were controlled in our consults, patients with hydrocortisone substitutive treatment were excluded. Twenty healthy control women were matched by age, BMI, blood pressure, glycemia, hiperlipidemia (LDL-c) and pharmacological treatment (metformin and drugs to control blood pressure) with normocortisolic women with cured Cushing's syndrome. Anthropometric and metabolic laboratory parameters were assessed. Soluble parameters to measure oxidative/nitrosative status (SOD, GPx, GRx, GST, CAT, TAS, Vit E, Prot Carb, MDA, LDLox, TPerox, NO, ET-1) and endothelial activation/inflammation (sCD40L, VCAM-1, SP-Selectin, PCR-US, IL6, TNF- α , Adiponectin) were evaluated. Data were expressed as mean and SD or as median range and as percentages respectively. Comparisons between groups were performed using a non parametric test (Mann-Whitney's U test). Tests were two-tailed and a $P < 0.05$ was considered significant.

The results show significant differences in BMI and systolic and diastolic blood pressure, between active Cushing and both, cured and healthy controls. A significant difference in biochemical metabolic parameters between active and cured Cushing and healthy controls was found in HOMA and LDL-c; $p < 0.05$. No differences were found between cured Cushing and healthy control except for LDL-c (159 ± 65 vs 116 ± 25 mg/dL); $P < 0.05$. There were significant differences for some parameters of oxidative/nitrosative status and endothelial activation/inflammation between untreated Cushing and healthy controls, untreated and cured and finally between cured and healthy controls.

The most relevant result shows not only differences between the three groups but also shows the persistence of higher inflammatory parameters between the cured Cushing's patients and healthy controls. These results agree with authors that support a persistent and increased cardiovascular risk in cured Cushing's patients.

Nothing to Disclose: AL-C, AC-P, AL-J, AS-M, AM-A, EV-M, RIF, IC, LJ-J

P3-634

Post-Surgical Recovery in Patients with Cushing's Syndrome: Results of an Open-Ended Survey.

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Despite biochemical normalization, health-related quality of life (HRQL), as judged by the SF-36 survey, remains impaired after successful surgical treatment of Cushing's syndrome (CS). To understand HRQL during recovery better, members of the Cushing's Support and Research Foundation were invited to complete an open-ended survey about their experiences as patients after surgical treatment of CS. Of 94 respondents (79 females, 12 males and 3 of unknown gender), 69% (n=65) had a pituitary and 31% (n=29) had an adrenal etiology of CS; responses of each group were similar and are reported together. Most (73%, n=69) were no longer taking glucocorticoid replacement therapy (GRT). Among those providing information about GRT discontinuation (n=53), 81% (n=43) underwent a gradual GRT tapering process, 11% (n=6) never took GRT post-operatively and 8% (n=4) took GRT but then stopped abruptly. Patients describing their post-surgical care plan (n=43) expressed that tapering was dictated by their physician (n=17), self-initiated (n=14), or mutually decided (n=12). The median time of GRT use among patients off steroids was 11 months (range: 0-108 months), but the self-reported time to recovery was longer (median 20 months, range 0-108 months). Most patients (84%, n=79) characterized their overall recovery experience as negative but 13% (n=12) had positive experiences (3 did not respond). Although the survey did not ask about symptoms, 35% (n=33) of patients complained of lethargy, 33% (n=31) of joint pains and 33% (n=31) of some form of mental illness including depression during the recovery process. Seventeen percent (n=16) stated that they were not given sufficient information about the recovery experience. When asked about coping mechanisms used after surgery, patients cited reported family or friends (36%, n=34), support groups specifically for those affected by CS (25%, n=23), physicians (17%, n=16), analgesia (20%, n=19), exercise or physical therapy (21%, n=20), returning to their normal daily activities (16%, n=15), and resting (16%, n=15). In summary, from the patient's perspective, the recovery period following surgery for CS may be long and challenging. We speculate that improved physician understanding of this subjective experience, and additional patient and family education might improve patient satisfaction and self-perceived HRQL during recovery from Cushing's syndrome.

Sources of Research Support: In part by the NIH intramural program of NICHD.

Nothing to Disclose: BSA, NMN, KC, LKN

P3-635

Late-Night Salivary Cortisol Measurements with an Automated Immunoassay System in the Diagnosis of Cushing's Syndrome.

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Introduction: Measurement of late-night salivary cortisol (LNSC) is increasingly used as a screening test in suspected Cushing's syndrome (CS). Nevertheless, this test has not been routinely used in our hospital so far. An automated cortisol immunoassay has specified saliva as a suitable sample material (Roche Cobas Cortisol).

Aims: The aim of the study was to establish LNSC reference-range data for this test and to affirm its usefulness in the diagnosis of CS.

Subjects and methods: 72 healthy volunteers participated in the study (24 male/48 female; 18-67 years). Saliva samples were obtained at midnight in 3 different days with cotton salivette® (Sarstedt) and the mean of the values was determined for each one. In 67 patients (20 male/45 female; 22-77 years) evaluated for the diagnosis or follow-up of CS, at least 2 measurements of LNSC were done being at least one saliva sample collected in the same day of other diagnostic test. CS was considered present if clinical features and at least 3 different tests were positive (urinary free cortisol, 1-mg overnight dexamethasone suppression test, longer low-dose DST, or midnight serum cortisol test) or if the patient had CS confirmed by immunohistological findings. CS was excluded in the patient group if there were 2 different tests negative. CS was thereby diagnosed in 20 (CS group) and excluded in 47 patients.

Results: The mean±sd, 5th and 95th percentiles of LNSC were 0.15±0.08, 0.04, 0.30 ug/dL for the control (n=72), 0.72±0.58, 0.2, 1.85 ug/dL for the CS group (n=20) and 0.12±0.09, 0.02, 0.29 ug/dL for patients without CS (n=47). Patients with proven Cushing's syndrome had significantly higher mean LNSC than controls and patients in which CS was excluded (p<0.001). The cut-off used for the LNSC was 0.32ug/dL (mean+2sd, control group). All the patients with CS had at least one value of salivary cortisol >0.32ug/dL. Only 1 patient with CS had a mean LNSC below 0.32 ug/dL. Only 2 of the 47 patients in whom CS was excluded had a mean LNSC >0.32ug/dL. Considering having a mean LNSC >0.32 ug/dl as a positive test, we found a sensitivity of 95%, a specificity of 96%, a negative predictive value of 98% and a positive predictive value of 90%.

Conclusions: These preliminary data favour the use of Late-Night Salivary Cortisol with this automated immunoassay which can represent an interesting alternative to laborious radioimmunoassay systems for the diagnosis of CS.

Nothing to Disclose: AO, DC, EV, MA, JTG, JLM

P3-636

Subclinical Cushing's Syndrome in Outpatients Attending a University Hospital.

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Preclinical Cushing's syndrome is defined as endogenous cortisol excess in the absence of a cushingoid appearance. We screened 59 adult patients with a) diabetes mellitus (DM) with poor glycemc control (DM type1 n=7, 2 men, 5 women, aged 29-52 y.o. ; DM type 2 n=23 , 11 men and 12 women, 52-64 y.o); b) incidental adrenal masses (n=12; 3 men and 9 women, 38.0-68.0 y.o.); c) high blood pressure and central obesity (n=10, 4 men and 6 women ,18-69 y.o), d)hirsutism (n=5, 24-63 y.o) and e) kidney stones(n=2, 1 man and 1 woman, 20 and 30 y.o).None of them had clinical appearance of Cushing's Syndrome (CS) and were free of drugs interfering the hypothalamic- pituitary -adrenal function and/or dexamethasone metabolism. All subjects collected two 24 hour urine specimens for total urinary cortisol (UFC) and creatinine measurements. Salivary samples were obtained at 8 h and 23 h in two non-consecutive days for salivary cortisol assessment (SAF₈ and SAF₂₃, respectively). In all non diabetic patients morning salivary (SAF_{dex})and serum cortisol (F_{dex}) was determined after overnight oral 1 mg dexamethasone suppression test. Salivary ,serum and urinary cortisol were assayed by RIA (1,2).

Reference values obtained from 121 healthy volunteers and 21 confirmed CS were UFC < 248 nM/day; SAF < 18 nM; SAF₂₃< 3.8 nM; SAF_{dex} ≤ 2.0 nM; F_{dex} ≤ 50nM.

Results: Cortisol excess was detected in 3 women. Data are displayed in Table 1.

Table 1

| Patient# | Age | UFC (nM/day) | SAF8 (nM) | SAF23 (nM) | SAF dex (nM) | Fdex(nM) |
|----------|-----|---------------|-----------|------------|--------------|----------|
| 1 | 29 | 363.0; 240.0 | 6.5;6.0 | 5.0;4.5 | 7.0 | 414.0 |
| 2 | 34 | 2004.0; 650.0 | 14.0;15.0 | 13.0;12.0 | 6.0 | 275.0 |
| 3 | 49 | 200.0; 190.0 | 6.5;7.0 | 1.5;0.8 | 2.0 | 63.0 |

≠1 and≠2:high blood pressure and central obesity;≠3: incidental adrenal mass.

ACTH values were 20 pg/ml (# 1) and 27 pg/ml (# 2) . Petrosal sinus sampling confirmed the central source of ACTH in both . Transphenoidal pituitary exploration showed the presence of a microadenoma in #1 and hyperplasia in #2. They became hypocortisolemic after surgery. ACTH was less than 10 pg/ml in patient#3. After right adrenalectomy histology described a cortical adrenal adenoma.

This study stresses the importance of searching cortisol excess in outpatients with non-specific symptoms of CS. In our experience the initial evaluation with more than one first line screening test improved the diagnostic performance.

Assessment of corticoadrenal reserve through salivary steroids. CardosoE, Persi G,Arregger AL,Contreras L.N.The Endocrinologist 2002,13:459-464.

Diagnostic value of salivary cortisol in Cushing's syndrome.Cardoso EML, Arregger AL, Tumilasci O, Contreras LN. Clin Endocrinol(Oxf)2009,70.516-521

Nothing to Disclose: ALA, EMC, AE, EGMT, LNC

P3-637

Spectroscopy on 3Tesla-MRI in Patients with Cushing's Syndrome.

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Introduction: Patients with Cushing's syndrome (CS) present a variety of brain alterations.

Aims: To study spectroscopy on 3TMRI in patients with CS, as compared to controls (C), and long-standing major depressive disorder (MDD), as known to present hypercortisolism.

Material and methods: 13 patients with CS (3 active), 14 controls and 19 MDD patients were evaluated. MRI was obtained using a 3T Philips Achieva facility (software version 2.1.3.2), the SENSE 8-channel head-coil, and a specifically designed acquisition protocol (3D-MPRAGE whole brain sequence, Turbo Field Echo, TR=6.7, TE=3.1, Voxel size=1x1x1.2) on which 1H-Magnetic Resonance Spectroscopy (MRS) was obtained from the ventromedial region and bilateral medial temporal regions. Raw data were exported and postprocessed using the LC Model, an external reference method that provides concentrations (mmol) of the most important brain metabolites: total N-Acetyl Aspartate, Creatine, Glutamate, Glutamine and Choline.

Results: Choline levels were significantly lower in CS compared MDD (P=0.002) and controls (P=0.007) in the ventromedial prefrontal cortex, but not different between controls and CS. Additionally, Choline levels were lower in the right medial temporal regions of CS patients compared to MDD (P=0.006). Glutamate levels were higher in the RMT regions of CS patients compared to MDD (P=0.002) and controls (P=0.001) and higher in the left medial temporal (LMT) regions of CS patients compared to MDD (P=0.001). Glutamate levels were not different in CS and controls at the RMT and LMT.

Conclusions: CS patients show a distinct pattern of spectroscopic alterations in the ventromedial prefrontal and medial temporal cortex, as observed on MRI analyses, from controls and major depressive disorder. These findings could influence the cognitive performance of CS patients.

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Disclosures: SW: Speaker, Novartis Pharmaceuticals; Study Investigator, Novartis Pharmaceuticals.

Nothing to Disclose: ER, JY, AS, EG, MP, VP, OL-M, YV, BGA

P3-638

Modern Treatment of Cushing's Shows a High Cure Rate and a Low Mortality.

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Introduction: Cushing's syndrome (CS) is a rare but severe disease, which is potentially fatal if untreated.

Aim: To assess the cure rates and the mortality in a large series of patients with (CS) during a long-term follow-up period.

Patients and Methods: Subjects diagnosed with CS who presented/followed-up in our Department between 01/1967-06/2009 were studied.

Results: 217 patients were identified (163 females/54 males). The median age at diagnosis was 40 years (range 10-81) (females: 40 (13-81)-males: 40.5 (10-76)). 182 (83.8%) subjects had pituitary disease, 12 (5.5%) ectopic CS and 23 (10.6%) adrenal CS (8 of them with adrenal cancer). The mean follow up was 10.9 years (0-45.8). At last assessment, 68.6% of the patients were cured (undetectable post-operative serum cortisol after pituitary surgery or adrenalectomy or ACTH deficiency after radiotherapy) and 31.4% had active syndrome. A total of 33 deaths were found; 25 of these subjects had disease (25/182, 13.7%), 4 had ectopic syndrome (4/12, 33.3%) and 4 (3 with cancer) had adrenal CS (4/23, 17.4%). The 10 years survival rates were 89% for the whole group, 86% for those with disease, 78% for those with ectopic and 96% for those with adrenal origin. There was no significant difference in the survival rates between the three aetiologies of the CS.

Conclusions: In this one of the largest series of patients with CS managed in a tertiary referral centre, we showed that with modern treatment nearly 70% of the patients will be finally cured and nearly 90% of them will survive at 10 years follow-up.

Nothing to Disclose: GN, TS, JK, NK, JAHW

P3-639

Laparoscopic Adrenalectomy for Bilateral Adrenal Tumors.

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Bilateral adrenal lesions (BAL) are associated with ACTH-dependent and ACTH-independent hypercortisolemia, pheochromocytoma as a part of multiple endocrine neoplasia syndrome and metastases to the adrenal. The aim of the study is to present the incidence of this problem and proceedings.

Material and methods:

From 29.10.1997 to 31.12.2009 in the Department of General, Vascular and Transplant Surgery WUM 529 patients underwent 547 laparoscopic adrenalectomies (LA). Imaging studies revealed bilateral adrenal tumors in 80 (15,1%) patients. Among them 71 (13,4%) bilateral lesions were diagnosed at the time of diagnosis and were caused by Cushing disease - 4/71 (5,6%), Cushing syndrome - 14/71 (19,7%), PreaCushing syndrome - 14/71 (19,7%), pheochromocytoma - 7 (9,9%), Conn syndrome - 5 (7,1), incidentaloma - 27(38%). In 9 (1,7%) cases the final diagnosis of bilateral tumors was made after unilateral adrenalectomy in the past, among them were Cushing syndrome - 2/9 (22,2%), PreaCushing syndrome - 1/9 (11,1%), pheochromocytoma - 6/9 (66,7%).

Results:

Patients underwent 9 (11,2%) simultaneous bilateral laparoscopic adrenalectomies (SBAL), 2 (2,5%) simultaneous bilateral LA with sparing adrenalectomy on one side, 15 (18,7%) two-stage bilateral (7 classical and laparoscopic, 1 retroperitoneal and laparoscopic and 7 only laparoscopic) adrenalectomies with interval time (7 days to 20 years). Unilateral LA were made in 64 (80%) patients with the observation of the contralateral lesion. In these patients none of clinical symptoms recurred. Conversion was necessary in 1 (1,2%) case. For simultaneous LA were qualified pheochromocytoma and exceptionally incidentaloma suspected for malignancy. In the remaining two-staged or unilateral LA with the observation of clinical symptoms and fenotype changes in imaging studies of the contralateral lesion were preferred. In case of two-staged LA the priority had lesion regarding tumor size, growing rate and density. The mean operating time of the SBAL was 304 minutes.

Conclusions:

Accepted tactic of laparoscopic treatment of patients with bilateral lesions confirmed its safety and efficiency. Operative tactic depends on the etiology of adrenal lesions and surgical team experience.

Nothing to Disclose: MO, JD, MJ, AJ, MP, JS

P3-640

The Repercussions of Cortisol Normalization on Cognitive Functions in Patients after Successful Cure of Cushing's Syndrome.

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Introduction: Cushing's syndrome (CS) has been associated with moderate to severe deficits in mnemonic functions. These patients have difficulty consolidating and recovering previously learned material (functions which depend on the hippocampus and adjacent areas) (Starkman et al., 1992). CS has also been linked to impairments in working memory (WM) (Leon-Carrion et al., 2009), a function which is dependent on extrahippocampal regions (prefrontal cortex). Studies on patients with CS after normalization of hypercortisolism suggest irreversible mnemonic deficits. However, given that no studies have been done on changes in memory impairments dependent on extrahippocampal structures, our objective is to evaluate this function in patients after successful cure of CS (normalization).

Patients and Methods: Our sample included 12 females with CS (mean age 33,75; range = 53-15) (9 non-invasive macroadenomas, 2 adrenal adenomas, and 1 ectopic adenoma). Patients were first assessed with hypercortisolism, and then again after normalization (months between assessments = 12.42; range = 17 - 6). Working memory was assessed using the revised Luria's memory curve (BNS: www.neurobirds.com).

Results: The data did not show changes in any of the test's indexes: Mnestic volume ($P = 0.7$); mnemonic contamination ($P = 0.27$); self-awareness ($P = 0.34$); mnemonic consolidation ($P = 0.38$); mnemonic loss of information ($P = 0.22$).

Conclusions: Our data suggests that certain deficits in WM found during the acute phase of the disease persist after normalization. Our results also show that the reported neurotoxic effects of prolonged exposure to pathological levels of cortisol on the hippocampus are similar to those found in extrahippocampal areas. Further studies including a larger patient sample and neuroimaging are needed to corroborate these results.

Nothing to Disclose: AM-A, JFM-R, MAM-C, AS-M, EV-M, AL-J, JL-C, AL-C

P3-641

Evaluation of Adrenal Masses: Analysis of 137 Consecutive Patients.

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Introduction: Adrenal masses is an issue of clinical importance and always involves extensive evaluation. The incidence of adrenal masses has increased because of increased imaging and is now seen in about 10% of patients more than 50 years of age. Differential diagnoses can include 43 kinds of diseases (including hyperplasia, benign and malignant lesions). Diagnostic work up should involve measurements of hormones and imaging studies. Masses greater than 4 cm should undergo surgery because they can produce hormones or become malignant.

Results The results from this study are derived from a consecutive series of 137 cases presenting with an adrenal mass over the last 5 years. There were 97 females and 40 males, median age was 68,2 ± 9,8 . We performed a complete analysis of adrenal steroids ,cortisol, aldosterone, DHEA,DHEAS, renin, ACTH, 17hydroxyprogesterone, , catecholamines, methanephrines, suppression of cortisol after dexamethasone 1mg and stimulation after synthetic ACTH (250mcg/IV). Imaging studies included MRI in all patients and the size and volume of lesions were detected. We excluded patients who were taking corticosteroids and tamoxifen which can interfere with steroids measurements, masses more than 4 cm and biopsies were performed in those with a high suspicion of malignancy.

Table 1 Results of evaluation

| | |
|--------------------------------|----|
| Adrenal carcinoma | 2 |
| Metastases | 2 |
| Congenital adrenal Hyperplasia | 2 |
| Primary lymphoma | 3 |
| Aldosteronism | 7 |
| Liposarcoma | 1 |
| Pheochromocytoma | 6 |
| Hyperplasia | 25 |
| Myelolipoma | 5 |
| Addison disease | 4 |
| Cystic lesion | 2 |
| Cushing adrenal | 3 |
| Adenoma non funct | 53 |
| Histoplasmosis | 1 |
| Cushing disease | 3 |
| Excluded | 9 |

Discussion: The evaluation of adrenal masses should be extensive and involve the measurements of many steroids, hormones and imaging tests. Older people with bilateral lesions and adrenal insufficiency should undergo biopsy because of the higher risk of malignancy. MRI imaging can help to detect Pheochromocytoma and can distinguish adenomas from carcinomas better than computerised tomography. Before performing surgery for large masses or cystics lesions it is important to exclude masses with overproduction of hormones.

Nothing to Disclose: MKH, LH, CEK

P3-642

Adrenal Cortical Carcinoma: A Portuguese Retrospective Multicentric Study.

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The adrenal cortical carcinoma is a rare endocrine malignancy with a poor overall survival. The diagnosis is usually suspected at an advanced stage after imaging studies, although patients may present with hormone excess or a local mass effect. The diagnosis confirmation and the prognosis evaluation are based on the histopathology results. The evaluation of the therapeutic efficacy is limited in each center by the reduced number of patients and their rapid evolution. **Aims:** To evaluate the multicentric portuguese experience on the diagnosis and treatment of these patients. **Patients and Methods:** A retrospective study of the clinical files in different Endocrinology Departments was performed and the clinical expression, treatment and evolution were analyzed. **Results:** Nine centers participated, with a total of 57 cases (70% female), diagnosed from 1989 to 2009. At diagnosis: mean age 49.3±15.2 years; the more frequent clinical presentations were hypercortisolism (32%), pain (32%) and incidentaloma (23%). According to McFarlane classification patients were staged as: I - 2; II- 27; III- 7; IV - 21. Only 17 (29.8%) patients were diagnosed before surgery, being the only ones with an endocrinological evaluation. Surgery was done in 53 patients, with a surgical description of total tumoral removal in 83%. The registered tumoral weight ranged between 43g to 2100g (median 285g). The pathological characteristics descriptions were scarce. In 24 patients (mean age 42.6±16 years), mostly on stage II (54%) and stage IV (25%), mitotane was prescribed, beginning with 1 to 7 g daily (2.6±1.8). Maximum dose was 1 to 12.5 g daily (5.6±3.0). Mitotane was done in 11 patients. The mean duration of treatment was 16.5±15 months (1-66) with a median survival of 14.5 months (2 to 141). In the other 33 patients (aged 53.1±15 years, in stage IV-45% and stage II-43%) mitotane treatment was not prescribed. Twenty nine of those were operated (only 9 with a previous diagnosis) with a median survival of 21 months (0 to 212). **Conclusions:** In a large number of cases the diagnosis was made after surgery, without a previous endocrine evaluation. Nevertheless, rising referral is being verified. We could observe a large survival period in some patients, wich is an uncommon feature. We conclude that a more frequent and exhaustive application of the histopathological and immunohistochemical internationally established criteria are needed to properly evaluate, classify and treat these patients.

Nothing to Disclose: IP, AV, JC, AM, PM, MM, FL, HS, IS, MP

P3-643

Epidemiological Evaluation of 152 Patients from 28 Years of Experience in Cushing's Syndrome Management Contributing to the Paradigm of Its Etiology.

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Background: Cushing's syndrome is a complex endocrine condition with potential serious complications if misdiagnosed or untreated. The etiological diagnosis of Cushing's syndrome still challenges the specialists as the most perplexing and controversial problem in endocrinology. **Objectives:** Evaluate and describe epidemiological, clinical and laboratorial findings of Cushing's syndrome patients from an important health care hospital in Brazil. **Methods:** In order to scientific analyze 152 patients attended in our service from 1982 to 2010, they were allocated into two different groups based on their diagnoses: ACTH-dependent (AD) and ACTH-independent (AI) Cushing's Syndrome. The first group included patients with Cushing's disease (n=108), ectopic ACTH (n=5) and indetermined ACTH-dependent (n=4). By the other hand, the second group was formed by patients diagnosed with adrenal carcinoma (n=16), adrenal adenoma (n=17) and pigmented nodular adrenal hyperplasia (n=2). They were analyzed based on sex, age at diagnosis, comorbidities, ACTH level and urinary free cortisol/24h (UFC) volume. **Results:** The AD group consisted in 117 patients and the AI group included 35 patients. There was 74,4% (n=87) women in the first and 77,1% (n=27) in the second (p = 0,439). Stratifying the patients by groups of age at the moment of diagnosis, AD contained 12% (n=14) of patients under 20 years old and AI had 20% (n=7). Between the ages of 20 and 40 years, we found 52,1% (n=61) and 40% (n=14). Patients over 40 years old were 35,9% (n=42) and 40% (n=14). There was no significant difference between these groups (p = 0,335). The analyses of comorbidities such as hypertension, abnormal glucose, psychiatric disorders and heart diseases showed no statistical significance between the groups. The median of ACTH level were 60 pg/mL (39,1 - 87) in the AD group and 10 pg/ml (9 - 10) in the AI group (p < 0001). Furthermore, the medians of UFC levels were almost equal [355 µg/dL (187,5 - 631,73); 354,53 µg/dL (158 - 1033,75); p = 0,732]. **Conclusion:** On the basis of etiological diagnosis, the significant values of ACTH levels found in the two analyzed groups are explained by the physiology process of Cushing's syndrome. Our database research showed no statistical difference between the other variables evaluated, reinforcing that on CS diagnosis we must use a serial of laboratorial tests and imaging for solve the paradigm of its etiology.

Nothing to Disclose: FC, VB, PBDL, MAC, GR

P3-644

Current Practice and Outcomes in Glucocorticoid Replacement Therapy - A Worldwide Patient Survey.

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Recent data suggest that outcomes in patients with adrenal insufficiency (AI) can be suboptimal under current therapeutic practice for glucocorticoid replacement. Quality of Life (QoL) has been shown to be compromised, bone mineral density reduced, cardiovascular morbidity and overall mortality rate increased.

AIM: To survey patients with AI on current practice in glucocorticoid replacement therapy, their self-perceived health status and outcomes.

METHOD: Patients were recruited via patient organizations (e-mail contact lists and newsletters) to respond anonymously to a web-based survey. Unique survey entries were set up for each patient organisation thus enabling geographical localisation of the entries. The survey was open from September 12th to December 19th 2008.

RESULTS: Responders were 1245 patients with adrenal insufficiency. 84% with primary AI, 11% with secondary AI and 5% unsure. 64% were from the USA, 20% from Europe and 15 % from the ROW. Hydrocortisone was used by 75%, Prednisone/Prednisolone by 11%, Cortisone Acetate by 6% and Dexametasone by 4% of respondents. Dosing regimens were 10% on OD, 42% on BID, 32% on TID and 17% on other. Multiple dosing is experienced as a problem by 38%. A total of 64% report reduced and compromised QoL necessitating changes to physical activity, social life, work life and family life. Moreover, 40% report being absent from work in the three months preceding their participation in the survey and 29% of those reported more than three weeks' absence. Work or school absenteeism was more marked among those with 2° AI. Irrespective of diagnosis, 76% were concerned about long term side effects of their replacement therapy such as osteoporosis, obesity, fatigue and cardiovascular morbidity, in that order.

CONCLUSION: Glucocorticoid replacement therapy among AI patients participating in the survey consisted primarily of hydrocortisone administered two or three times daily. A large majority of subjects reported that their disease and its current treatment have an impact on QoL leading to alterations in physical activity, social life, work life and family life. Three quarters reported concerns about long-term side-effects from their treatment. These data demonstrate - from the patients' perspective - a need for improvement in glucocorticoid replacement therapy.

Sources of Research Support: DuoCort Pharma AB.

Nothing to Disclose: MF, GB, SS, GJ

P3-645

The Use of Opioids To Control Pain in the Peri-Operative Period Does Not Seriously Impair Hypothalamic Pituitary Adrenal (HPA) Function.

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Background: Multiple studies have demonstrated that chronic opiate use impairs HPA function. While acute administration of opiates was reported to decrease ACTH and cortisol levels, studies examining their short-term use are limited. As opiates are commonly used to control pain in the peri-op period, examining their impact on HPA function is clinically important. **Methods:** We examined HPA function in patients with NL HPA axis undergoing transsphenoidal pituitary adenomectomy. We followed an established protocol that stipulates that patients with normal HPA axis are not given glucocorticoids unless clinical or biochemical evidence for impaired HPA function are evident. Patients who had diseases or were taking medications that can influence HPA function were excluded. Plasma/serum levels of ACTH and cortisol were measured repeatedly during the first 48 postop hrs in the 56 patients (27F, 29M) included in the study. Normal HPA function for that period was defined as multiple serum cortisol levels of > 15ug/dL. As different opiates were used, the opioid intake was converted to morphine-equivalent doses. **Results:** During surgery, 90% of patients received opiates (mean dose:52±30 mg). In the first 5 postop hrs, 79% of patients received opiates (26±16mg)and nearly 50% of patients required opiates during the ensuing 24 hours. As shown below, mean serum cortisol levels increased appropriately in the peri-op period and the levels were <15 ug/dL in only 4/56 during the first 5 postop hrs, and all were receiving narcotics.

Hours After Adenomectomy

| | 0-5 Hours | 6-10 Hours | 11-20 Hours | 21-28 Hours |
|--|-----------|------------|-------------|-------------|
| Mean serum cortisol (ug/dL) for the group | 30±9.1 | 38.8±15.1 | 33.9±15.1 | 19.8±9.5 |
| No. of patients/ total with serum cortisol < 15 ug/dL | 4/56 | 2/56 | 3/56 | 14/56 |
| Mean morphine-equivalent dose in patients with a cortisol < 15 ug/dL | 55.6±32.2 | 0 | 18±9.1 | 18±7.1 |

However, by the 28th postop hr, 14/56 patients had serum cortisol levels of < 15 ug/dL and all were receiving narcotics. Even though serum cortisol levels decreased briefly in some patients to < 5 ug/dL, there were no associated clinical manifestations and all were discharged without ever receiving glucocorticoids. Opiate use did not influence plasma ACTH levels. **Conclusions:** The data indicate that the stress of surgery and the postop period can overcome the potential suppressive effects of opioids on the HPA axis and are reminiscent of the poor suppressibility of the axis with dexamethasone in this setting (1).

Arafah BM J Clin endocrinol metab 2006; 91:3725.

Nothing to Disclose: KN, RA-A, BMA

P3-646

Improved Quality of Life in Patients with Primary Adrenal Insufficiency by Using a Novel Once-Daily Dual Release Hydrocortisone Tablet: A Randomised Controlled, Cross-Over Trial.

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Patients with adrenal insufficiency (AI) receiving current available glucocorticoid replacement therapy have compromised outcome with increased mortality rate from cardiovascular disease and impaired quality of life (QoL) (1, 2). Novel biopharmaceutical techniques have allowed the development of a dual release tablet that can more closely mimic the physiological diurnal serum cortisol time profile using a once-daily administration (3).

AIM: The aim was to compare QoL during treatment with a once daily dual release oral hydrocortisone tablet (OD) compared with thrice daily (TID) administration of a standard hydrocortisone tablet.

PATIENTS: 64 patients with primary AI on stable hydrocortisone replacement doses were included. 63 were eligible for an intention-to-treat analysis (37 men) with a mean age of 47.3 (SD 13.7) years and BMI of 26.2 (4.0) kg/m².

METHODS: This was a randomised, two-period 12-week cross-over multi-centre trial comparing OD with TID using the same daily dose of hydrocortisone. Four questionnaires for QoL; SF(Short Form)-36, PGWB (Psychological General Well-Being), FIS (Fatigue Impact Scale) and a diurnal VAS scale were used at baseline, 4 weeks and at 12 weeks.

RESULTS: Using the same daily dose of hydrocortisone, OD had an increased cortisol exposure during the first 4 hours after intake in the morning and reduced exposure in the late afternoon as compared with TID. After 4 weeks there was a trend for reduced QoL during OD as compared with TID. After 12 weeks, however, OD was associated with a perceived improvement in cognitive function ($p < 0.05$), psychosocial function ($p < 0.05$) and a trend for improvement in the total FIS score ($p = 0.08$) as compared with TID. Well-being improved ($p < 0.05$) and total PGWB score demonstrated a trend for improvement ($p = 0.06$) on OD as compared with TID. Patients reported less afternoon moodiness on OD ($p < 0.05$). SF-36 detected no statistical significant differences between the two treatment regimens.

CONCLUSION: The new hydrocortisone regimen and its produced serum cortisol profile was safe, well accepted and tolerated, and accompanied by improved QoL and well-being. The open trial design is not optimal for assessing treatment effects on QoL. However, as a placebo effect is most likely to occur early in a trial the observed biphasic mode of action indicates a true improvement on QoL induced by a more physiological cortisol exposure profile.

(1) Hahner S et al. J Clin Endocrinol Metab 2007;92:3912-22.

(2) Lovas K et al. Clin Endocrinol (Oxf) 2002;56:581-8.

(3) Johannsson G et al. Eur J Endocrinol 2009;161:119-30.

Sources of Research Support: DuoCort Pharma AB.

Disclosures: GJ: Founder, DuoCort Pharma. HL: Founder, DuoCort Pharma. SS: Founder, DuoCort Pharma.

Nothing to Disclose: AGN, RB, PAB, BEE, BE, PD, MR, OR, JW-T

P3-647

Hydrocortisone Administration for Prevention of Adrenal Crisis in Stress - Pharmacokinetics of Different Administration Modes in Adrenal Insufficiency and Comparison to Surgical Stress.

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Patients with adrenal insufficiency (AI) require adjustment of their hydrocortisone (HC) dose to avoid life-threatening adrenal crisis during severe stress (eg surgery or sepsis). On the other hand, inappropriately high doses of HC offered perioperatively may impair glucose homeostasis and immune function increasing susceptibility to infection and delaying wound healing. Studies assessing the optimal steroid cover perioperatively in patients with AI are lacking and HC replacement regimens in stress have been selected on an empirical rather than on a rational basis, with huge variability in choice of doses and administration modes.

We have examined the pharmacokinetics and bioavailability resulting from different HC administration modes in AI subjects and compared results to cortisol levels produced by non-AI patients under surgical stress conditions. Nine patients with primary AI underwent 4 study days (in random order) separated by an interval of 1 week: Day 1, 50 mg HC per os every 6 hrs (HC-PO); Day 2, 50 mg HC im every 6 hrs (HC-IM); Day 3, 50 mg HC per iv injection every 6 hrs (HC-IVI); Day 4 200 mg HC per continuous iv infusion (HC-IVC). Thirteen patients undergoing elective surgery [intermediate stress procedures (thyroidectomy, parathyroidectomy)] were recruited as controls. All participants had frequent blood sampling over 24 hours, in surgical controls after induction of anaesthesia. Serum cortisol concentrations were measured by liquid chromatography/tandem mass spectrometry. Statistical analyses: t-test, Mann-Whitney rank sum test, trapezoidal integration for areas under the concentration curve (AUC).

Average minimum (c_{min}), mean (c_{mean}) and maximum (c_{max}) serum cortisol concentrations were: surgical controls 58/251/517 nmol/L; AI patients HC-PO 315/767/1504, HC-IM 333/765/1163, HC-IVI 226/694/1434, HC-IVC 249/668/861 nmol/L. The AUC_{0-6h} [mean(\pm SEM)] in the surgical controls was 1950 ± 524 nmol/L*h, and was significantly higher during all four HC administration modes: HC-PO 4379 ± 195 , HC-IM 4141 ± 130 , HC-IVI 3574 ± 284 , HC-IVC 5055 ± 258 nmol/L*h (all $p<0.001$ vs. surgical controls).

The highest maximum and lowest minimum serum cortisol concentrations after iv bolus HC (HC-IVI) are the least suited to maintain steady cortisol levels; the most steady levels are provided by HC-IVC. Our results convincingly demonstrate that currently recommended HC doses are much higher than actual cortisol demands in increased stress associated with intermediate surgery.

Nothing to Disclose: NK, AET, SK, DAV, DMH, JK, CJM, JAHW, WA

P3-648

Sexuality, Fertility and Androgens in Women with Addison's Disease.

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The aim of this study was to evaluate sexual function, fertility, and androgen levels in a large group of women with well-characterized Addison's disease.

Background: Although primary ovarian insufficiency is associated with Addison's disease, it is not known whether Addison's women have affected sexual function. Moreover, the fertility of the patients has not been studied systematically. The concentration of circulating androgens may not reflect effects on the cellular level; the androgen activity in peripheral tissues may be better reflected by the level of the androgen metabolites.

Design: Female Addison's patients (n = 269, age range 20-79 yr) were contacted and asked to complete the Sexual Activity Questionnaire (SAQ). Their blood samples were analyzed for the androgen metabolite 5 α -androstane-3 α , 17 β -diol-3-glucuronide (3 α -Diol-G) and adrenal autoantibodies. Control subjects for the SAQ ratings were 740 women. Control subjects for 3 α -Diol-G measurements were 114 female blood donors aged 20 to 70 years.

Results: SAQ was answered by 65% (174/269). The Addison's women were significantly more sexually active, and they scored significantly better on both sexual pleasure and discomfort compared to normal controls. Androgen status was evaluated by 3 α -Diol-G levels in 153 females with Addison's disease and 114 blood donor controls. Addison's patients (n = 140) not taking DHEA had significantly lower 3 α -Diol-G than blood donors (mean 0.49 vs. 2.2 μ g/ml, P<0.0001). The 3 α -Diol-G levels did not correlate significantly with age. Patients taking DHEA (n = 13) had significantly elevated 3 α -Diol-G levels compared with the blood donors (mean 5.4 μ g/ml vs. 2.2 μ g/ml, P=0.002). Women with self-reported history of premature menopause had significantly lower values of 3 α -Diol-G than women without such a history (mean 0.34 vs. 0.53 μ g/ml, P<0.0001). Before their diagnosis of Addison's disease the women had 229 children, whereas 236 were expected, yielding a standard incidence ratio (SIR) for birth of 0.97 (CI 0.845-1.095). For the fertile years after the diagnosis was made, the observed number of births was 54, with expected 87.5, yielding SIR for birth of 0.69 (CI 0.52-0.86).

Conclusion: Despite androgen depletion, female patients with Addison's disease do not self-report impaired sexuality. The fertility is however reduced after the diagnosis is made, but the reasons for this observation remain uncertain.

Nothing to Disclose: MME, ESH, AAD, KL

P3-649

Optimal Glucocorticoid Replacement and Quality of Life in Severely Adrenocorticotropin (ACTH) Deficient Hypopituitary Subjects.

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The optimal and physiological replacement regimen of hydrocortisone (HC) in severe ACTH deficiency remains unknown and there is a lack of data regarding the effect of different HC regimens on quality of life (QoL) in hypopituitary patients. We aimed to define the dose regimen of hydrocortisone which results in a 24hour cortisol profile that most closely resembles that of healthy controls, without adverse impact on QoL.

10 male hypopituitary patients with severe ACTH deficiency (basal cortisol $\leq 100\text{nM}$ and peak response to stimulation $< 400\text{nM}$) were enrolled in a crossover study of 3 HC dose regimens given at 8 am and 4 pm. Following 6 weeks of each regimen, patients underwent 24hour cortisol sampling studies and QoL assessment using the Short Form 36 and the Nottingham Health Profile questionnaires. The measurements were compared to those of healthy, matched, HC naïve controls using analysis of variance.

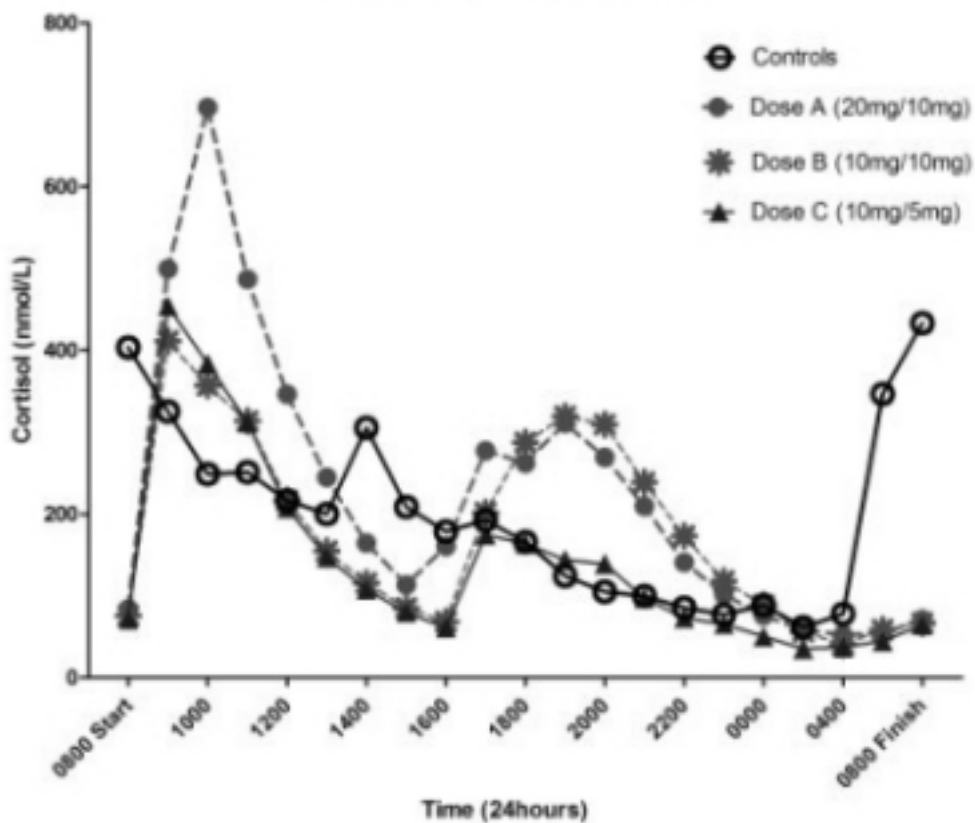
Results are depicted in figure 1. Regimen C (10mg mane, 5mg tarde) produced the 24hour profile which most closely resembled that of the controls when compared to the other two doses. In addition, both regimen A (20mg/10mg) and B (10mg/10mg) produced supraphysiological peaks but not regimen C. (Table 1) There was no difference in QoL in patients between dose, however energy level was significantly lower across all dose regimens compared to controls ($p < 0.001$).

Mean (SD) Peak and Trough Cortisol (nmol/L)

| | Dose A | Dose B | Dose C | Controls |
|-------------------------|------------|------------|-----------|-----------|
| Peak Cortisol 8am-4pm | 811 (168)* | 540 (87) | 555 (124) | 411 (113) |
| Trough Cortisol 8am-4pm | 84 (46)* | 62 (33)* | 59 (22)* | 159 (64) |
| Peak Cortisol 5pm-2am | 417 (100)* | 411 (110)* | 222 (102) | 226 (91) |
| Trough Cortisol 5pm-2am | 43 (28) | 55 (44) | 34 (21) | 51 (24) |

*significant deviation from control $p < 0.01$

Mean 24hour Cortisol Profile



The lower dose of HC (10 mg/5 mg) produced a more physiological cortisol profile, without any deterioration in quality of life, compared to higher doses still used in clinical practice. This may result in a lower cardiometabolic risk in a group of patients known to have excess cardiovascular mortality. Further studies are underway to assess this hypothesis.

Nothing to Disclose: LAB, BR, MJH, PO, WT, DS, CJT, AA

P3-650

Randomized, Double-Blinded, Cross-Over Study Comparing Various Glucocorticoid Substitution Schemes in Secondary Adrenal Insufficiency.

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Background: In secondary adrenal insufficiency (AI), glucocorticoid replacement remains a challenge, with various compounds and dosing schemes being used. Biochemical markers like urinary free cortisol and salivary cortisol day profiles are controversial. Therefore, doses are adjusted on the basis of subjective parameters both by the patient and the treating physician.

Methods: Patients with proven AI (n=18) received three different glucocorticoid replacement regimens for each 4 weeks in a randomized, double-blinded, cross-over study design, with doses applied at 7.00, 12.00,15.00,18.00:

(A)Hydrocortisone 10mg-placebo-5mg-placebo,

(B)Hydrocortisone 10mg-5mg-placebo-5mg,

(C)Prednisolone 5mg-placebo-placebo-placebo.

After every replacement regimen, quality of life (SF-36) and psychological distress (BSI) were assessed along with diurnal changes of current well-being (Bf-S) and alertness (SSS) using validated questionnaires. Inclusion criteria were an investigation of the adrenal function by insulin tolerance test within the last 12 months, and stable substitution of any affected pituitary axes for >3 months. Exclusion criteria were a known depression, pregnancy, difficulties in language, and prior history of hypercortisolism. Control groups included patients with pituitary disease and adrenal sufficiency (AS; n=20), and age- and sex-matched healthy subjects (C; n=21).

Results: AI showed improvements of physical quality of life (i.e. SF-36 physical function, p<0.05; SF-36 physical role function, p<0.05) and current well-being (at 18:00, p<0.05) under scheme A (hydrocortisone 10-0-5-0 mg) compared to the other replacement regimens. Quality of life and current well-being were significantly impaired compared to healthy controls.

Conclusions: Although the differences in psychological parameters were comparatively small, our results indicate beneficial effects of hydrocortisone replacement twice a day. Psychological parameters should be considered as additional criterion for treatment decisions.

Nothing to Disclose: SB, PN, NU, SE, MS, KM, SP

P3-651

Cortisol, Testosterone and Soccer; Modulation of Adrenal and Testis Function during a Competitive Season.

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Activation of hypothalamic-pituitary-adrenal axis as well as hypothalamic-pituitary-testis axis usually occurs after physical or/and psychological stress. Competitive soccer can be definitely considered as a stressor and many studies have investigated the dynamic modulation of adrenal and testis. Cortisol, DHEA-S and testosterone have been studied and their variations have been related to variables such as intensity of effort, winning or losing outcome, home-away match ecc. However, with respect to both cortisol and testosterone levels, several studies have shown significant increase after a soccer match while others have failed to show any significant change. Furthermore, few studies have considered changes of testosterone and cortisol throughout a whole season. The availability of salivary assays has allowed us to reliably monitor a male soccer team during both trainings and matches. A significant increase of cortisol levels has been shown in all but one games, while testosterone levels significantly decreased in 8/12 matches. Testosterone/cortisol ratio significantly decreased in 10/12 occasions. When evaluating trainings, significant increase of cortisol levels has been shown in 3/9 sessions, while a significant increase of testosterone levels has been shown in 2/9 sessions. Testosterone/cortisol ratio significantly decreased in 1/12 session and that was the only morning training session; in all the other trainings no significant change has been shown. When comparing training and match values, mean levels of basal cortisol were significantly higher at the beginning of match than at the beginning of training although the latter were significantly increasing throughout the study; mean values of basal testosterone were not different throughout the study between matches and trainings. We finally compared the testosterone/cortisol ratio; the pre-training ratio was not significantly different than pre-match ratio although the pre-training ratio was progressively and significantly reducing throughout the study. In conclusion, our data show that evaluation of adrenal and testis function in soccer athletes have to take into account differences between the intensity of efforts in trainings and matches (when the psychological and physical stress is definitely higher) as well as the period of the season under study; indeed changes at the beginning of the season are significantly different from those obtained at the end of the season.

Nothing to Disclose: VP, IB, CG, ADB, RV, FM, GN

P3-652

Pseudo-Pheochromocytoma: False Positive Uptake of 123I-Metaiodobenzylguanidine in Accessory Spleen.

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Background: 123I-Metaiodobenzylguanidine (MIBG) is a radiopharmaceutical which has high affinity for neuroendocrine tumors (NET). Its main purpose is localizing pheochromocytoma (p). Its sensitivity for adrenal p is approx. 85% vs. 58% for extraadrenal p. Several drugs decrease MIBG uptake such as prochlorperazine, diltiazem and labetalol. However, its specificity is still high. MIBG accumulation is seen almost exclusively in NETs and reports in other lesions are limited (1). **Clinical case:** A 39-yr AA woman presented with severe HTN, nausea and frequent headaches for 1 yr. PMHx includes pituitary adenoma resection and splenectomy for hemolytic anemia. ICU admission with nitroprusside and esmolol infusions were initiated since her HTN was unmanageable despite maximal doses of multiple oral medications. Her evaluation showed a crea of 0.7 (0.6-1.0 mg/dl), plasma free MN of 61 (0-57 pg/ml), plasma free NMN of 246 (0-148 pg/ml), 24 hr urinary MN of 98 (19-140 mg/24 hr), 24 h urinary NMN of 495 (52-310 mg/24 hr), plasma aldo of 4 (4-31 ng/dl), and plasma renin of 1.6 ng/ml/h. Selective renal angiogram showed large and patent renal arteries bilaterally. MRI of the abdomen showed a 4.7- cm mass with smooth lobular contour anterior to the upper pole of the left kidney. MIBG revealed focal increased uptake in the LUQ of the abdomen. She received appropriate preoperative tx for 2 wks including phenoxybenzamine before surgery. Six hrs postop, she developed hypotension and tachycardia. Vital laboratory and fluid resuscitation was begun. She suddenly and unexpectedly had cardiopulmonary collapse. Cardiopulm resuscit was attempted but was unsuccessful. Her autopsy revealed extravasation of blood within the surgical site. Pathologic findings revealed an accessory spleen and some benign pancreatic tissue but no pheochromocytoma.

Conclusion: MIBG uptake is determined by the norepinephrine transporter (NOET) and is reported in lung, heart, spleen, thyroid, non-NETs and renal pathologies (2). We found one report on a false positive MIBG scan due to accessory spleen (3). The mechanism is not entirely understood but may be related to the presence of the NOET. In cases of positive MIBG uptake located in the vicinity of the spleen, the upper pole of the left kidney or tail of the pancreas, one should consider an accessory spleen or non-pheo tissue and performing additional imaging studies before surgical intervention.

1. Cecchin, D., Lumachi, F., Marzola, M C., et al. A meta-iodobenzylguanidine scintigraphic scoring system increases accuracy in the diagnostic management of pheochromocytoma. *Endocrine-Related Cancer*. 2006; 13:525-533.
2. Elgazzar AH, Gelfand MJ, Washburn LC, Clark J, Nagaraj N, Cummings D, Hughes J, Maxon HR 3rd. I-123 MIBG scintigraphy in adults. A report of clinical experience. *Clin Nucl Med*. 1995 Feb;20(2):147-52.
3. Granata, C., Carlini, C., Conte, M., et al. False Positive MIBG Scan due to Accessory Spleen. *Med Pediatr Oncol*. 2001; 37:138-139

Nothing to Disclose: NC, AHB, CAK, GU

P3-653 Pheochromocytoma Presenting as Stress-Induced Cardiomyopathy; a Case Report.

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Introduction Stress-induced cardiomyopathy, also called broken heart syndrome is characterized by transient left ventricular dysfunction that mimics myocardial infarction. It has been shown in studies that plasma catecholamines are significantly higher in persons with stress induced cardiomyopathy [1].

Case Report- A 78 year old lady with a history of coronary artery disease, paroxysmal atrial fibrillation, hypertension and hyperlipidemia presented with chest pain and dyspnea and was found to have elevated troponin levels. The left heart catheterization revealed patent stents to the circumflex artery. Her exercise stress test was normal. The echocardiogram showed an ejection fraction of 25 % which improved spontaneously to 55 % over the next few months. She had a repeat episode of hospitalization for dyspnea and congestive heart failure and the ejection fraction again declined to 30 % but once again improved to 50 % over the course of a few days. She was diagnosed as stress-induced cardiomyopathy. During the course of hospitalization for an episode of chest pain, dizziness and palpitation, a 24 hour urinary metanephrines obtained to screen for Pheochromocytoma was found to be very high. Repeat 24 hour urinary metanephrines under stable conditions in endocrine clinic was 4009 µg/24 hrs (35-460). Plasma Metanephrines was 844 pg/ml (0-62), Plasma Aldosterone Concentration (PAC) was 6.3 ng/dl (1.0 - 16.0), and Plasma Renin Activity (PRA) 4.76 ng/ml/hr. CT scan of the abdomen showed 5.4 x 4.1 x 5.7 cm right adrenal mass which was peripherally enhancing with central necrotic and cystic changes.



The patient then underwent excision of the right adrenal mass. The histopathology confirmed Pheochromocytoma. The patient is now symptom free and her blood pressure is well controlled with no medications.

Conclusion Pheochromocytoma is a catecholamine secreting tumor of the adrenal medulla that presents with the triad of hypertension, headache and diaphoresis, but it may stay silent for many years. Pheochromocytoma should be suspected in a patient with idiopathic dilated cardiomyopathy.

(1)Kassim TA, Clarke DD, Mai VQ, Clyde PW, Mohamed Shakir KM. Catecholamine- induced cardiomyopathy. *Endocr Pract.* 2008 Dec; 14(9):1137-49.

Nothing to Disclose: PS, PV, AK, TFS, AE

P3-654

Pheochromocytoma Presenting as Cardiogenic Shock Due to Reversible Cardiomyopathy.

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The classical presentation of pheochromocytoma is a triad of headache, palpitations, and severe hypertension. Cardiovascular manifestations such as rhythm disturbances, angina pectoris, myocardial infarction, acute heart failure, dilated cardiomyopathy, and cardiogenic shock have also been reported in the literature, but are uncommon(1). We report the case of a 46-year-old woman who presented in cardiogenic shock following elective surgery and was found to have a pheochromocytoma.

Three years previously the patient presented to an emergency room with chest pain and was found to have elevated troponin level and left ventricular dysfunction. Coronary angiography was normal and the symptoms abated. Two years previously she again presented with chest pain and dyspnea and was found to have left ventricular dysfunction. She was diagnosed with myocarditis and LV ejection fraction by echocardiography eventually returned to 55%. One and half years previously she developed palpitations and underwent ablation for an AV nodal reentrant tachycardia. Two days before admission to our hospital she underwent elective robotic hysterectomy and bladder resuspension. Postoperatively she developed hypotension, tachycardia, and respiratory distress. She was found to be in cardiogenic shock with cardiac index of 1.5 L/min/m². On admission to UT Southwestern she was found to have a left ventricular ejection fraction of 10-15%. She was treated with pressors, inotropes, dialysis, and supportive care. Plasma epinephrine was measured at 19,972 pg/mL and plasma norepinephrine was 37,000 pg/mL. Plasma metanephrine was 70.1 nmol/L and plasma normetanephrine was 128 nmol/L. CT of the abdomen showed 7.4 cm ovoid mass in the right adrenal with internal cystic changes. After weaning from pressor and inotropic support, she was treated with alpha blockade and one month later readmitted for right adrenalectomy. Pathologic examination showed a 10 x 6.1 x 4.9 cm pheochromocytoma. Postoperatively she remained normotensive without treatment and in follow-up over four months had normal plasma and urine metanephrines on two occasions. Left ventricular ejection fraction by echocardiogram has remained stable at 55%. Catecholamine-induced cardiomyopathy, as above, is a well-known entity. Cases in the literature have ranged from myocarditis to cardiogenic shock to sudden death, though rare. For a patient with cardiomyopathy and no obvious cause, pheochromocytoma should always be considered (2).

(1)Wu et al. Inter Med 2008; 47:2151

(2) Kassim et al. Endocr Pract 2008; 14:1137

Nothing to Disclose: MZ, EED

P3-655

A Catecholamine Crisis on Mount Kilimanjaro: A Hypoxia Effect?.

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Background: Paragangliomas are very rare vascularized tumors that arise from the autonomic nervous system. High-altitude low oxygen pressure seems to be a risk factor for sporadic head and neck paragangliomas. Exposure to high-altitudes location is reported to have short- and long-term effects in the sympathoadrenal system, characterized by increased 24-hour excretion of urine norepinephrine. We describe an individual with a sympathetic paraganglioma and catecholamine crisis while reaching the summit of Mount Kilimanjaro in Africa.

Clinical Case: A 59-year-old man was found in 2004 with a norepinephrine-producing, right atrial paraganglioma. The tumor was surgically removed. Genetic testing for *SDHB* and *SDHD* gene mutations was negative. He remained normotensive and asymptomatic for three years. Plasma and urinary catecholamines and metanephrines were normal. In 2007, he climbed Mount Kilimanjaro (9,340 ft / 5895 meters) in Tanzania. After reaching the summit; he developed palpitations, throbbing headaches, diaphoresis, tremulousness, anxiety, panic attacks, and intense oppressive chest pain. The patient did not exhibit nausea, vomiting, anorexia, or altered mental status. He was descended to a local hospital where his symptoms persisted. His blood pressure was 180/110 mmHg and his pulse was over 100 per minute. A myocardial infarct was ruled out. A 24/hr urine collection for normetanephrines revealed 10,563 ug/24 hr (< 900 ug/24hr). He was treated with alpha and beta blockers. The patient underwent an abdominal CT scan that showed liver and bone metastasis, in addition to metastatic abdominal and pelvic lymph nodes. Back in the United States, a biopsy of the pelvic bone confirmed a metastatic paraganglioma. He completed 16 cycles with Cyclophosphamide, Vincristine and Dacarbazine exhibiting stable disease. One year after finishing chemotherapy his disease has progressed.

Conclusions: High altitude is associated with an elevation of the sympathetic activity. In individuals with catecholamine-secreting pheochromocytomas and paragangliomas, the exposure to a high altitude low oxygen pressure may induce or exacerbate a catecholamine crisis.

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

P3-656

Pheochromocytoma and Cushing's Disease: A Rare Combination with Unclear Etiology.

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Background: Multiple endocrine neoplasias have been described in the literature but no syndrome has described pheochromocytoma with Cushing's disease.

Clinical Case: A 28-year-old Caucasian woman presented with one year of palpitations, anxiety, excessive sweating, and uncontrolled hypertension. She reported 6 months of secondary amenorrhea and weight gain. There was no family history of adrenal disorders or endocrinopathies. On examination, blood pressure was 167/80 mm Hg and heart rate 95 beats per minute despite antihypertensive therapy. Examination also showed moon face, acne, central obesity, and purple striae. Laboratory tests included plasma free metanephrine 15.1 nmol/L (<0.5), normetanephrine 12.7 nmol/L (<0.9), 24-hour urine free cortisol of 77 micrograms (0-50) and 8 am cortisol of 4.5 mcg/dl and ACTH of 16 pg/mL after an 8 mg overnight dexamethasone. A 6.5 cm heterogeneous left adrenal mass was seen on abdominal computerized tomography and had intense uptake on MIBG scan. The possibility of pheochromocytoma with ectopic ACTH production was raised. After medical preparation, she underwent left open adrenalectomy confirming a 6.5 cm pheochromocytoma with vascular invasion, but immunohistochemical staining for ACTH was negative. Marked improvement in her hypertension, anxiety, and palpitations was seen post operatively, but Cushing's symptoms persisted.

Repeat tests 3 months later showed 8 AM salivary cortisol of 194 ng/dL (100-750), midnight salivary cortisol of 265 ng/dL (<100), 24-hour urine free cortisol of 63 micrograms, 8 AM cortisol of 7 mcg/dL after 1 mg dexamethasone at midnight, and 2 mcg/dl after 8 mg dexamethasone at midnight. MRI of the sella revealed a 0.6 cm hypointense area in the right side of the pituitary suggestive of microadenoma. Inferior petrosal sinus sampling was declined and transphenoidal resection was performed. The resected tumor showed expanded acini and loss of acinar pattern with immunoreactivity to ACTH staining suggestive of an ACTH-producing pituitary microadenoma. Genetic evaluation for MEN types 1 and 2 was negative.

Conclusion: This unusual case of two symptomatic endocrine tumors does not fit the description of known multiple endocrine neoplasias. Based on history, it appears to be a sporadic case but it is still unclear if there is any genetic or molecular explanation for this combination of two uncommon endocrine tumors.

Nothing to Disclose: TB, TAR, NDP, IEM, CJ, MAH

P3-657

Adrenal Medullary Hyperplasia Presenting with the Clinical Features of Pheochromocytoma.

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Introduction:

Adrenal medullary hyperplasia is a distinct and rarely reported disorder. Prior to histopathological evaluation, the clinical features of this disorder can resemble pheochromocytoma.

Clinical Case:

A 51 yr/o male presented for evaluation of episodic palpitations and excessive sweating. He was otherwise asymptomatic, except for recent initiation of lisinopril for newly diagnosed hypertension. Physical Examination: HR 82 bpm and BP 133/90 mmHg. His examination was otherwise normal, including no features of Cushing's syndrome. Laboratory: Normal plasma aldosterone to renin activity ratio and 24-hour urine cortisol level. Urine and plasma metanephrines were mildly elevated on several samplings (Table 1& 2).

Table 1: 24 - Hour Urinary Metanephrines

| | 1st Sample | 2nd Sample | 3rd Sample | Lab Reference |
|--------------------------------|------------|------------|------------|---------------|
| Metanephrine (mcg/24hr) | 211 | 146 | 221 | 26 - 230 |
| Normetanephrine (mcg/24hr) | 793 | 556 | 639 | 44 - 540 |
| Metanephrines Total (mcg/24hr) | 1004 | 702 | 860 | 90 - 690 |
| Creatinine (g/24hr) | 2.55 | 2.22 | 3.01 | 0.63 - 2.50 |
| Volume (mL) | 1100 | 1100 | 1450 | |

Table 2: Plasma Metanephrines

| | 1st Sample | 2nd Sample | 3rd Sample | Lab Reference |
|-------------------------|------------|------------|------------|---------------|
| Metanephrine (pg/mL) | 64 | 51 | 91 | 0 - 57 |
| Normetanephrine (pg/mL) | 112 | 175 | 173 | 0 - 148 |

CT and MRI of the adrenals demonstrated an 8mm left adrenal nodule with increased uptake on MIBG scan. He underwent an uncomplicated left adrenalectomy, and pathology revealed adrenal medullary hyperplasia. Six weeks later his symptoms had resolved and he remained normotensive without any medications. His post-operative plasma and urine metanephrines were normal on repeated occasions.

Conclusion:

This case resembled pheochromocytoma in several aspects. Ultimately, histopathological examination diagnosed adrenal medullary hyperplasia, which typically features a nodular or diffuse increase in medullary tissue volume and a decrease in cortico-medullary ratio. There is typically medullary expansion into the adrenal alae or tail. While classically thought to be a bilateral phenomenon, there are rare reports of unilateral adrenal medullary hyperplasia. This supports the theory that the two diagnoses are more closely related than initially thought, and may be part of a continuum of catecholamine excess. Pheochromocytoma, therefore, may represent an advanced presentation of nodular adrenal medullary hyperplasia.

Nothing to Disclose: PZM, RCJ, TDH, VQM, PWC, KMMS

P3-658

Importance of Life-Long Follow-Up of Patients with Pheochromocytoma: A Case of Recurrence 48 Years after Initial Diagnosis.

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Background: Pheochromocytomas are malignant in about 10% of cases, and the pathologic diagnosis of malignancy requires the presence of distant disease. We report here a case illustrating that long-term follow-up is indicated after surgery for all pheochromocytomas, including apparently benign tumors.

Clinical case: We report the case of a 65 year-old woman who was operated at age 17 for a 6-cm right pheochromocytoma. There was no evidence of associated hereditary syndrome. There was no follow-up. The patient was recently investigated for high blood pressure. Her urinary levels of norepinephrines were 2372 nmol/d (N<440) and normetanephrines 10 622 nmol/d (N<240). Her plasma normetanephrines were increased to 29.6 nmol/L (N<0.9). Abdominal MRI revealed a 10-cm T2 hyperintense mass in the right adrenal bed that showed increased MIBG uptake. The tumor was highly hypermetabolic on FDG PET/CT scan with a SUV of 7.6. Hypermetabolic retroperitoneal lymph nodes were also demonstrated. She underwent surgical debulking of the tumor. Histopathology showed malignant pheochromocytoma with a minor component of ganglioneuroma. There was liver, right kidney and diaphragm invasion with positive resection margins and a positive lymph node. Genetic testing for *RET*, *VHL*, *SDHB*, *SDHC* and *SDHD* genes was negative. Ten months after surgery, her urinary metanephrines are twice the normal limit. The MIBG scan revealed a small persistent hypercaptation in the tumoral bed. Palliative treatments are under consideration.

Conclusions: To our knowledge, 48 years is the longest reported recurrence time for pheochromocytoma. This illustrates that malignancy may occur a long time after the initial diagnosis of apparently benign pheochromocytoma, and underlines the importance of life-long follow-up of all patients operated for pheochromocytoma.

Nothing to Disclose: CL, ML, PP, CC, IB

P3-659

Atypical Presentation of Pheochromocytoma.

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Objective: To describe an atypical presentation of pheochromocytoma.

Case: We present a case of a 77 year old male with a history of coronary artery disease and COPD who fell at home. He was diagnosed with a subdural hematoma and an ST elevation myocardial infarction at a community hospital and was transferred to our facility. He required mechanical ventilation and enteric nutrition, but was unable to tolerate due to high gastric residuals and abdominal distension. A CT scan showed small bowel obstruction and areas of bowel ischemia. Incidental adrenal masses were noted, measuring 4 cm on the right and 0.7 cm on the left. The patient had several episodes of extreme fluctuations in blood pressure from 78/46 to 220/97. Phentolamine was administered and blood pressure stabilized. Twenty-four hour urine metanephrine was 5097 ug/d, normetanephrine was 1970 ug/d, plasma metanephrine was 3.71 nmol/L, and plasma normetanephrine was 3.01 nmol/L. A I-123 MIBG scan was consistent with right adrenal pheochromocytoma. Right adrenalectomy was planned but the patient suffered cardiac arrest and expired. Autopsy confirmed the existence of right adrenal pheochromocytoma.

Discussion: Pheochromocytomas are rare tumors and classic symptoms include headaches, palpitations, and diaphoresis. Hypertension is the most common sign and may be sustained or paroxysmal. Clinical presentation can be highly variable and nonspecific, ranging from fatigue and anxiety to diarrhea and constipation. Some patients with pheochromocytoma are asymptomatic. The patient we described presented with a myocardial infarction, ileus, and labile blood pressure when the diagnosis of pheochromocytoma was suspected and made.

Conclusion: Diagnosis of pheochromocytoma can be difficult due to its low prevalence and variable and nonspecific presentation. Due to high mortality one must always maintain clinical suspicion.

Nothing to Disclose: YBG, SC, MV

P3-660

Pheochromocytoma: An Unpredictable Tumor.

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Background: Pheochromocytoma is a rare endocrine tumor of great clinical importance. It usually manifests in the fourth to fifth decades of life, though familial forms can present earlier. It is rarely seen in the elderly and less than 10% of patients are asymptomatic.

Case: A 77-year-old white male presented with respiratory distress. Past history was significant for a retro-peritoneal tumor diagnosed five years ago as pancreatic carcinoma on a needle biopsy. Subsequent abdominal CT scans, however, were suggestive of left adrenal tumor. He received no treatment for this. Physical examination was unremarkable except for very labile systolic blood pressures, varying between 40-300 mm Hg, needing intermittent infusions of dopamine, phenylephrine, nitroglycerin and esmolol. He did not have hypertension in the past, and was never on anti-hypertensives. Cardiac enzymes were positive and echocardiogram revealed an ejection fraction of 35% without non-specific regional wall motion abnormalities. A pheochromocytoma was suspected and 24-hr urine fractionated catecholamines and metanephrines were ordered. Urine metanephrines were as high as 61,614 µg/24 hours (about 130 times the normal) and plasma free metanephrines were >85.9nmol/L (180 times normal); this was considered significant despite the fact that he was on vasoactive drugs. Repeat urine metanephrines on days four and thirty after the patient was weaned off vasoactive medications were elevated at 14 times normal. MRI of the abdomen and MIBG scan were consistent with a 11cm left adrenal pheochromocytoma. Review of prior pathology slides of the tumor confirmed it to be a pheochromocytoma.

During the hospitalization, prazosin was added followed by beta-blocker which stabilized blood pressures to normal limits. Repeat echocardiogram showed normalization of left ventricular function. The initial cardiac abnormalities were attributed to stress and catecholamine toxicity. Elective resection of tumor was done 2 weeks later. Surgical pathology results were consistent with pheochromocytoma.

Conclusions: Pheochromocytomas are clinically unpredictable with variable presentations. Unusual features in our patient include late presentation in the eighth decade of life, huge catecholamine surge, being asymptomatic for a long time despite attaining a large size and surviving a biopsy without hemodynamic crisis. Necrosis leading to catecholamine surge could have resulted in the clinical presentation.

Nothing to Disclose: RA, JG, VA, AD, CD, PD, EF

P3-661

Glycyrrhizin Induced Hypokalemic Hypertension: An Unexpected Source.

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Introduction

Chronic licorice ingestion is a well-known cause of hypokalemic hypertension with low levels of plasma renin and aldosterone. The main compound of licorice, glycyrrhizin, is hydrolyzed after ingestion to its active component 18 β -glycyrrhetic acid (18 β GA). This 18 β GA specifically inhibits 11 β -hydroxysteroid dehydrogenase type 2, the enzyme catalyzing the conversion of biologically active cortisol into inactive cortisone thereby protecting the mineralocorticoid receptor from activation by cortisol.

Clinical case

A 49-year old woman was referred to our clinic for a third opinion concerning a chronic hypokalemic hypertension. No underlying cause had been identified despite elaborate investigations elsewhere including (ad)renal imaging, endocrine tests for primary aldosteronism and Cushing's syndrome. Urinary excretion of 18 β GA was positive on several occasions, but history taking by a dietician was negative for consumption of glycyrrhizin containing products. She also suffered from recurrent syncope for which no explanation had been found despite evaluation by a cardiologist and neurologist. Her medical treatment consisted of amlodipine, triamterene, potassium chloride, and an oral contraceptive. Laboratory results in plasma: Na⁺ 138 mmol/L (ref 135-145), K⁺ 4.4 mmol/L (3.5-5.0) creatinine 73 mmol/L, aldosterone 0.03 nmol/L (0.04-0.35), renin activity 0.7 nmol/L/h (1.2-3.5); 24-hr urine analysis (GC-MS): (THF + allo-THF)/THE: 2.4 (0.6-1.1), glycyrrhetic acid 69 μ g/l (<5). Thorough history taking now revealed that she consumed one cup of 'Star mix tea' daily since a few years. She was advised to stop drinking this tea. Amlodipine, triamterene and potassium chloride could be stopped, while the patient remained normotensive and normokalemic. Repeated urinary analysis showed glycyrrhetic acid <5 μ g/l and (THF + allo-THF)/THE: 1.7. Analysis of the 'Star mix tea' by the Dutch Food and Consumer Product Safety Authority revealed that each 'One-Cup bag' contained 47 mg glycyrrhizin. Thus, the patient's daily consumption did not exceed the generally recommended maximal daily intake of 100 mg glycyrrhizin per day.

Conclusion

If a patient denies licorice consumption, glycyrrhizin intoxication should still be considered as a cause of hypokalemic hypertension. Assessment of urinary 18 β GA is helpful and should therefore be assessed early in the work-up. However, the specific source of glycyrrhizin in the diet can remain enigmatic for a long time.

Nothing to Disclose: HLL, MJM, PHG, JL, MNK

P3-662

A Novel Method for the Diagnosis of Glucocorticoid Remediable Aldosteronism without a Long Polymerase Chain Reaction.

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Background: Glucocorticoid remediable aldosteronism (GRA) is a rare form of inherited hypertension characterized by hyperaldosteronism, high levels of urinary 18-hydroxycortisol (18-OHF) and 18-oxocortisol (18-oxoF), all of which are under ACTH control. This disorder is caused by the presence of a chimeric gene originating from a recombination between the 11-hydroxylase (*CYP11B1*) and aldosterone synthase (*CYP11B2*) genes. The recombination site between two genes usually cannot be identified because of their high nucleotide homology. Therefore, the chimeric gene has a theoretical recombination region, sitting anywhere from the start of intron 2 to the end of exon 4 in the chimeric gene (Fig). So far a long polymerase chain reaction (PCR) for amplifying a part of the chimeric gene (3.9 kb) (Fig) is a gold standard for the diagnosis of GRA. Another PCR and sequencing are, however, required to identify a recombination site.

Objective: To evaluate whether a standard PCR allows definitive diagnosis of GRA.

Patients: The clinical and laboratory characteristics of affected children are shown on the Table. GRA was clinically highly suspected.

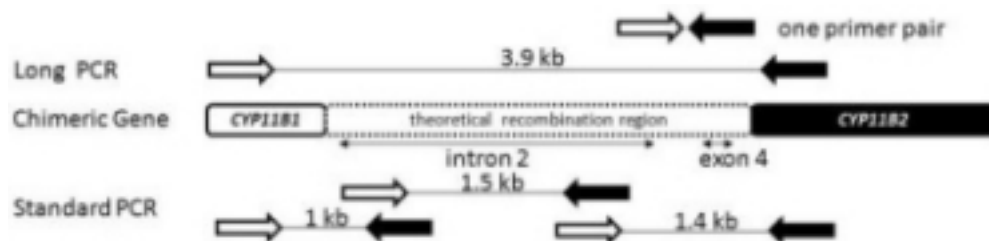
Methods: We performed a long PCR using one primer pair as previously described. We also tried a standard PCR using newly designed three primer pairs which cover all theoretical recombination regions (Fig).

Results: The long PCR yielded a 3.9 kb size of PCR product in each patient. The standard PCR did 1 and 1.5 kb size of products in patient 1 and 2, respectively. Sequencing those standard PCR products demonstrated that each possessed a recombination site in intron 2 of a chimeric gene, confirming the diagnosis of GRA.

Conclusion: We established a novel method for the diagnosis of GRA with a standard PCR. The newly designed primer pairs could cover every theoretical recombination site in a chimeric gene.

| Patient No/Age/Sex | Patient 1/12Y/Male | Patient 2/10Y/Male |
|--------------------------|--------------------|--------------------|
| Blood pressure(mmHg) | 192/131 | 156/110 |
| Renin | <2.0 pg/ml* | <0.2 ng/ml/hr** |
| Serum aldosterone(pg/ml) | 553 | 406 |
| Urinary 18-OHF(mg/g•Cr) | 0.38 | 1.72 |

*Active renin concentration **Plasma renin activity



Nothing to Disclose: MH, TI, SN, NA, TK, SS, TH

P3-663

A Young Female with Acute Renal Failure Post-Aldosteronoma Resection Precipitated by Transient Hypoaldosteronism.

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Background: Most patients with aldosterone-secreting tumors are medically managed preoperatively with antihypertensives, K⁺ supplementation and aldosterone(Aldo) receptor antagonists. Commonly, after surgery, these medications are reduced or eliminated and blood pressure normalizes or improves substantially. We describe the unusual case of a young woman who postoperatively developed hypotension and subsequently acute renal failure related to transient hypoaldosteronism.

Case: 44-year-old female with 1ary hyperaldosteronism and HTN for over 15 years caused by an aldosteronoma was evaluated in the clinic in preparation for unilateral adrenalectomy. Her laboratory tests months prior to preparation for surgery and off of medications were as it follows: Aldo 433 ng/dl, Plasma renin activity (PRA) 0.3 ng/mL/h, K⁺ 3.3 mg/dl.GFR 58 mL/min/1.73m².

Besides following a low salt diet, she was on the following medication regimen: Eplerenone, Benzapril, Amlodipine and K⁺ supplementation.

Preoperatively her BP was 148/88.

She underwent laparoscopic adrenalectomy. In the immediate postoperative period, her medications were weaned off within 1 week. She developed dizziness, hypotension (80-95/ 40-60). Consequently her renal function deteriorated. She had a GFR: 22 mL/min/1.73m², K⁺ 5.6 mg/dl, PRA: 0.4 ng/mL/h and Aldo: 2 ng/dl.

Five weeks after surgery her ACTH stimulation test showed: Basal PRA: 0.6 Aldo: 3ng/dl, Stimulated Aldo: 3ng/dl. She increased her salt intake. She was treated with fludrocortisone 0.05 mg daily weaned off over 2 months with improvement in her blood pressure:110-120/65-75 , K⁺ :4.8 mg/dl and symptoms. Her kidney function remained unchanged: GFR 22 mL/min/1.73m²

Conclusion: We have brief reports in the literature describing transient hypoaldosteronism after unilateral aldosteronoma resection¹. As demonstrated in this case it can lead to hypotension and end-organ hypoperfusion. These complications can be prevented if in the immediate postoperative period salt intake increases and mineralocorticoid supplementation (fludrocortisone) is temporarily used². There is body of evidence demonstrating an improvement in kidney function several months post-aldosteronoma resection indicating that not all the effects of hyperaldosteronism on the kidneys are irreversible⁴.

(1)Bravo E et al., Journal of Clinical Endocrinology & Metabolism 1975; 41:612-618

(2)Mattsson C et al., Nature Clinical Practice 2006; 2:198-208

(3)Sawka AM et al., Annals of Internal Medicine 2001; 135:258-261

(4)Sechi LA et al., JAMA; 2006; 295:2638-2645

Nothing to Disclose: AV, HZ, RP, CVV

P3-664

Overestimated Renal Function and Delayed Detection of 'Masked' Chronic Kidney Disease by Aldosterone Excess: A Lesson from a Case of Primary Aldosteronism with Cardiorenal Complications.

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Background: Patients with primary aldosteronism (PA) have been shown to have significantly higher incidence of cardiorenal complications than those with essential hypertension. Recently, chronic kidney disease (CKD) has been attracting clinical attention to prevent progression into end-stage kidney diseases.

Clinical case: A 51-year-old man was referred to undergo laparoscopic adrenalectomy (ADX) for his left adrenal tumor. While he was diagnosed as having PA with a left adrenal tumor at the age of 33, he had taken medication including spironolactone (SPL) 50 mg/day. Baseline aldosterone level of 178.2 ng/dl with plasma renin activity below a level of detection (<0.1 ng/ml/h), captopril test, and adrenal venous sampling all supported the diagnosis of PA with aldosterone excess from left adrenal side. Computed tomography scan revealed a left adrenal tumor of 30 mm and incidentally a left renal tumor of 45 mm. At this point, his serum creatinine level was 0.8 mg/dl with estimated glomerular filtration rate (eGFR) of 80.1 ml/min/1.73m². His BP was 154/98 mmHg with irregular pulse of 70 bpm, and EKG showed atrial fibrillation. In addition, both diffuse hypokinesia and mildly elevated BNP levels were consistent with heart failure. Human ANP analogue, carperitide, was administered and the dosage of SPL was gradually increased to 150 mg/day to control levels of serum potassium and blood pressure before the left ADX and left partial nephrectomy. Just before the surgery, his eGFR was reduced to 47 ml/min/1.73m², indicating that he had stage 3 CKD. Histopathological examination confirmed that there were aldosterone-producing adenoma and renal cell carcinoma. Examination of non-tumorous attached renal tissue also showed global glomerulosclerosis with interstitial fibrosis and intima-media thickening of renal arterioles, which were consistent with the diagnosis of hypertensive nephrosclerosis. After the surgery, his eGFR levels showed no significant decrease during a follow-up period of up to 9 months.

Conclusion: The eGFR might be overestimated under the influence of excess aldosterone in patients with primary aldosteronism. Earlier diagnosis of PA with appropriate pharmacological and/or surgical intervention might be necessary to prevent progression of 'masked' CKD in patients with apparently 'normal' renal functions.

Nothing to Disclose: YI, RM, MK, OM, HS, HS, SI, FS

P3-665

Cushing Disease and Cryptic 21-Hydroxylase Deficiency in the Mother of Two Daughters with Nonclassic Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency.

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Background: Clinical features of both nonclassic congenital adrenal hyperplasia (NC CAH) and Cushing disease can be subtle, making diagnosis challenging. Rarely, corticotropinomas have been reported among patients with CAH, presumably due to hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis (1,2).

Clinical Cases: The index patient was a 6.9 year-old Ashkenazi Jewish female referred for endocrinology evaluation for possible hypoglycemia. The patient had no symptoms of androgen excess. Her physical exam and bone age x-ray were within normal limits, but a random 17-OHP was 3069 ng/dL. ACTH stimulation testing confirmed the diagnosis of NC CAH. Subsequently, the index patient's older sister was referred for evaluation. Again, ACTH stimulation testing confirmed the diagnosis of NC CAH. Due to rapidly advancing bone age, she was treated with hydrocortisone, luprolide acetate, and growth hormone. Following her daughters' diagnosis, the mother began to question her own health. She was relatively short (153.7 cm), and our observation was of subtle central obesity. The mother complained of fatigue, gradual weight gain, acne and hirsutism. She was eventually diagnosed with both NC CAH and Cushing disease. After her initial pituitary surgery failed to effect a cure, she had definitive transsphenoidal surgery with removal of a single ACTH-positive tumor and subsequent hypocortisolism. There was no pituitary hyperplasia. She is now doing well on maintenance glucocorticoid. Genetic testing showed that both daughters and mother were homozygous for the *CYP21A2* V281L mutation. The girls' father was heterozygous for this mutation.

| Subject | Age (yrs) | 17-OHP_0 (ng/dL) | 17-OHP_30 (ng/dL) | Genotype |
|--------------|-----------|------------------|-------------------|-------------|
| II-2 | 6.9 | 1249 | 5870 | V281L/V281L |
| II-1 | 8.8 | 1373 | 6680 | V281L/V281L |
| I-1 (mother) | 43 | 675 | 6440 | V281L/V281L |

Conclusions: Given the relatively common prevalence of *CYP21A2* mutations in certain ethnic groups, the possibility of disease status should be considered in the parents of affected children. Chronic ACTH stimulation and impaired negative feedback may increase the risk of tumor formation in patients with CAH. The family presented here highlights the complex phenotype observed in some patients with 21-hydroxylase deficiency.

(1) Charmandari E, et al. *J Pediatr Endocrinol Metab* 2005;18:97-101

(2) Boronat M, et al. *J Endocrinol Invest* 2004;27:583-90

Nothing to Disclose: ACF, DPM, LKN, SFW

P3-666

A Case of Double Adenomas in a Single Adrenal Gland Associated with Primary Aldosteronism and Hypercortisolism - Analysis Using the Bilateral Adrenal Venous Catheterization and Immunohistochemical Evaluation of Steroidogenesis.

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A simultaneous occurrences of the two distinct adrenocortical disorders, primary aldosteronism (PA) and hypercortisolism in unilateral or bilateral adrenals, have been reported in several cases. These cases have not necessarily been fully evaluated from the standpoints of pathological and endocrinological aspects. Therefore, we report a case harboring two separate each-hormone-secreting adrenocortical adenomas in the left adrenal. We evaluated the case by 1) hormone secreting dynamics using bilateral adrenal venous catheterization technique (AVS) supported by ACTH loading, and 2) immunohistochemical analysis of steroidogenic enzymes in the resected specimens.

A 36-year-old female with Cushingoid features and symptoms was admitted for an evaluation of two discrete masses in the left adrenal gland identified by CT scan. Endocrinological evaluations revealed basal ACTH <1 pg/ml, cortisol (F) 17.5 µg/dl, and PRA 0.3 ng/ml/h. Basal PAC level was not elevated (4.9 ng/dl), but stimulated PAC levels by ACTH provocation were elevated (peak PAC/F=1.2), which lead to the clinical diagnosis of hypercortisolism and PA syndrome. Subsequent AVS aimed at localizing the each-hormone-secreting sites demonstrated that only the left adrenal gland secreted both hormones. The basal and stimulated levels of PAC/F in each adrenal vein were 6.6/257 and 4140/969 at the left, and 586/58 and 1123/71 at the right, respectively. Removed left adrenal gland contained two independent tumors (25 and 12 mm). The first one was consisted of light clear cells (60%) with scattered dark compact cells (40%). Both c17 and 3BHSD were expressed in an abundance. The latter one was composed of mostly light clear cells, and 3BHSD but not c17 immunoreactivity was detected in the tumor cells. Non-neoplastic attached adrenal was markedly atrophic with little DHEAST immunoreactivity, and so-called paradoxical hyperplasia of the zona glomerulosa with scarce 3BHSD expression was also detected. All of these findings indicated that 1) The bigger tumor was F-producing adenoma and the smaller one aldosteronoma (APA), 2) suppressed aldosterone production from the APA with a possible strong ACTH-sensitivity might have been induced by low ACTH level due to co-existing hypercortisolism, and 3) the low basal PAC level detected in the left adrenal vein might be due to both suppressed activity of the APA and decreased aldosterone synthesis from attached zona glomerulosa of the tumor, with the altered enzyme expression.

Nothing to Disclose: DK, KS, MS, NF, YS, KN, YY, ME, HS, NM, EA

P3-667

Virilizing Adrenocortical Adenoma with Subclinical Cushing's Syndrome in a Middle Aged Woman.

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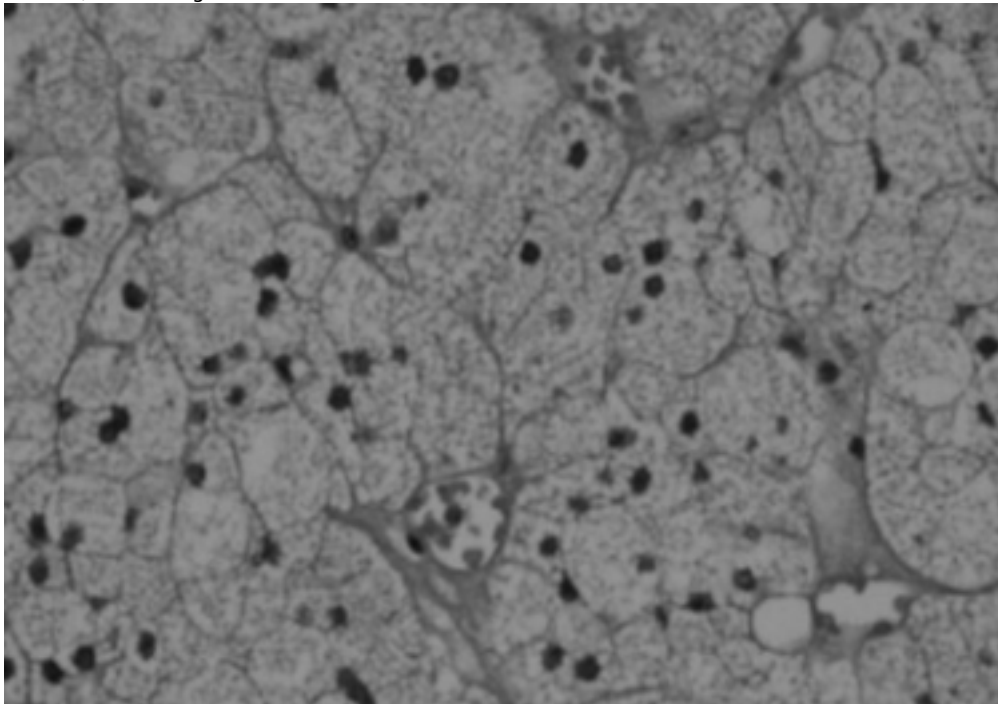
INTRODUCTION

Virilizing adrenocortical tumors are classified into benign adenomas and carcinomas. Carcinoma is more common in children, but adenoma and cancer are both seen in adult women. We report a very rare case of virilizing adrenocortical adenoma associated with subclinical Cushing's syndrome.

CLINICAL CASE

A 45 year old woman presented with 1 year history of increasing hirsutism, loss of scalp hair and amenorrhea for 2 years. There was no change in voice. The patient had no other past medical history, nor was taking any medications or herbal supplements. She has 3 children. Physical exam was notable for prominent terminal facial and abdominal hair, also present on her extremities and significant hair loss on the scalp. Mild clitoromegaly was noted on pelvic exam. Laboratory data were pertinent for an elevated total testosterone level 326 ng/dL (nl 15-70ng/dL), free testosterone 29.8pg/mL (nl 1.0- 8.5pg/mL); and gonadotropins (LH 39.5IU/L and FSH 37.4IU/L) in postmenopausal range. Prolactin was 6.5ng/mL (nl 3.0- 30ng/mL) and ACTH undetectable at <5ng/L (nl 9-52ng/L). Serum cortisol after overnight 1mg dexamethasone test was elevated at 7.8mcg/dL and 24 hr urine free cortisol 10 days later was high at 55.2µg/24hr (nl 5.0-50.0 µg/24hr). Transvaginal ultrasound revealed normal ovaries and CT abdomen with contrast showed a 2.6 cm low density mass in the right adrenal gland.

Laparoscopic right adrenalectomy was performed. Tumor pathology was consistent with a benign 3.5 cm adrenal adenoma histologically composed of homogenous population of cells with Zona fasciculata-like appearance with no necrosis, hemorrhage or mitosis.



CONCLUSION

The most common causes of hyperandrogenemia in adult women are polycystic ovary syndrome (75%), idiopathic hirsutism (15%) and congenital adrenal hyperplasia (3%). Ovarian and adrenal androgen secreting tumors are very rare, accounting for only 0.2% of cases of androgen excess. High degree of clinical suspicion, early diagnosis with appropriate work up and complete resection carry a favorable prognosis.

1. Del Gaudio AD, Del Gaudio GA. Virilizing adrenocortical tumors in adult women. Report of 10 patients, 2 of whom each had a tumor secreting only testosterone. *Cancer* 1993;72:1997-2003.

Nothing to Disclose: CK, MDS, RA, RA, IAW, GV, FAS

P3-668

Adrenocortical Carcinoma in a Patient Carrying a *BRCA2* Mutation.

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Background: Although most cases of adrenocortical carcinoma (ACC) are sporadic, a small percentage may be a component of hereditary cancer syndromes like Li Fraumeni syndrome (LFS). LFS which may be caused by a germline mutation in the *TP53* gene predisposes to early onset of various cancers including sarcomas, breast cancer and, ACC. **Clinical case:** A 50 yo French Canadian male was evaluated for a palpable lesion in his left flank. Ultrasound showed a retroperitoneal mass of 23 x 17 x 13 cm. The lesion was resected and the pathology confirmed an ACC of 23 cm with negative margins. He was referred to us post-operatively. The endocrine work-up was negative except for increased levels of DHEA-S (9.9 umol/L N:0.5-5.5). However, PET scan, CT-scan and bone scintigraphy showed a unique lytic lesion of the right acetabulum suggesting a bone metastasis. A biopsy confirmed the diagnosis of ACC metastasis that was resected and treated with radiotherapy. Mitotane treatment was started and increased up to 6g per day. The patient was then free of disease for 9 months. Then PET-scan showed 2 new hepatic lesions and 3 pulmonary nodules suspect of metastasis. The patient is now under chemotherapy treatment.

Family history: The patient's mother was diagnosed with breast cancer at 53 yo. Two maternal aunts developed breast cancer at 46 and 53 yo respectively and another one had ovarian cancer at 61 yo. Seven maternal cousins were affected with breast cancers between 29 and 53 yo with 2 diagnosed < 35 yo. A maternal cousin was suspected to be affected with an osteosarcoma at 11 yo. The French Canadian founder mutation 8765delAG *BRCA2* was found to be associated with predisposition to breast cancers in the maternal branch.

Genetic analysis: The patient consented for genetic analysis of *TP53* and *BRCA2* genes. He was found to be a carrier of the familial germ-line mutation 8765delAG *BRCA2*. *TP53* gene sequencing revealed a previously reported polymorphism in exon 4 (c.215C>G, p.Pro72Arg).

Conclusions: To our knowledge, we report here the first case of ACC associated with germ-line *BRCA2* mutation in a family where cancer history was compatible with a Li Fraumeni syndrome. Allelic loss analyses are currently performed on the adrenal tumor to better identify the relationship between this mutation and the ACC tumor. The role of the *TP53* polymorphism as a modifier of risk of other cancer in association with a *BRCA2* mutation has to be investigated.

Sources of Research Support: Grant FRSQ-15907 from Fonds de la Recherche en Sante du Quebec.

Nothing to Disclose: VP, SG, SC, CMM, SN, IB

P3-669

Bilateral Selective Endovascular Embolization of Adrenal Arteries as a Preparative Step for Adrenalectomy in a Patient with Life-Threatening Hypercortisolemia and Ketoconazole-Induced Liver Injury.

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Background: The treatment of choice of ACTH-dependent Cushing syndrome is a transsphenoidal hypophysectomy. If, by any circumstance, the procedure cannot be performed or it is ineffective, other means have to be chosen control hypercortisolemia. In many cases, bilateral adrenalectomy is the only possible and curative approach. However, uncontrolled hypercortisolemia greatly increases the risk of complications.

Clinical case: 49-years old male patient has been admitted to our department because of severe hypercortisolemia caused by recurrent pituitary adenoma. At admission the patient presented with several life-threatening complications including cardiac insufficiency, supraventricular and ventricular tachyarrhythmias, hypoproteinemia and hypokalemia. The patient was diagnosed with ACTH-dependent Cushing syndrome 3 years before. Then, the patient presented with typical clinical and biochemical features of the disease, with 15mm pituitary adenoma infiltrating left cavernous sinus. Due to its extrapituitary expansion leading to incomplete surgical procedure, adjuvant stereotactic radiotherapy was performed. The partial biochemical remission was achieved, and medical treatment was introduced before the delayed effect of radiotherapy could have been achieved. Rosiglitazone and cabergoline at typical doses, were proven to be ineffective, and ketoconazole had to be withdrawn because of acute cholestatic liver injury with jaundice and SGOT and SGPT levels exceeding reference value over 20 times. Other causes of the condition were ruled out. Selective embolization of adrenal arteries was chosen as a safe procedure to reduce hypercortisolemia. After CT angiography one dominant left artery and three right were indicated for the procedure. The left and right arteries were obliterated during two separate procedures. Cortisol levels rose up to 300% immediately after the embolizations, with a subsequent decline to 40-60% of the initial values. General condition and metabolic disturbances of the patient improved greater than could have been expected from cortisol reduction alone, and successful bilateral endoscopic adrenalectomy was performed.

Conclusion: Bilateral selective endovascular embolisation of adrenal arteries can be a safe method of partial reduction of hypercortisolemia in selected cases, where other approaches failed.

Nothing to Disclose: KM, RJ, JW-S, MT-Z, MJ, RW, WK, JS

P3-670

Uneventful Pregnancy after Incidental Mitotane Exposure.

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Background:

Mitotane is an adrenocorticolytic drug used for treatment of adrenal carcinoma (AC) and refractory Cushing's disease. It acts on adrenal cortical cell mitochondria and inhibits CYP11B1 (11-beta hydroxylase) and cholesterol side chain cleavage enzymes (CYP11A1) leading to mitochondrial destruction and subsequent adrenocortical cell necrosis. The mutagenic potential of mitotane is unknown and animal reproductive studies have not been conducted. We report a case of a pregnant woman who was exposed to mitotane during pregnancy with no apparent adverse effects on the infant.

Clinical case:

A 40 year old Hispanic woman who reported a prior tubal ligation, presented with one year history of increasing abdominal girth. CT scan revealed a 30 x 23 cm right sided retroperitoneal mass. PET scan showed uptake in lung and liver consistent with metastasis. Biopsy of the mass was consistent with adrenal carcinoma. She underwent laparotomy with resection of right retroperitoneal mass and right adrenal gland. Pathology was consistent with a stage III AC lesion with extraglandular extension. She was started on mitotane 3000 mg 3 x daily and tolerated therapy well. After 10 months on mitotane she was found to be pregnant with ultrasound confirming a gestation age of 25 weeks with no fetal abnormalities. Mitotane was discontinued. She was evaluated by high risk maternal fetal medicine and started on replacement hydrocortisone. After extensive discussion it was decided to restart therapy with mitotane as the patient had reached late second trimester, beyond the period of organogenesis, and workup was negative for fetal abnormalities. She was closely followed and delivered a healthy 3789 grams baby girl at term. Due to history of cesarean sections, elective surgery was performed.

Conclusion:

Safety of cytotoxic drugs in pregnancy is largely derived from case series and case reports. Mitotane is associated with glucocorticoid and mineralocorticoid insufficiency and is labeled as category C for use during pregnancy due to the potential for permanent fetal adrenal toxicity. Our report of a successful delivery of a healthy fetus after exposure to mitotane suggests that termination of pregnancy may not be mandatory for all cases of accidental exposure of mitotane during early pregnancy.

Nothing to Disclose: SA, MSB, DD, KF-S, MI, LO, NBW

P3-671

Coexistence of Hyperparathyroidism and Adrenocortical Hypersecretion.

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Background: Although the association of hyperparathyroidism (HP) and hypercorticism is rare (1, 2), a relationship between PTH and adrenocortical function has been reported. PTH stimulates both steroid and aldosterone production (3-6), while abnormalities in cortisol and aldosterone levels are frequent in HP (7) and PTH and aldosterone levels are correlated (8). On the other hand, hyperaldosteronism enhances PTH level (9, 10) and adrenal tumor cells are able to synthesize PTHrp (11). In MEN 1, adrenocortical lesions occur frequently but are mostly non functional.

Patients: We report here 6 patients with HP and adrenal overfunction (3 primary hyperaldosteronism (PA), 1 subclinical Cushing syndrome (SCS) and 2 SCS associated to PA).

Case 1: A 48-yo man was investigated for PA. Despite the presence of 2 left adenomas on adrenal CT, adrenal vein sampling (AVS) demonstrated bilateral source of aldosterone secretion. He had a history of nephrolithiasis and a primary HP was diagnosed with scintigraphy suggesting an adenoma. Despite surgical removal of a parathyroid adenoma mild HP persists.

Case 2: A 61-yo woman was first investigated for primary HP. The surgical resection of a parathyroid adenoma normalized calcium and PTH levels. Two left adrenal adenomas were incidentally found. The endocrine investigation revealed SCS and PA.

Case 3: Bilateral adrenal adenomas were incidentally found in a 66-yo man. Diagnosis of SCS and PA were performed. In addition, he had a history of nephrolithiasis and osteopenia associated with HP.

Case 4: A 54-yo woman was followed for HP-induced osteoporosis and nephrolithiasis. She was operated twice but surgery failed to normalize hypercalcemia. Bilateral adrenal adenomas were incidentally found and endocrine work-up revealed SCS.

Case 5 and 6: Two patients (40-yo man and 62 yo-woman) were referred for PA. Adrenal CT and AVS were discordant. In addition, the presence of mild elevated calcemia led to the diagnosis of HP.

No other clinical features of MEN-1 were present in these patients and the search for MEN-1 gene mutations have been negative in 1 patient and are under way in others.

Conclusion: We report the unusual association of parathyroid and adrenal overfunctions in 6 patients. It is unclear whether this association results from a link between PTH and adrenal function or from unidentified common genetic factor other than MEN-1 syndrome.

1. Barkan Aet al., J Clin Endocrinol Metab 1980;51(1):144-7.
2. Ferriss J.B et al., Acta Endocrinol (Copenh) 1983;103(3):365-70.
3. Isales C.M et al., Endocrinology 1991;129(1):489-95.
4. Mazzocchi G et al., Am J Physiol Endocrinol Metab 2001;280(2): E209-13.
5. Rafferty B et al., Endocrinology 1983;113(3):1036-42.
6. Rosenberg J et al., J Endocrinol 1987;112(3): 431-7.
7. Rajput R et al., Indian J Med Res 2009;130(4):418-22.
8. Brunaud L et al., Surgery 2009;146(6):1035-41.
9. Resnick L.M. et al., Am J Med 1985;78(3):385-90.
10. Selektor Y et al., Am J Med Sci 2008;335(2):105-10.
11. Rizk-Rabin M et al., Cancer Epidemiol Biomarkers Prev 2008;17(9):2275-85.

Sources of Research Support: Grant MT-13189 from the Canadian Institutes of Health Research.

Nothing to Disclose: SG, TLM, IB, AL

P3-672

Neuroendocrine ACTH Producing Carcinoma of the Thymus - Experience with 10 Patients.

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Neuroendocrine ACTH-producing tumor (NET) of the thymus gland is a rare cause of Cushing's syndrome. The literature is mainly single case reports. To understand better the presentation, treatments and outcomes we reviewed 10 cases (7 men and 3 women) seen at the National Institutes of Health from 1986-2010. Patients presented with classic Cushing's features at relatively young age (7-51 years, median 23). Four were children aged 7-17. 24-hour urinary free cortisol was high (median 50 times the upper limit of normal, range 16-104 fold elevated). Lab results were consistent with ectopic ACTH production with a lack of peripheral to central gradient on inferior petrosal vein sampling. Initial chest CT was performed 5-55 months (median 10) after the onset of Cushing's syndrome and localized a thymic tumor in 9/10 cases. In 1 case, imaging 2 years later localized the tumor. All patients underwent thymectomy and 7/8 tumors examined stained for ACTH. Tumor volume ranged from 1-512cm³, median 30cm³. Two tumors were Masaoka stage I (localized), 3 stage II (positive nodes) and 4 stage III (adjacent tissue invasion). Two stage III patients were treated adjuvantly – one with chemoradiation and one with radiation. Six patients had symptomatic recurrence 20-28 months after surgery (median 25), including the 2 treated with adjuvant therapy, with metastases to the neck and mediastinum in 5 and liver in one. 4/5 treated with radiation also received chemotherapy. All recurrent patients received ketoconazole; 3 later had bilateral adrenalectomy. Five recurrent patients died 50-90 months after thymectomy (median 57). At last review 5 patients were alive 3-60 months (median 21) after thymectomy. Thymic ACTH-producing NET can be an aggressive disease that may occur in children and young adults. While the tumors are usually large they may be as small as 1cm³ making localization challenging. We suggest that thymic NET be considered in patients with features of ectopic ACTH production and rapid clinical presentation, particularly in children.

table1

| Case | Presentation age | Months to diagnosis | Tumor volume (cm ³) | Stage | Adjuvant therapy | Alive/dead | Months after surgery |
|------|------------------|---------------------|---------------------------------|---------|------------------|------------|----------------------|
| 1 | 13 | 27 | 512 | III | - | alive | 3 |
| 2 | 17 | 9 | 81 | II | - | alive | 4 |
| 3 | 50 | 10 | 5 | I | - | alive | 21 |
| 4 | 24 | 72 | 15 | II | - | alive | 39 |
| 5 | 7 | 25 | 1 | I | - | alive | 90 |
| 6 | 51 | 7 | 280 | III | - | dead | 50 |
| 7 | 27 | 41 | 160 | III | chemoradiation | dead | 56 |
| 8 | 40 | 7 | 3 | II | - | dead | 57 |
| 9 | 21 | 9 | 30 | III | radiation | dead | 72 |
| 10 | 14 | 36 | unknown | unknown | - | dead | 90 |

Sources of Research Support: In part by the NIH intramural programs of NICHD and NCI.

Nothing to Disclose: NMN, AL-C, AMB, BSA, CAS, GG, LKN

P3-673

Five Cases of Ectopic ACTH Syndrome.

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Background. Ectopic ACTH secretion (EAS) tumor is a wide spectrum of tumors from isolated lesions to widespread metastatic malignancies.

Clinical cases. We presented 5 patients (3 female, 2 male) 43,0 [18,0; 54] years old with EAS tumors observed in our clinic. EAS was diagnosed in 2001 for one patient and in 2008-2009 for others. Physical examination revealed at first observation moon-like face, muscle wasting, weakness and purple striae (in all patients); significant weight gain and centripetal obesity (4); edema in the lower extremities and ostealgia (3); acanthosis nigricans (2), hypertrichosis (2), alopecia (2), acne (2), psychiatric disorders (2); significant weight lost (1), skin hyperpigmentation (1). After clinical examination severe hypertension (5), pyoinflammatory process (5), diabetes mellitus (3) and osteoporosis (2) have been revealed. Laboratory assessment revealed high serum cortisol level 1704,5 [1465-2087] nmol/l, high plasma ACTH level 46,35 [22,4-347] pmol/l, hypokalemia (4 cases) and hypercholesterolemia (2). Predisposition to thrombosis on the coagulogram was revealed in 3 cases.

Imaging tests were used to determine the EAS localization.

Surgical intervention on the primary tumor was performed to 4 patients. Completely tumor removal achieved only in 2 cases. One patient was inoperable at the moment of hospitalization. The patient died in several days after diagnosis have been made (pancreatic islet cell tumor). Histological assessment has been revealed ACTH-producing cell in small cell lung cancer, bronchial carcinoid tumor, thymoma, pancreatic islet cell tumor and medullary carcinoma of the thyroid. Proliferation indexes (Ki 67) were = 9 [2; 13,5]%. Metastasis were revealed in 2 cases (pancreatic islet cell tumour – metastasis were in liver and kidneys; medullary carcinoma of the thyroid - in bones). After surgical tumor removal, clinical symptoms improved in 3 patients. In one patient adrenal enzyme inhibitors has been prescribed for a long period of time because of impossibilities of completely tumor removal, refused from adrenalectomy and severe hypercortisolism progression. On adrenal enzyme inhibitors his cortisol level is in normal range.

Conclusion: Only 1 patient with EAS died during observation period. EAS caused severe hypercorticism in all patients. Completely removal of tumor can lead to resolve severe hypercorticism. Adrenal enzyme inhibitors can be prescribed in other cases with good results.

Nothing to Disclose: IVK, AVD, OAN, TAB, AVM, EIP

P3-674

A Case of ACTH-Independent Cushing's Syndrome Due to Primary Pigmented Nodular Adrenocortical Disease.

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Background: Primary pigmented nodular adrenocortical disease (PPNAD) is a rare cause of ACTH independent Cushing's syndrome (CS), seen in about 1% of cases. This is diagnosed mainly in children and young adults. PPNAD is most commonly seen in patients with the Carney Complex (CNC). We report a case of CS due to PPNAD in a child with positive family history of hypercortisolism but without features of CNC.

Clinical Case: A 10 year old boy presented with weight gain and increased facial and pubic hair over the past few months. Family history was significant for CS in his mother diagnosed at age 12, and managed by bilateral adrenalectomy. No pathology records were available for review. His initial tests were consistent with ACTH-independent CS: elevated urine free cortisol (UFC) of 165 mcg/24h and 190 mcg/24h (Ref range 1 - 45) and a suppressed plasma ACTH level of <5 pg/ml (Ref range: 7-50). A low dose Dexamethasone suppression test was done with 0.5mg q 6hrs for 2 days which revealed an 8am Cortisol of 21.7 mcg/dl with an ACTH of <5 pg/ml. Abdominal CT scan and ultrasound showed normal adrenal glands bilaterally. The Liddle's test was performed and showed lack of suppression of AM cortisol with low dose and high dose Dexamethasone, and an increase in UFC on days 5 and 6 following administration of high dose Dexamethasone (405 ug/g and 414.29 ug/g, normal <37). Thyroid ultrasound and cardiac echocardiography did not reveal any features of CNC. Genetic testing for alterations in the PRKAR1A gene, seen commonly in cases of PPNAD with CNC, did not show any deletions or duplications. The patient underwent a laparoscopic bilateral adrenalectomy and the adrenal glands were reported as being normal in appearance. Pathology revealed bilateral PPNAD. He is now doing well on replacement glucocorticoid and mineralocorticoid therapy.

Conclusion: PPNAD presents with typical features of ACTH-independent CS. Up to 90% of cases of PPNAD may be associated with CNC and a mutation in the PRKAR1A gene. Screening for cardiac and thyroid manifestations of CNC is imperative in all patients with PPNAD at diagnosis and future visits. There have been case reports of isolated PPNAD which also have alterations in the same gene. However, our patient displayed isolated PPNAD with a positive family history of CS, but with no identifiable mutation in PRKAR1A. This indicates that there may be unidentified mutations in cases of isolated PPNAD that still have a familial component.

Nothing to Disclose: SRP, LR, MWH

P3-675

Illicit Expression of Glucagon Receptors (GR) in Adrenal Glands (AG) of a Patient with Cushing's Syndrome (CS) Related to ACTH-Independent Macronodular Adrenal Hyperplasia (AIMAH).

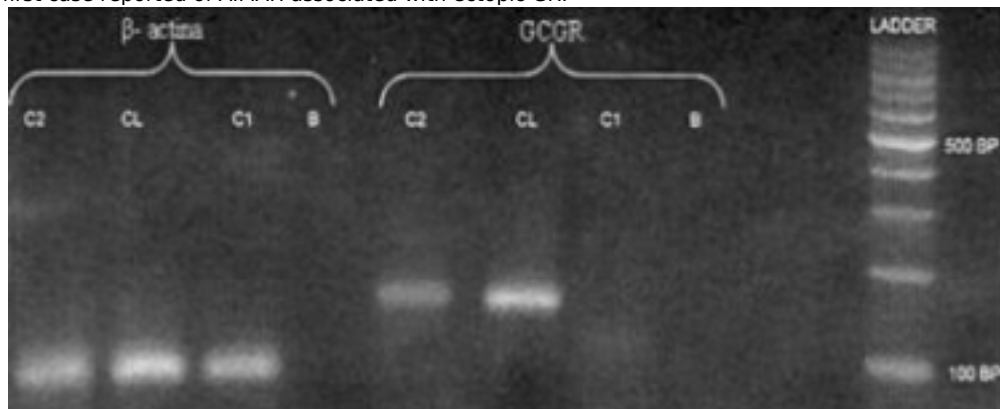
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Various aberrant receptors functionally coupled to steroideogenesis in AIMAH pathogenesis related to CS have been described.

A 64-year old man presented with typical CS. Biochemical studies revealed: elevated 24- hr urinary free cortisol (UFC) 250 µg (<100), elevated late- night salivary cortisol: 24 nmol/L (0.7- 5) and abnormal 1- mg overnight dexamethasone suppression test: 32 µg/dl (< 5). Morning plasma ACTH was undetectable: <10 pg/ml (10 - 46), confirmed in three assays. An abdominal computed tomography scan showed bilateral macronodular adrenal hyperplasia. To screen for aberrant adrenal expression of G-protein coupled receptors, mixed meal and LHRH, ACTH an glucagon tests were performed . The results showed increased levels of serum cortisol more than 300% after stimulation with glucagon. Hypercortisolism was successfully managed with ketoconazole treatment. 4-month treatment with octreotide LAR was also able to reduce cortisol secretion. CS was cured after bilateral adrenalectomy. Histological examination showed macronodular adrenal hyperplasia of the adrenal cortex. After laparoscopic removal, adrenal cortical tissue was processed, total RNA was extracted from the patient AG. cDNA was obtained using RT polymerase Improm II (Promega) and used as a template for RT-PCR assay. Hepatocarcinoma cell line, normal adrenal from a control subject and β-actin were used as positive, negative and amplification controls, respectively. RT-PCR was performed with specific glucagon receptors (GR) primers. As shown in figure 1, the PCR product corresponding to GR mRNA was present in patient AG but absent in normal control gland.

These results suggest that the mechanism of AIMAH causing CS involves the illicit activation of adrenal GR. This is the first case reported of AIMAH associated with ectopic GR.



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Electrophoresis in agarose gel

Control β-actine, a PCR product of 122 bp: line 1: patient AG(C2); line 2: hepatocarcinoma cel line(CL), line 3: normal AG (C1); line 4: negative control assay (B). PCR product of 148 bp RNAm GCGR is present in line 5/C2, line 6/CL, absent in line 7/C; line 8/B

Nothing to Disclose: VCdM, MAR, MLV, MK, MG, AB, PFD

P3-676

Remission of ACTH-Independent Macronodular Adrenal Hyperplasia (AIMAH) by Unilateral Adrenalectomy.

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AIMAH, a rare cause of Cushing's syndrome (CS) is a macronodular, bilateral adrenal disease characterized by aberrant adrenal receptors. It is usually sporadic, although autosomal dominant familial clustering is reported. Bilateral adrenalectomy has been the treatment of choice, but in several reported cases, unilateral adrenalectomy resulted in normal adrenal function up to 7 years after surgery. In all cases the larger gland was removed; in one case, removal was based on adrenal vein sampling (AVS). We report a second case of unilateral adrenalectomy after AVS resulting in a short-term "cure".

A 31 y.o. female with a 2 year history of infertility and 80 lb weight gain had classical signs of CS; hypertension, buffalo hump, centripetal obesity, and striae. Her grandmother underwent bilateral adrenalectomy for CS at the NIH ~30 years ago. Labs: a.m. pl cortisol (PC): 24 ug/dl, a post-1 mg dexamethasone PC: 27 ug/dl, 24 h UFC: 267 mcg/24h and ACTH < 5 pg/ml. MRI revealed multiple large bilateral adrenal nodules. The largest one, in the L adrenal, was 2.1 x 2.5 cm and on the right was 1.8 x 2.2 cm. Pituitary MRI was normal. Preoperative AVS results:

Table 1

| | Cortisol | Aldosterone |
|-------|-----------|-------------|
| Left | 378 ug/dl | 373 ng/dl |
| Right | 941 ug/dl | 1141 ng/dl |
| IVC | 103 ug/dl | 129 ng/dl |

Despite the higher R adrenal PC, a left laparoscopic adrenalectomy was performed because the largest adrenal nodule was in the left gland. Surgical Path (L adrenal): a 56 gm, 12 x 4 x 1.5 cm gland. Sectioning revealed multiple round/yellowish, poorly encapsulated nodules with the largest one measuring 3 cm in diameter.

The pt did surprisingly well following the L adrenalectomy in light of the higher cortisol level in the R adrenal gland at AVS. At one month post-op, she had already lost 25 pounds and had a normal BP. Her am PC had decreased to 15.3 mcg/dl and her 24 h UFC decreased to 171mcg/24h. Five months postop, the patient's lab values had normalized (PC: 12.0 mcg/dl, UFC 11 mcg/24 h), she had lost more weight and most of her buffalo hump disappeared. This case demonstrates several important points:

- a) Unilateral adrenalectomy may result in prolonged remission of CS
- b) Pre-operative AVS, while possibly misleading in this case, deserves further study.

Conclusion: Unilateral adrenalectomy should be considered as initial treatment for AIMAH. In the event of clinical remission, the patient will still require prolonged observation but may be spared the problems associated with life-long Addison's disease.

(1) Kirschner MA et al. JCEM 1964; 24: 947-955

(2) Iacobone M et al. World J Surgery 2008; May 32 (5): 882-889

Nothing to Disclose: SAP, NHE, MR, MJ, DB

P3-677

Hemi-Adrenalectomy and Propranolol Administration as Effective Treatments in ACTH-Independent Macronodular Adrenocortical Hyperplasia with Subclinical Cushing's Syndrome.

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Background ACTH-independent macronodular adrenal hyperplasia (AIMAH) is an extremely rare disorder characterized by cortisol over-secretion from the adrenal cortex independent of ACTH. The cortisol secretion has been shown to be mediated by the aberrant expression of receptors for a variety of hormones. The optimal therapeutic strategy for the metabolic disorders in AIMAH with subclinical Cushing's syndrome has been remain uncertain.

Clinical Case A 56-year-old man with hypertension was referred for the evaluation of multiple bilateral adrenal tumors. While Cushingoid features were not apparent, an elevated cortisol level (6.1 µg/dL) in response to a 1-mg dexamethasone suppression test (DST), and a weak response to a cosyntropin-releasing hormone (CRH) provocation test were observed. Furthermore, the serum cortisol level increased in response to a posture test, isoproterenol infusion, or vasopressin administration. Accordingly, the patient was diagnosed as having AIMAH with subclinical Cushing's syndrome associated with the aberrant expression of β-adrenergic and vasopressin receptors. After right adrenalectomy, the serum cortisol level in response to 1-mg DST showed 2.9 µg/dL. In addition, the cortisol level by 1-mg DST decreased to 2.2 µg/dL after one year of propranolol therapy. The responses of serum cortisol levels due to isoproterenol infusion or vasopressin administration decreased compared to before the therapies. The homeostasis model assessment (HOMA-IR), which is an index of insulin sensitivity, was improved after adrenalectomy and propranolol therapy (2.6 to 1.7).

Conclusion This is an extremely rare case of AIMAH accompanied by subclinical Cushing's syndrome associated with the aberrant expression of β-adrenergic and vasopressin receptors. Furthermore, unilateral adrenalectomy and propranolol inhibited cortisol hypersecretion in the present case. Thus, it is thought that unilateral adrenalectomy and propranolol might be a potential therapeutic option for AIMAH accompanied by subclinical Cushing's syndrome associated with β-adrenergic receptor expression.

Nothing to Disclose: MM, KO, TS, ND, TA, SN, KY, NK

P3-678

Clinical and Molecular Studies of Macronodular Adrenocortical Hyperplasia of the Zona Reticularis, a New Syndrome.

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A 29 YO Caucasian male was evaluated for a 5 year history of progressive bilateral adrenal enlargement, which was incidentally discovered on a computed tomography (CT) scan for nephrolithiasis. He had fathered two children and had no family history of adrenal disorders or intersex children. Physical exam showed no features of Cushing syndrome or hyperandrogenism. The 24 hour urinary free cortisol, serum testosterone, and plasma ACTH were normal, and the serum cortisol rose to >20 mcg/dL with normal 17-hydroxyprogesterone and 11-deoxysteroids after cosyntropin administration. These screening tests excluded ACTH-independent hypercortisolism and congenital adrenal hyperplasia. Repeat CT scan showed massively enlarged, heterogeneously enhancing adrenal glands (right 13.8 x 11.2 x 9.2 cm, left 10.5 x 9.5 x 11.4 cm). After dexamethasone, 2 mg/d for 4 days, the cortisol suppressed to <1 mcg/dL, but the DHEA and DHEAS remained markedly elevated at 22,893 ng/dL and 2,036 mcg/dL, respectively. The patient underwent bilateral adrenalectomy for compressive symptoms and gave written informed consent for use of the adrenal tissue in research studies. Histology showed macronodular cortical hyperplasia. Incubation of tissue homogenates with [³H]-labeled pregnenolone, progesterone, and 17-hydroxyprogesterone showed robust 17-hydroxylase and 17,20-lyase activities but low or absent 21-hydroxylase and 3 β -hydroxysteroid dehydrogenase/isomerase activities. Immunoblot and microarray analyses confirmed high CYP11A1 and CYP17A1 protein and mRNA but low or absent HSD3B1, HSD3B2, and CYP21A2 expression. Expression of cytochrome *b₅* and AKR1C3, markers of the zona reticularis, was markedly elevated. These studies demonstrate that this patient had macronodular hyperplasia of the adrenal zona reticularis, a new clinical syndrome. We speculate that this condition can be clinically silent in men but might cause severe hyperandrogenemia in women.

Sources of Research Support: Burroughs-Wellcome Fund, Clinical Scientist Award in Translational Research #1005954 to RJA.

Nothing to Disclose: HKG, JR, LMW, FEN, WER, RJA

P3-679

Sudoreses as First Manifestation of Cushing's Syndrome ACTH-Independent Macronodular Adrenal Hyperplasia.

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Background-ACTH-Independent macronodular adrenal hyperplasia (AIMAH) is a rare cause of endogenous Cushing's syndrome (CS), in which clinical features usually become apparent only after several decades of life. This form of adrenal hyperplasia typically produces excess cortisol with overt or subclinical CS, however, in asymptomatic individuals in whom the AIMAH is incidentally discovered, the HHA axis is usually disrupted. The diagnosis is suspected by bilateral adrenal nodules on incidental imaging studies or following the demonstration of ACTH-independent hormonal hypersecretion (1). Most AIMAH cases, cortisol secretion is aberrantly regulated by hormones such as GIP, AVP, adrenergic agonists, LH/hCG and serotonin, acting through their specific receptors. The molecular mechanisms responsible for ectopic expression of such hormone receptors and/or their aberrant coupling to steroidogenesis are unknown (2).

Case: A 38-year woman exhibited mild hypertension and excessive sudoreses. Her BMI was 27,5Kg/m². Initial tests corroborated the diagnosis of ACTH independent CS: elevated 24hr urinary cortisol secretion (670;732 and 618 mcg/24hr, n< 140/24hr), cortisol after 1 mg dexamethason overnight test (19,9 and 22,7 mcg/dl, n<1,8 mcg/dl), elevated midnight serum cortisol (14,4 mcg/dL n<7 mcg/dL), measurement of suppressed plasma ACTH by immunometric assay concentrations below(< 5ng/l). An abdominal CT-scan showed bilateral macronodular adrenal hyperplasia (diameter of adrenal glands 23x21 mm left and 19x17 mm right). A screening protocol of abnormal hormone receptor revealed no change in cortisol secretion in response to GNRH, TRH, food, posture, metoclopramide, Glucagon, and terlipressina. The patient was submitted to unilateral laparoscopic adrenalectomy, the histological examination showed macronodular hyperplasia of the adrenal cortex. Immune-histochemic was positive to inibin, melan A and sinaptofisina. Hypercortisolism and sudoreses persisted as bothering manifestations even after surgery and beginning of the pharmacological treatment with Ketoconazol.

Conclusion: Patients with AIMAH show a big heterogeneity of clinical manifestations, for example, the presented case showed an atypical sudoreses as one the first manifestations. Aberrantly expressed receptors in the adrenal cortex appear to play a central role in the hormonal hypersecretion in this disease, but in some cases, like the one presented herein, other unknown mechanisms are possibly implicated.

(1) Costa MHS, Lacroix A: Cushing's Syndrome Secondary to ACTH-Independent Macronodular Adrenal Hyperplasia. *Arq Bras Endocrinol Metab* 2007; 51/8

(2) Lacroix, A.,N'diaye, N.,Tremblay, J.,Hamet, P. Ectopic and abnormal hormone receptors in adrenal Cushing's syndrome. *Endocr. Rev.* 2001;22;75-110

Nothing to Disclose: MPF, MSN, DRdM, APCN, LAP

P3-680

Too Much of a Good Thing: Polycythemia and Aromatase Inhibitors.

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Background:

Aromatase inhibitors such as letrozole are used with increasing frequency to treat pubertal males with short stature and restricted height prediction. Concerns about the untoward effects of these medications have been raised. We describe two boys in whom letrozole administration was associated with substantial polycythemia.

Case Reports:

Patients 1 and 2 were Caucasian males with intrinsically limited growth potentials. Patient 1 was evaluated at 11:1y, height age (HA) was 7:9y; genital development was Tanner stage II; bone age (BA) was 8y; predicted adult height was 165cm. By age 12:6y, male genital development was Tanner stage III; BA was 11:6y. Letrozole (2.5 mg/day) was begun. After 24 months of letrozole administration, height velocity accelerated (from an average of 6.8cm/yr to 9cm/yr) and the rate of bone age maturation declined (Δ CA/ Δ BA from 0.4 to 1.73 yrs). Hemoglobin and hematocrit values increased coincident with supraphysiologic levels of testosterone and modest hyperbilirubinemia (Table).

Patient 2 was initially evaluated at 7:6y when HA was 5:3y; there was Tanner stage I male genital development; BA was 6y; predicted adult height approximated 160cm. He had a normal prepubertal growth rate for 18 months but then failed to return. At age 14:3y, male genital development was Tanner stage III; BA at 13:5y was 12:6y. Letrozole (2.5 mg/day) was begun. During 13 months of letrozole administration, height velocity accelerated (from 6cm/y to 8.8cm/y), and BA matured 1y. Hemoglobin and hematocrit levels had increased coincident with supraphysiologic levels of testosterone and slight hyperbilirubinemia (Table).

| | Patient 1 | | Patient 2 | |
|--------------------|-----------|------|-----------|------|
| | Pre | Post | Pre | Post |
| Hgb g/dL | 14.1 | 17.5 | 14.3 | 16.0 |
| Hct % | 41 | 50 | 40 | 46 |
| LH mIU/mL | 1.6 | 9.6 | 2.5 | 10.7 |
| FSH mIU/mL | 1.8 | 7.3 | 1.1 | 0.9 |
| Testosterone ng/dL | 321 | 1240 | 109 | 1450 |
| Estradiol ng/dL | 0.44 | 0.7 | 0.24 | 0.14 |
| Bilirubin mg/dL | 0.5 | 2.4 | 0.5 | 1.2 |

Conclusion:

The use of aromatase inhibitors in peripubertal males leads to significant increases in testosterone levels. Androgens exert a direct stimulatory effect on differentiation of a pluripotential stem cell to erythroid precursors leading to polycythemia. Treatment of these patients with an aromatase inhibitor resulted in elevated hemoglobin and hematocrit values. We recommend that in addition to determining lipid levels and bone mineralization, erythropoiesis be monitored in adolescent males receiving an aromatase inhibitor.

Nothing to Disclose: ADTD-T, DISS, AWRR

P3-681

Follow up of Pseudoprecocious Puberty Associated with Functional Ovarian Cysts.

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We report the findings and clinical course of six girls aged 1.2 to 6.9 years with precocious pseudopuberty due to functional ovarian cysts. All patients presented with breast development and vaginal bleeding. Five of six girls had a unilateral ovarian cyst detected by ultrasound at the first episode. The sizes of cysts were 17-46 mm. Plasma estradiol concentration was raised (25.7-382 pg/mL) in five patients, but was within the normal prepubertal range (<15 pg/dL) in two.

Following the initial episode the secondary sexual characteristics of all patients regressed spontaneously without treatment at the latest after 2 to 3 months. Follow up lasted for up to seven years with recurrent episodes of variable frequency and severity in all six patients. Three patients presented recurrent functional ovarian cysts. In two of three girls, the bone age was rapidly advanced. But they did not develop central precocious puberty after repeated episodes of precocious pseudopuberty. None of the patients showed any of the pigmented skin lesions or bone dysplasia characteristic of McCune-Albright syndrome.

Functional ovarian cysts represent a rare, self-limiting endocrine disorder in prepubertal girls. This episode may be a single event or there may be recurrences with similar clinical features at unpredictable interval. Sustained estrogen activity can accelerate skeletal maturation. Most patients with recurrent ovarian cysts require a conservative approach. In general, surgery is not indicated but should be very conservative in case of ovarian torsion.

Nothing to Disclose: IJC, HJK, JHY

P3-682

Sclerosing Stromal Tumor of the Ovary with Masculinizing in a Premenarchal Girl.

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Background: Sclerosing stromal tumor of the ovary is an extremely rare benign neoplasm that is mainly in the second and third decades of life with a mean age of 27. Patients have pelvic pain or palpable abdominal mass. Hormonal effects include menstrual cycle disturbance or masculinizing are usually uncommon. There have been fewer than 100 case reports of this rare tumor and most of cases have described women who have undergone menarche.

Clinical case: An 11-year old premenarchal girl presented with deepening of voice. She showed clitoromegaly and hirsutism with a male suprapubic hair pattern. Endocrine evaluation revealed elevated testosterone, androstenedione and dehydroepiandrosterone-sulfate levels. CYP21A2 gene encoding steroid 21-hydroxylase showed normal. Ultrasound examination showed a heterogeneous left ovarian tumor consisting of predominantly solid tissue. Pelvis MRI showed an 8.9x6.2x6.6 cm sized mass on the left ovary. Left oophorectomy was performed and microscopic examination confirmed sclerosing stromal tumor. Immunohistochemical studies showed a positive vimentin reaction and smooth muscle actin stains. Following surgery, the hormones level recovered to normal range and the hirsutism resolved.

Conclusion: We present the unique case of sclerosing stromal tumor of ovary with virilization occurring in a premenarchal girl.

Nothing to Disclose: CJK, YJW

P3-683

Vaginal Bleeding during Treatment of Central Precocious Puberty with a Long-Acting Gonadotropin-Releasing Hormone Agonist.

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Background : Treatment of central precocious puberty with a gonadotropin-releasing hormone agonist can cause complications like growth retardation, postmenopausal syndrome-like symptoms, and transient vaginal bleeding. Transient vaginal bleeding can occur after the first or second injection. However, no case of vaginal bleeding in the middle of the treatment has been described. We encountered a case of transient vaginal bleeding in the middle of the treatment with a long-acting gonadotropin-releasing hormone agonist.

Clinical case : A girl aged 7 years-10-months was admitted for breast budding and acne. She was at the 97th percentile in height and the 75th to 90th percentile in weight. Her breast development was of Tanner stage 2. Laboratory findings revealed that her bone age was equivalent to that of a 10-year-old and LH, FSH, and E2 levels were 1.68 IU/L, 2.46 IU/L and 26 pg/mL, respectively. The volume of the ovaries was 5.6 and 3.6 cm³, and the uterus measured 4.8 × 1.5 cm. The gonadotropin stimulation test revealed that the luteinizing hormone (LH) level peaked at more than 5 IU/L. The patient was treated with 3.75 mg (110 mg/kg) of long-acting leuprolide acetate for central precocious puberty. For the seventh injection, leuprolide acetate was replaced with 3.75 mg (110 mg/kg) of triptorelin due to the complain of the high cost. After the tenth injection, the patient developed vaginal bleeding and lower abdominal pain that persisted for 3 days. No abnormal mass or cyst was detected by pelvic ultrasonography. At this time, the volume of her ovaries was 3.9 cm³ and 4.6 cm³, and her uterus size was 3.2 × 1.6 cm, without endometrial thickening. The hormone levels remained unremarkable, and a gonadotropin stimulation test revealed a low LH response (peak LH level, <5 IU/L). Triptorelin was once again replaced with leuprolide acetate, and the vaginal bleeding ceased.

Conclusion : Vaginal bleeding may occur in the middle of the treatment of central precocious puberty with a long-acting gonadotropin agonist. However, other causes of vaginal bleeding, apart from treatment with a long-acting gonadotropin agonist, should be explored.

Nothing to Disclose: SHP, MHJ, WKC, BKS

P3-684

Central Precocious Puberty Presenting with Vaginal Bleeding.

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Introduction: Central precocious puberty results from activation of the hypothalamic-pituitary-gonadal axis and can be diagnosed with basal LH levels over 0.6 IU/L, FSH levels over 2 IU/L and stimulated peak post GnRH agonists LH levels over 6.9 IU/L and FSH levels over 5 IU/L. (1) Isolated menses is uncommon. It has been suggested that increased endometrial sensitivity to estrogen and/or ovarian stimulation may be the cause for this bleeding.(2)

Clinical Case: 2-3/12 year old AA healthy female born full-term presents with 3 month history of vaginal bleeding. Bleeding occurs monthly with bright red blood and lasts for 2 days. Initial day is described as bleeding that will fill a pull-up diaper and the second day is spotting. No breast development, estrogen exposure, or height acceleration is noted. No known history of abuse. Family history is significant for father starting puberty between 9-10 years of age and mother achieving menarche at 10 years of age. Prior evaluation included normal renal and bladder ultrasound. Cystoscopy and vaginoscopy with an almost occluded hymen and somewhat open cervix. Physical examination revealed height at the 90th percentile, weight and BMI over the 97th percentile and otherwise normal except for a large cafe-au-lait spot (5.5-2.5 cm) on the left neck. Tanner 1 breasts and pubic hair with prepubertal vaginal mucosa. Initial evaluation as follows: pediatric LH= 0.14 mIU/mL (<0.21), FSH= 1.47 mIU/mL (0.5-4.5), ultrasensitive estradiol= 9 pg/mL (<16), PRL = 20.7 ng/mL (<25), normal TFT's, bone age of 3 years, and pelvic ultrasound revealed prepubertal uterus with 3mm endometrial stripe, right and left ovaries measured 0.4 mL. Vaginal bleeding continued monthly and Leuprolide stimulation test was done. Baseline LH was 0.1 mIU/mL and baseline FSH was 2.9 mIU/mL. 90 minute LH was 5 mIU/mL and FSH was 26.7 mIU/mL. MRI of the brain was negative. Because vaginal bleeding continued, 7.5 mg Lupron depot injections were started. 1 month after therapy patient was diagnosed with a seizure disorder with a repeat negative MRI of the brain. Two months after initiation of therapy, vaginal bleeding had stopped.

Conclusion: Our patient presented with isolated menarche despite not meeting the strict criteria for central precocious puberty, it appears as if patient does have the diagnosis since vaginal bleeding has stopped with GnRH agonist therapy. It is possible her underlying undiagnosed seizure disorder was the cause of her precocious puberty.

(1)Sperling et al., Pediatric Endocrinology. 2nd Edition. 483-488

(2)Blanco-Garcia M et al., Isolated Menses in Prepubertal Girls. Pediatrics 1985; 43-47.

Nothing to Disclose: MSD, JGB

P3-685

Rational Approach to the Diagnosis of Severe Growth Hormone Deficiency in the Newborn.

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Context and objective

Severe congenital growth hormone deficiency (GHD) of the newborn is a rare disease, which can cause life-threatening hypoglycemias beginning in the first week of life. Reviews and consensus papers on the diagnosis of GHD repeatedly state the lack of a practical evidence-based approach to the diagnosis of GHD in the newborn. Here, we provide for the first time sound reference values and a diagnostic cut-off for the growth hormone (GH) levels in newborns at the age between the days 3 to 5.

Design, Setting and Patients

GH was measured in the eluate from 314 filter papers of the newborn screening test performed in our University Hospital by using a highly sensitive hGH-ELISA. Reference data are compared with measurements from 9 newborns with very high likelihood of having severe GHD, and cut-offs for the diagnostic work-up are defined.

Results

In the presence of clinical evidence, the diagnosis of neonatal GHD can be confirmed during the first week of life by a single randomly taken GH level < 7µg/L with 100 % sensitivity and 98 % specificity on the basis of our assay method. GH content in newborn screening cards stored for almost three years were not different to the content found in recently used screening cards indicating high immunological stability of GH over time. Therefore, the diagnostic approach can utilize stored screening cards. In addition, we observed a clear gender dichotomy in respect to GH with healthy female newborns having significantly higher GH levels than males. Cigarette smoking during pregnancy was associated with higher, transient tachypnea of the newborn with lower GH levels.

Conclusions

We provide the first rational approach to the diagnosis of severe GHD in the newborn and evidence for gender dichotomy of the neonatal GH axis.

Disclosures: GB: Research Funding, Novo Nordisk, Pfizer Global R&D; Speaker, Lilly USA, LLC, Novo Nordisk, Pfizer Global R&D. ML: Employee, Mediagnost Company.

Nothing to Disclose: MW, GB, KW, AKF

P3-686

A Common Polymorphism in the Insulin-Like Growth Factor 2 Gene Is Associated with Late Antenatal Growth, Birth Size and Size at 3 Years of Age.

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Insulin-like growth factors (IGF) play an important role in antenatal and postnatal growth. Genotype differences in IGF-2 have been associated with weight differences in adult populations and cord IGF-2 concentrations are associated with birth size. We analysed the impact of differing IGF-2 alleles (Apa I; alleles AA;AG;GG) on ante and post natal growth in a cohort of children drawn from the UCL Fetal and Postnatal Growth Study.

We genotyped 449 cord blood samples chosen at random from a cohort of 1650 singleton, white Caucasian pregnancies who delivered at term and related antenatal biometric data as well as measures of birth size and size at 3 years of age to the IGF-2 Apa I genotype. One way analyses of variance (ANOVA), with the Tukey honest significant differences (HSD) post hoc test was used to test significance.

The IGF-2 Apa I AA genotype was significantly associated with birth size and size at 3yrs of age (Table). IGF2 Apa I genotype was not significantly associated with cord IGF-2 concentrations and biometric markers of antenatal growth as well as placental weight.

| IGF2 ApaI SNP | GG | AG | AA | p |
|---|-------------------|-------------------|--------------------|--------|
| 3rd trimester Abdominal Circumference (cm) | 28.7 (18) | 28.6 (16) | 28.1 (16) | 0.26 |
| Birth weight SDS | 0.16(0.9) (n=236) | 0.13(0.9) (n=180) | -0.22(0.78) (n=33) | 0.025 |
| Birth Length SDS | -0.10(1) | -0.13(1.2) | -0.52(0.97) | 0.04 |
| Cord IGF2 (ng/ml) | 526.4(118) | 520(123) | 494(109) | 0.371 |
| 3 years Weight SDS | 0.15(1.0) (n=118) | -0.03(0.9) (n=76) | -0.7(0.9) (n=22) | 0.0005 |
| 3 year Height SDS | 0.2(1.1) | 0.2(0.9) | 0.04(1.0) | 0.44 |

Data shown as mean with standard deviation in parentheses.

These data suggest that the IGF-2 Apa I polymorphism does not influence antenatal growth up to 30 weeks of gestation. Between 30 weeks and term the polymorphism may influence fetal growth in terms of weight and length with further influences out to 3 years of age. This polymorphism is associated with a 205g difference in birth weight (similar effect size to smoking in pregnancy) and a 1.7 kg difference in weight at 3 years of age suggesting an amplification of effect size with age. The lack of effect on cord blood IGF2 concentration and placental weight suggests an effect mediated more at the tissue/organ level or that the polymorphisms are a marker for other changes in nearby growth loci.

Nothing to Disclose: RP, SB, REH-D, JCA, SEH, PCH

P3-687

Pharmacokinetics (PK) of rhGH/rhIGF-1 Co-Administration Therapy Given to Short, Prepubertal Children with Low IGF-1 Levels.

B Reiner MD¹, JW Frane PhD² and GM Bright MD³.

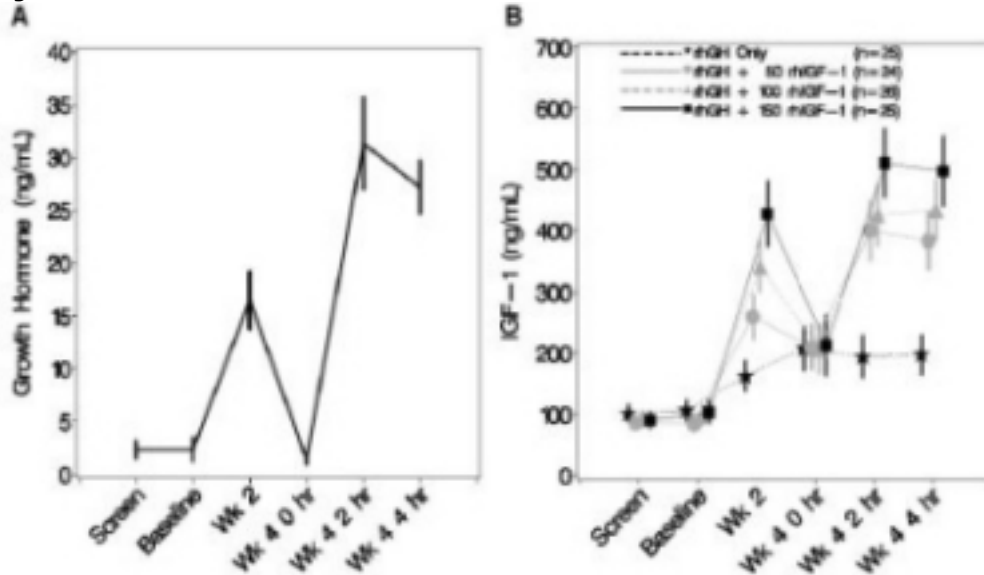
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Introduction: This PK study describes the serum changes in IGF-1 and GH when rhGH/rhIGF-1 co-administration (co-admin) or rhGH monotherapy is given to short, prepubertal children with low IGF-1 and normal GH levels (IGFD).

Methods: This is an ongoing randomized, open-label study of co-admin versus rhGH alone in treatment-naïve, prepubertal children with height SDS ≤ -2 , IGF-1 SDS ≤ -1 and maximum stimulated GH ≥ 10 ng/mL. Subjects receive morning subcutaneous injections of either rhGH alone (45 μ g/kg once-daily) or co-admin (45 μ g/kg rhGH and 50, 100 or 150 μ g/kg rhIGF-1 as two injections) with meals. Subjects were treated with half of the assigned doses (weeks 0-2), and then they received the full dose (weeks 2-4). Serum GH and IGF-1 samples were collected from 100 subjects at two pre-treatment time points, a median of 2 hours after half of the assigned dose at week 2 and at 0, 2 and 4 hours after a full dose at week 4. Baseline GH and IGF-1 samples were collected at screening and pre-treatment visits. GH and IGF-1 were measured at Esoterix (Calabasas Hills, CA).

Results: The mean and its 95% CI for GH concentrations for all dose groups over time, and the mean and its 95% CI for IGF-1 concentrations for rhGH alone and co-admin groups over time are shown (Figure 1A and 1B, respectively).

Figure 1. GH and IGF-1 concentrations over time



Mean GH concentrations were proportional to dose between half and full doses, peaked <4 hours post-dose and returned to pre-treatment levels 24 hours post-dose. GH concentrations were similar for the rhGH alone and the co-admin dose groups (data not shown). In contrast, mean IGF-1 concentrations did not return to pre-treatment levels within 24 hours. For the rhGH alone group, mean IGF-1 concentrations were similar at 0, 2 and 4 hours post-dose. Mean IGF-1 concentrations increased with increasing rhIGF-1 dose but did not exhibit strict dose proportionality.

Conclusions: Mean IGF-1 concentrations increased with rhGH alone, but there were further dose-dependent increases with co-admin of rhIGF-1. rhIGF-1 does not appear to influence the PK of GH since GH concentrations were similar for the rhGH alone and co-admin groups.

Sources of Research Support: Ipsen, US and Genentech, Inc.

Disclosures: BR: Speaker, Ipsen; Study Investigator, Ipsen; Medical Advisory Board Member, Ipsen. JWF: Consultant, Ipsen, Genentech, Inc. GMB: Employee, Ipsen.

P3-688

An Open Label 1-3 Year Clinical Trial on the Effects of the Aromatase Inhibitor Anastrozole Combined to the LHRH Analogue Leuporelin in Girls with Compromised Growth Potential.

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Context: Third generation aromatase inhibitors have been used to increase predicted adult height in boys with CDGP, ISS and GHD, but in young females only in the context of MAS. **Objective:** To investigate whether anastrozole in adjunction to leuporelin could improve adult height prediction in girls with compromised growth. **Methods:** Five girls aged 6.3-11.5 yrs received leuporelin 0.3 mg/Kg/month s.c. and anastrozole tabl 1 mg/day p.o. for 1-3 yrs. Cases 1-2 were on treatment with leuporelin for idiopathic precocious puberty 1 yr prior to inclusion but without improvement in adult height prediction. Cases 3-4 had rapid-tempo early puberty with bone age acceleration. Case 5 is a Silver-Russell syndrome and was also on hGH 0.04 mg/Kg/day. **Results:** There was a net increase in the predicted adult height of 4.5 cm the 1st yr (Table 1). Completion of the 2nd yr resulted in loss of 0.8 cm of the 1st year's gain. Patient 5 had only a minor gain the 2nd and 3rd yr additively. Height velocity diminished by 9.25% the 1st yr but seemed to stabilize in cases 2, 5 thereafter. There were no significant changes in BMI, in biochemical and lipid profiles. Calcium metabolism was optimal or optimized in all subjects. BMD evaluated by DXA scans showed z-scores within normal range for bone age and without significant inter-patient changes throughout treatment. Basal LH was <1, FSH<2 IU/L, E2<15 pg/ml and T<0.5 ng/ml in all cases. Uterine length and ovarian volume were or returned within the pre-pubertal range. None of the girls developed ovarian cysts or other side effects. **Conclusions:** At least one year of combined treatment with anastrozole and leuporelin seems to significantly increase the adult height potential of girls with idiopathic precocious puberty, rapid-tempo early puberty and growth disorders who would not benefit substantially from an LHRH analogue alone. Gain in adult height and safety of this approach needs to be confirmed.

Table 1

| Age (yrs) | Height (SDS) | Bone Age (yrs) | Predicted Adult Height (cm) (Bayley-Pinn eau) | Target height (cm) | Gain in Predicted Adult Height (cm) [1st-2nd-3rd year] | | |
|--------------|--------------|----------------|---|--------------------|--|-------------|------|
| 6.27 | +1.1 | 10.50 | 145.4 | 169.8 | +6.9 | - | - |
| 6.30 | +0.44 | 8.50 | 156.9 | 162.0 | +4.5 | -1.2 | - |
| 10.78 | -1.64 | 12.25 | 144.6 | 161.0 | +2.4 | - | - |
| 11.38 | -1.39 | 12.00 | 149.1 | 155.8 | +4.9 | - | - |
| 11.47 | -4.49 | 8.66 | 135.9 | 157.0 | +4.4 | -0.5 | +1.5 |
| Median 10.78 | Median -1.39 | Median 10.50 | Median 145.4 | Median 161.0 | Median +4.5 | Median -0.8 | |

- (1) Mavras N et al., J Clin Endocrinol Metab 2008; 93: 823-831
- (2) Mavras N, Endocrinol Metab Clin N Am 38 (2009); 613-624
- (3) Dunkel L, Horm Res 2009;71(suppl 1):57-63

Nothing to Disclose: DTP, AP

P3-689**The Consequences of Catch-Up Growth in Infancy on Cardiometabolic Profile and Adiponectinemia Persist in Young Healthy Adults Born Small for Gestational Age.**

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Introduction: Subjects born small for gestational age (SGA) present unfavorable metabolic outcome later in life. In children and adolescents born SGA the occurrence of catch-up growth (CUG) in infancy is associated with insulin resistance, adiposity and lower adiponectinemia. However, it is less clear whether CUG outcome on adiponectinemia and cardiometabolic profile persists in adult life. **Aim:** To ascertain the association between CUG of weight (CUGW), adiponectinemia and cardiometabolic profile in SGA adults. **Subjects and Method:** 87 SGA subjects longitudinally followed from birth to 23-25 y were grouped according to the presence (CUGW: n=65; 33F/32M) or absence (non-CUGW: n=22; 7F/15M) of CUGW. CUGW was defined as a change in weight z-score for age and gender above 0.67 between birth and 8-10 y. Adiponectin (RIA) and cardiometabolic variables were evaluated at 23-25 y. Statistics: one-way ANOVA adjusted by BMI and gender, Fisher's exact test and odds ratio and Spearman's correlation test; $P < 0.05$. **Results:** CUGW was associated with higher chance of being overweight at 8-10 (OR=12.7; 95%CI: 0.7 to 222; $P=0.01$) and at 23-25 y (OR=13.3; 95%CI: 1.6 to 107; $P=0.002$). The prevalence of overweight or obesity was higher in CUGW young adults than in non-CUGW (38% vs. 4.5%; $P=0.002$). At 23-25 y, CUGW subjects presented significantly higher adiposity (BMI: 24.6 ± 3.7 vs. 19.7 ± 2.8 kg/m²; $P < 0.001$ and abdominal circumference: 83.8 ± 7.3 vs. 68.8 ± 6.2 cm; $P < 0.001$), higher blood pressure (systolic: 121 ± 15 vs. 111 ± 11 mmHg; $P < 0.001$ and diastolic: 72 ± 9 vs. 65 ± 4 mmHg; $P < 0.001$) and HOMA-IR (1.6 ± 1.3 vs. 0.9 ± 0.7 ; $P < 0.001$) than non-CUGW. CUGW subjects also presented unfavorable lipid profile (total cholesterol: 171 ± 39 vs. 149 ± 25 mg/dL; $P=0.007$; LDL-cholesterol: 103 ± 33 vs. 85 ± 22 mg/dL; $P=0.01$, HDL-cholesterol: 48 ± 14 vs. 51 ± 13 mg/dL; $P=0.01$ and triglycerides: 100 ± 66 vs. 62 ± 27 mg/dL; $P < 0.001$). Adiponectinemia was negatively correlated with BMI ($r=-0.23$; $P=0.03$), abdominal circumference ($r=-0.28$; $P=0.01$), blood pressure (systolic: $r=-0.22$; $P=0.04$ and diastolic: $r=-0.23$; $P=0.03$), HOMA-IR ($r=-0.29$; $P=0.008$) and positively with HDL-cholesterol ($r=0.37$; $P=0.02$). Adiponectinemia tended to be lower in CUGW subjects (9.5 ± 4.5 vs. 11.2 ± 6.6 µg/mL; $P=0.06$). **Conclusion:** CUGW adults presented higher adiposity, blood pressure, HOMA IR, less favorable lipid profile and a tendency to lower adiponectinemia. Therefore, the metabolic consequences of the CUGW in infancy persist in adult life.

Sources of Research Support: Fundacao de Amparo a Pesquisa do Estado de Sao Paulo - FAPESP (grants: 07/58105-1 and 07/50713-2).

Nothing to Disclose: ACB, ARE, FLF-R, MC, ACM, HB, MAB, SRA

P3-690

Growth Hormone Therapy in Short Children Born Small-for-Gestational-Age: Effects on Abdominal Fat Partitioning and on Circulating Follistatin and High-Molecular-Weight Adiponectin.

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Context & Objective: A high sensitivity to insulin and a low amount of subcutaneous fat are among the hallmarks of short children born small-for-gestational-age (SGA). We studied the effects of growth hormone (GH) therapy on fat partitioning (including in the abdominal region) and on circulating levels of triacylglycerol and newly identified adipokines, such as follistatin and high-molecular-weight (HMW) adiponectin, in short SGA children.

Setting: University Hospital.

Patients: 35 short SGA children (mean age 7 yr, height -3.1 SD).

Design: All children received GH but they were randomized for an early start (GH for 4 mo) versus a delayed start (untreated for 4 mo, then GH for 4 mo). Mean GH dose after 4 mo was 36 mcg/Kg/d.

Main outcomes: fasting serum glucose, insulin, triacylglycerol, HMW adiponectin, follistatin; body size and composition; abdominal fat partitioning.

Results: GH therapy was accompanied by robust changes towards the norm (height; weight; lean mass; follistatin), but also by changes away from the norm (low HMW adiponectin; high triacylglycerol). Some baseline anomalies were amplified (more deficit of subcutaneous fat, both at total-body level and in the abdominal region), whereas other baseline anomalies were over-corrected (from a highly insulin-sensitive state to an insulin-resistant state).

Conclusion: GH therapy in short SGA children accompanied not only by a more normal body size and follistatinemia, but also by insulin resistance, hypo-HMW-adiponectinemia, hyper-triacylglycerolemia and an amplification of the deficit in subcutaneous fat.

Sources of Research Support: In part by a grant by Pfizer Spain.

Nothing to Disclose: LI, AL-B, MD, AJ, SM, FEZ

P3-691

The Relationship between Parents' Perception about Child's Height and Psychopathology in Community Children with Relatively Short Stature.

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Objective: This study investigated the relationship between height and psychopathology in community children with relatively short stature according to the parents' reports. Also, the matter of parental concern about child's height were explored. **Methods:** The Child Behavior Checklist (CBCL), The Brief Encounter Psychosocial Instrument (BEPSI) and The Child-Health Questionnaire-Parent Form 50 (CHQ-PF50) were administered to 423 parents (from elementary and middle school children's) in Gagnam, South Korea. Subjects were divided into three groups; (1) relatively short (n = 30), (2) average stature (n = 131), (3) relatively tall (n = 153). CBCL, BEPSI and CHQ-PF50 scores were compared among three groups. **Results:** There were no significant differences in psychosocial burden associated with short stature measured by BEPSI-K and CBCL-K scores among three groups. But general health perception score of relatively short was significantly lower than that of non-short on the CHQ-PF50. Also, They were more used complementary medicines, milk and growth hormone compared to the non-short. The parents' expected height of their children were 180.6 ± 3.5 cm for boys and 166.7 ± 3.5 cm for girls. This is respectively 90 percentile and 75-90 percentile for the Korean standard adult height. **Conclusion:** Our study shows that in Korea, parents tended to regard short children as having health problems. Also, the parental expectation for their child's attainable height is unrealistically tall, mostly due to lack of correct medical information.

Nothing to Disclose: JYS, KAY, JWH, CHS

P3-692

Risk Factors of Pituitary Insufficiency in Children and Adolescents with Rathke's Cleft Cysts.

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Purpose : This report was designed a study to evaluate clinical manifestations and risk factors of pituitary insufficiency in children and adolescents with Rathke's cleft cysts(RCC).

Methods : Total 44 patients with RCC younger than 19 years of age who were visited to Seoul national university children's hospital between January 1995 and September 2009 were enrolled. 15 patients were confirmed pathologically as RCC and 29 patients were diagnosed as RCC by brain MRI. Their clinical, hormonal and imaging features were reviewed retrospectively.

Results : Symptoms at presentation were as followings; headache(64.5%), endocrinopathy(61.3%), and visual disturbance(19.4%). Endocrinopathy included central precocious puberty(18.2%), diabetes insipidus(DI, 13.6%), general weakness(11.4%), and growth retardation(6.8%). After surgery, hyperprolactinemia resolved in 100%, but, insufficiency of growth hormone, hypothyroidism, and DI were not improved. Pathologically, RCC had simple cuboidal(26.7%), simple columnar epithelium(20.0%), and chronic inflammation on cystic contents(13.3%). There was correlation between severe headache, visual disturbance, general weakness, and cystic size and pituitary insufficiency except gonadotropin abnormality. Suprasellar extension of cysts and high signals of T2 weighted image in brain MRI were related to hypothyroidism, hypocortisolism, and DI. General weakness was shown only risk factor of pituitary insufficiency by multivariable linear regression analysis($R^2=0.549$).

Conclusions : General weakness is risk factor of pituitary insufficiency on RCC. Therefore, when the patients with RCC complain about general weakness, we should further evaluate pituitary function and consider surgical treatment.

Nothing to Disclose: HHL, MJK, ISY, JHK, SHL, YAL, CHS, SWY

P3-693

Evolutionary Conservation and Modification of a Growth-Limiting Genetic Program.

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We previously identified an extensive genetic program, which occurs in multiple organs postnatally and includes the downregulation of many growth-promoting genes, and we showed evidence that this program contributes to normal somatic growth deceleration in mice. We hypothesized that this program is conserved during mammalian evolution but is adjusted to progress at different rates, thereby accounting for differences in body size among mammalian species. Consistent with our hypothesis, we previously found that this genetic program occurs more gradually in rats than mice. To test the hypothesis further, here we first asked whether this program is conserved in a large mammal and second whether the program progresses at a slower rate than in small mammals.

We studied 6 genes (*Anln*, *Aspm*, *Cdk4*, *Ccnd2*, *Igf2*, *Smo*) that participate in the putative growth-limiting genetic program in rodents. These genes promote cellular growth or mitosis and are downregulated with age in juvenile mice and rats as body growth decelerates. mRNA levels were measured by real-time PCR in female Suffolk sheep liver and lung at fetal day 65, fetal day 90, 5 months (prepubertal, liver only), and 21 months (adult). In liver, none of these genes showed a significant decline in expression between fetal day 65 and fetal day 90. However, expression declined significantly between fetal day 90 and 5 months for *Anln* (12-fold decline, $P < 0.001$), *Aspm* (35-fold, $P < 0.001$), *Igf2* (7-fold, $P < 0.001$), *Smo* (6-fold, $P < 0.001$), but not for *Cdk4* (1.9-fold, NS) or *Ccnd2* (1.9-fold, NS). Similarly, mRNA levels in lung declined less than 2-fold between fetal day 65 and fetal day 90 but showed marked declines between fetal day 90 and 21 months for *Anln* (4-fold decline, $P < 0.001$), *Aspm* (17-fold, $P < 0.001$), *Cdk4* (2.7-fold, $P = 0.002$), *Igf2* (52-fold, $P < 0.001$), *Smo* (7-fold, $P = 0.002$), although not for *Ccnd2* (1.7-fold, NS).

In summary, we found that expression of these growth-promoting genes decreases with age in sheep but that the decline occurs later than in rodents; in sheep high expression persists for at least 3 months after conception, whereas in mice and rats the decline occurs largely by 2 months after conception. Our findings suggest that the putative growth-limiting genetic program identified in small mammals is conserved in large mammals but progresses at a slower pace, thus providing a possible explanation for the longer somatic growth period and greater adult body size of larger mammals.

Sources of Research Support: Intramural NICHD.

Nothing to Disclose: AD, JCL, GR, PF, TB, VP, JB

P3-694

An Animal Model of Pediatric Combined Pituitary Hormone Deficiency Disease.

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LHX3 is a LIM-homeodomain transcription factor that has essential roles in pituitary and nervous system development in mammals. Children that are homozygous for recessive mutations in the *LHX3* gene present with combined pituitary hormone deficiency disease (CPHD) characterized by deficits of multiple anterior pituitary hormones. Most *LHX3* patients also present with additional defects associated with the nervous system including a characteristic limited head rotation. However, of the ten described types of *LHX3* mutation, one mutation type (W224Ter) does not result in the limited head rotation, defining a new form of the disease. The W224Ter patients have CPHD but do not have the nervous system symptoms. Whereas other mutations in *LHX3* cause loss of the protein or its activity, the W224Ter mutation causes specific loss of the carboxyl terminal of the LHX3 protein – a region that we have shown to contain critical regulatory domains for pituitary gene activation. To better understand the molecular and cellular etiology of CPHD associated with *LHX3* gene mutations, we have generated knock-in mice that model the human *LHX3* W224Ter disease. The resulting mice display marked dwarfism, thyroid disease, female infertility, and reduced male fertility. Ongoing experiments are characterizing the effects of the mutation throughout development at the molecular and cellular level, an approach which is not feasible with the human patients. We have generated a novel mouse model of human pediatric CPHD. Our findings are consistent with the hypothesis that the actions of the LHX3 factor are molecularly separable in the nervous system and pituitary gland.

Sources of Research Support: NIH HD42024 to SJR.

Nothing to Disclose: SCC, REM, ADS, KWS, SJR

P3-695

Final Height and Clinical Characteristics in Children with Medulloblastoma Treated with Growth Hormone.

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PURPOSE: Brain tumor survivors are increasing because of improvement of treatment modality. Medulloblastoma is one of the most common highly malignant brain tumor. This study was performed to find out the clinical characteristics, growth status, and response to growth hormone(GH) treatment in children after treatment of medulloblastoma.

METHODS: Twenty-eight children after treatment of medulloblastoma were evaluated retrospectively. We evaluated serum insulin-like growth factor-1(IGF-1), and insulin-like growth factor binding protein-3(IGFBP-3) concentrations, and also observed their growth status and changes with GH treatment according to treatment modality.

RESULTS: GH deficiency was accompanied by 18 patients(64%). Twelve patients(43%) were diagnosed with thyrotropin deficiency, and 4 patients(13%) were diagnosed with adrenocorticotropin deficiency. Initial height at GH treatment was -2.35 ± 1.53 SDS and increased to -1.85 ± 1.28 SDS by 1 year, -1.64 ± 1.46 SDS by 2 years, -1.42 ± 1.49 SDS by 3 years after GH treatment. Final height was -1.67 ± 1.13 SDS. Initial IGF-1 concentrations were 173.38 ± 76.31 ug/L and IGF-1 increment after 1year GH treatment was correlated to height gain. Gender(male vs. female), surgical method(gross total resection vs. partial resection), tumor location(at fourth ventricle vs. outside fourth ventricle), tumor size(<4cm vs. >4cm in diameter) have no correlation with height gain. The younger age at GH treatment and cranial irradiation rather than craniospinal irradiation were correlated with height gain.

CONCLUSION: The age at GH treatment and type of radiation therapy were important prognostic factor for growth outcome. Serum IGF-1 increment was correlated to height gain during GH treatment. Early GH treatment and check up of serum IGF-1 might be helpful to improve final height.

Nothing to Disclose: HWC, YSP, DSK, HSK, DHK

P3-696

SHOX Gene Variants: Growth Hormone/IGF-I Status and Response to Growth Hormone Treatment.

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Background: Data regarding growth hormone (GH) and IGF-1 status in patients (pts) with *Short stature Homeobox-containing gene (SHOX)* variants (Vs) are lacking (1,2).

Objective: To define the GH and IGF-1 status in pts with *SHOX* Vs and assess their response to GH treatment.

Methods: We retrospectively analyzed pts with short stature and *SHOX* tested as part of clinical care. Pts with chromosomal or skeletal abnormalities were excluded. *SHOX* Vs included single nucleotide substitutions, partial gene deletions and duplications, previously described variants and novel mutations. Height (Ht) SDS and IGF-1 SDS were compared between pts with *SHOX* Vs (S+) and normal *SHOX* (S-). Pts tested for GH deficiency (GHD) (Arginine and L-Dopa) were classified as GHD (A, peak GH <7 ng/mL), partial GHD (B, peak GH ≥7-<10 ng/mL) and growth failure of unknown etiology (C, peak ≥10 ng/mL). Among GH treated pts, Ht SDS, IGF-1 SDS, Growth velocity (GV) and GH dose were compared at 6, 12 and 24 months of treatment. *SHOX* and IGF-1 were assayed at Esoterix (CA). ANOVA and Kruskal-Wallis tests were used for analyses.

Results: Among 403 pts tested for *SHOX* mutation, 48 did not meet inclusion criteria. Of 355 pts, 83(23%) were S+, with 19 different *SHOX* Vs, 3 not previously reported.

Baseline Comparison

| | S- (n=272) | S+ (n=83) | p |
|------------|--------------------|--------------------|----|
| Age {y}* | 10.56 (3.54) | 9.61 (3.01) | NS |
| Ht SDS* | -2.05 (0.76) | -1.94 (0.66) | NS |
| IGF-1 SDS° | -1.39 (-5.29,5.60) | -1.71 (-3.55,1.29) | NS |

* Mean (SD); ° Median (min, max); NS Not Significant

GH stimulation tests were performed in 147 S- and 54 S+ pts.

Baseline: GH Tested Pts

| | A | | B | | C | |
|------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------------|
| | S- (n=25) | S+ (n=8) | S- (n=31) | S+ (n=13) | S- (n=91) | S+ (n=33) |
| Age {y}* | 10.80(2.97) | 8.84(3.11) | 9.01(2.34) | 10.46(1.97) | 10.26(3.30) | 8.64(2.65) [~] |
| Ht SDS* | -2.06(0.56) | -1.89(0.85) | -2.20(0.60) | -2.23(0.83) | -2.22(0.69) | -1.82(0.53) ^ª |
| IGF-1 SDS° | -2.26(-3.43, 0.58) | -1.46(-2.38,-0.76) | -2.26(-3.76, 0.60) | -1.42(-2.41,-0.79) | -1.63(-5.29, 2.54) | -2.04(-3.55, 1.29) |

* Mean (SD); ° Median (min, max); [~] p=0.008, ^ª p=0.004

A total of 81/147 S- (55%) and 21/54 S+ (39%) pts were treated. Within all 3 groups, Ht SDS, IGF-1 SDS, GV and GH dose (0.25-0.29 mg/kg/wk) were not different between S- and S+ at 6, 12 and 24 months.

Conclusions: GH and IGF-1 characteristics were not different between S- and S+ pts. Response to GH was similar between S- and S+ pts for up to two years of treatment.

(1) Niesler et al., Hum Mutat. 2007; 28:933

(2) Rappold et al., J Med Genet. 2007; 44:306

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Nothing to Disclose: SS, MLK, MOR, YF, EJW, SJH, JHG, RR

P3-697

Regulation of Ghrelin, Obestatin and Adiponectin in Pediatric Patients with Chronic Renal Insufficiency and after Renal Transplantation.

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Background: Cachexia and growth retardation are common problems in paediatric patients with renal insufficiency and strongly predict morbidity and mortality. Disturbances of appetite-regulating hormones may contribute to this state. Acyl Ghrelin is a potent orexigenic hormone, whereas desacylated ghrelin is anorexigenic. Obestatin, an alternative splicing product of the ghrelin gene, has anorexigenic properties. The regulation of acyl ghrelin and its anorexigenic opponents and its role for the development of cachexia in renal insufficiency is unclear.

Methods: We measured plasma total ghrelin, acylated ghrelin, obestatin, leptin and adiponectin in children with chronic kidney disease stage III-IV (CKD) (n=29), children undergoing hemodialysis (HD) or peritoneal dialysis (PD) (n=29), children after renal transplantation (RTx, n=91) and healthy controls (n=27), and analysed the data with regard to auxological parameters (BMI, height).

Results: Patients with renal insufficiency had lower BMI compared to healthy and RTx children. Total ghrelin was elevated in CKD and PD patients (2387 ± 1401 pg/ml and 2986 ± 1661 pg/ml) compared to control subjects or transplant recipients (983.1 ± 580.5 pg/ml and 1414 ± 904.1 pg/ml; $P < 0.001$). As acyl ghrelin levels were not different between groups, the acyl ghrelin/total ghrelin ratio was reduced in uremic patients. Obestatin plasma levels were increased in patients with renal insufficiency compared to controls and RTx patients (512.1 ± 183.8 pg/ml, 581.4 ± 231.5 pg/ml, 553.7 ± 232.6 pg/ml vs. 364.9 ± 101.4 pg/ml and 362.5 ± 166.8 pg/ml; $P < 0.05$). Serum adiponectin levels were significantly increased in patients with CKD and PD compared to healthy controls, as well as in PD patients compared to transplant recipients.

Conclusions: In children, uremia leads to accumulation of anorexigenic hormones as desacyl ghrelin and obestatin. The orexigenic acyl ghrelin is not upregulated under uremic conditions resulting in a shift of the acyl/desacyl ghrelin ratio towards the anorexigenic desacyl ghrelin. In addition, acyl ghrelin action may be impaired by uremia-induced end-organ resistance. At present, it is unknown whether increased adiponectin concentrations in CKD and PD merely reflect decreased clearance or could be associated with a protective effect. In summary, disturbed balance between anorexigenic and orexigenic hormones seems to have a major impact on cachexia development in uremic paediatric patients.

Nothing to Disclose: AKB, RB, PFH, BPH

P3-699

Maintenance of Weight or Modest Weight Loss Following Lifestyle-Only Intervention Tends To Redistribute Body Fat, Decrease Lipid, CRP and Fibrinogen Levels and To Improve Parameters of Insulin Sensitivity in Obese Children.

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Objectives. To investigate whether lifestyle-only intervention in obese children and adolescents who either maintain their weight or lose modest weight redistributes parameters of body composition and reverses metabolic abnormalities. **Study design.** Changes in clinical, anthropometric and metabolic parameters were assessed in 126 obese children (aged 12.0±2.6 years, 53 males and 73 females) before and after 8 months of lifestyle intervention (dietary-behavioral and physical activity modifications). For analysis the data was subdivided into those patients who either maintained or lost 1-5% of their weight (n: 92; fifty one females and forty one males) or those who gained weight (n: 35; twenty two females and thirteen males) following intervention.

Results. Patients who either maintained their weight or had a modest weight loss presented with a significant decrease in Z-score BMI, waist circumference and waist/hip ratio (p<0.01) and in fat area (p<0.0001) following intervention. Physical activity increased as assessed either by patient and parental reporting or following the use of a pedometer (p<0.001). Total cholesterol (173.2±50.1 vs 155.6±35 mg/dl; p<0.05), LDL cholesterol (113.4±46 vs 97.9±28.5 mg/dl; p<0.01), triglycerides (123.1±64.1 vs 96.6±42.6 mg/dl; p<0.01), triglyceride/HDL ratio (3.5±2 vs 2.5±1.4; p<0.05), basal and postprandial insulin (11.9±8.5 vs 8.7±9.9 and 62.9±51.9 vs 44.2±69.6 uU/ml; respectively; p<0.01), HOMA (2.4±1.8 vs 1.8±1.4; p<0.01), CRP (3.1±1.5 vs 0.78±2.0 mg/L; p<0.01) and fibrinogen levels ((411.4±118.4 vs 321.6±177.0 mg/dl; p<0.05) decreased significantly during the 8 months period in these patients, while Quicki increased (0.3±0.04 vs 0.7±0.03; p<0.01). Conversely, patients who gained weight had an increase in waist circumference and waist/hip ratio (p<0.01), total and LDL cholesterol (p<0.05), triglycerides (p<0.05) and fibrinogen levels (p<0.01) between visits, while markers of insulin resistance remained stable.

Conclusions. Obese children who are able to either maintain their weight or lose a small amount of weight following modest lifestyle-only intervention tend to redistribute their body fat, decrease lipid, CRP and fibrinogen levels and to improve parameters of insulin sensitivity.

Nothing to Disclose: HM, MF, MP, MS, NC, RC, ZM, LV, RL

P3-700

Vitamin D Affects Components of Metabolic Syndrome in Obese Adolescent Females.

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Background: Vitamin D deficiency has been implicated as a risk factor for metabolic syndrome and type 2 diabetes. We aimed to determine the relationship of serum 25-hydroxyvitamin D (25(OH)D) with insulin- glucose dynamics, lipid profiles and liver transaminases in obese adolescent females and to assess if vitamin D treatment corrects any of the metabolic disturbances. **Methods:** 80 obese (BMI >95th centile for age and sex) postmenarchal adolescents [53 African American (AA) and 27 Caucasian (CA)] were evaluated with fasting measurements of serum 25(OH)D, PTH, lipid profile, C- reactive protein and transaminases [alanine transaminases (ALT), aspartate transaminases (AST)] followed by a 2-hour oral glucose tolerance test (OGTT) with glucose and insulin measurements at 0, 30, 60 and 120-minute intervals. A subgroup (n=14) of vitamin D deficient subjects [serum 25(OH)D concentration <20ng/ml (50nmol/liter)] were subsequently treated with 50,000 IU of ergocalciferol once a week for 8 weeks and reevaluated following treatment of vitamin D deficiency with OGTT along with follow-up measurement of serum 25(OH)D, PTH, serum calcium, CRP, ALT and AST .

Results: AA had a higher prevalence of vitamin D deficiency (85% in AA vs. 30% in CA). Among all subjects, 25(OH)D was inversely associated with fasting glucose ($r = -0.24, P=0.03$) and positively associated with LDL ($r=0.25, P=0.03$) independent of race and BMI. In analyses by race, after adjusting for BMI, 25(OH)D was inversely associated with fasting insulin ($r = -0.48, P= 0.01$), HOMA IR, ($r = -0.41, P= 0.04$), AST ($r = -0.5, P= 0.01$) and ALT($r = -0.45, P= 0.03$) and positively associated with LDL ($r = 0.4, P= 0.05$) in CA but not in AA. In the subgroup treated with vitamin D, fasting glucose improved after treatment with vitamin D (89.07 ± 8.3 mg/dl before and 84.34 ± 8.4 mg/dl after, $P = 0.05$).

Discussion: Serum 25(OH) D concentrations were inversely associated with fasting insulin and serum transaminases in obese adolescent females independent of BMI, although the relationship was only apparent in CA. Repletion of vitamin D status has beneficial effects on plasma glucose.

Sources of Research Support: Children's Center for Research and Innovation (CCRI grants), Children's Hospital, Birmingham, AL. AA is supported in part by the from Child Health Research Center Grant K12 HD043397 (T0909180013) and UAB Diabetes research training center P60 DK- 079626. JAA is supported by the American Heart Association (Greater Southeast Affiliate).

Nothing to Disclose: APA, JAA, KHS, BAG, KCM

P3-701

Vitamin D and Osteocalcin Are Associated with Insulin Sensitivity and Fat Mass in Healthy Middle School Children.

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CONTEXT: Nonclassic actions of vitamin D include potential regulation of immune and pancreatic beta cell function. Vitamin D deficiency has been associated with significantly higher HgbA1c and greater insulin resistance in obese adolescents. Like vitamin D, the osteoblast-derived protein, osteocalcin, has salutary effects in stimulating insulin release from islet cells and adiponectin from adipocytes.

OBJECTIVE: We hypothesized that both vitamin D and osteocalcin would correlate positively with measures of insulin sensitivity and negatively with body fat in healthy non-obese children.

DESIGN AND PARTICIPANTS: We analyzed anthropometric (BMI, % fat, and waist circumference), biochemical markers [lipids, inflammatory cytokines (TNF- α , Interleukin-6, C-reactive protein), glucose, and adiponectin], 25-OH vitamin D, osteocalcin (total and uncarboxylated) and intact PTH in 40 school children (20 males, 20 females), 11-15 yrs of age (mean 13.0 \pm 1 yrs) as a part of the ROAD Project. Insulin sensitivity was assessed by quantitative insulin sensitivity check index (QUICKi) and insulin secretory capacity was measured as acute insulin response (AIR, mean rise in insulin 3 and 5 minutes after I.V. dextrose) and glucose disposal index (GDI).

RESULTS: Concentrations of vitamin D were positively correlated with total osteocalcin ($r=0.35$, $p<0.05$), uncarboxylated osteocalcin ($r=0.34$, $p<0.05$) and insulin sensitivity (QUICKi $r=0.32$, $p<0.05$). There was a trend toward significance between vitamin D and AIR, but not GDI. BMI, percent body fat and total fat mass all correlated negatively with total osteocalcin, vitamin D and QUICKi. Among cytokines assayed, TNF- α showed the strongest positive correlation with total osteocalcin ($r=0.52$, $p<0.0008$), whereas IL-6 correlated negatively with iPTH, but not vitamin D or osteocalcin. Finally, ALT a marker for hepatic steatosis, correlated positively with uncarboxylated osteocalcin alone ($r=0.34$, $p<0.02$). There were no significant gender differences.

CONCLUSION: In our small multi-ethnic cohort of healthy school children we observed positive correlations of vitamin D and osteocalcin with direct and indirect measures of insulin sensitivity. Greater BMI, fat mass and percent body fat, known to be associated with insulin resistance, were also associated with lower levels of vitamin D and osteocalcin. These data suggest that metabolic bone factors are independent risk predictors for adiposity-related co-morbidities, even in the non-obese child.

Nothing to Disclose: CCB-B, PWS, DEC, SA, LA, SC, RC, DD, IF, AH, LI, FJJ, AMJ, BL, LM, KP, RR, WR, AR, ES, SS, ST, MR

P3-702

Normal Alanine Transferase: A Novel Independent Indicator of Adiposity-Related Comorbidity in Youth.

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Background: In select populations of obese children with elevated Alanine Transferase (ALT), ALT correlates with gender, BMI, waist circumference (WC), QUICKI, C-reactive protein, fasting glucose and insulin levels, and triglycerides (TG). Analyses of the relationship of non-preselected ALT to adiposity-related comorbidities (ARC) in children over a broad weight range are lacking.

Objective: Identify associations of ALT with risk factors for ARC in a heterogeneous group of children of varying ethnicities and body compositions.

Methodology: We analyzed anthropometric (BMI, % fat, and WC) and biochemical (ALT, preserved glucose, insulin, lipids, inflammatory cytokines) markers in 40 (20F) school children, ages 11-15 years (part of the ROAD consortium). Insulin secretory capacity was measured as acute insulin response (AIR, mean rise in insulin 3 and 5 minutes after 25 gm of intravenous dextrose) and glucose disposal index [GDI, log₁₀ (AIR x [fasting glucose] / [fasting insulin])]. Insulin sensitivity was measured using the quantitative insulin sensitivity check index {QUICKI, 1 / (log[fasting insulin μU/mL] + log[fasting glucose mg/dL])}. Significant correlations were also examined by multiple linear regression analysis to correct for age, sex, and % fat.

Results: The mean (SD) age was 13.0 (0.1) years. The range of BMI was 16.1-35.3. ALT [mean(SD)- 12.6(4.7) U/L, range 6-26] was normal (<30) in all subjects.

Significant correlations of ALT

| Variable | MEAN(SD) | r | P value | Semipartial r * | P value * |
|-------------------------|------------|-------|---------|-----------------|-----------|
| BMI(kg/m ²) | 22.6(4.8) | 0.56 | <0.001 | | |
| % Fat | 27.6(8.6) | 0.32 | 0.040 | | |
| Fat mass(kg) | 17(9.2) | 0.49 | 0.0015 | | |
| WC(cm) | 78.3(22.0) | 0.54 | 0.0004 | | |
| TG(mg/dL) | 76.1(47.6) | 0.43 | 0.006 | 0.31 | 0.036 |
| HDL(mg/dL) | 51.5(11.9) | -0.35 | 0.025 | -0.31 | 0.049 |
| Fasting insulin(uU/mL) | 10.4(9.8) | 0.51 | 0.0008 | 0.36 | 0.008 |
| QUICKI | 0.35(0.03) | -0.48 | 0.0019 | -0.39 | 0.020 |

*Semi-partial correlation coefficients and p values corrected for age, sex, and % fat.

Conclusion: ALT was significantly correlated with anthropomorphic markers (% body fat, WC) and biochemical markers (Insulin, QUICKI, TG, HDL) suggesting that even within the normal spectrum, ALT may be an easily accessible, early, and independent indicator of adiposity and insulin resistance in youth.

Sources of Research Support: AMDeC.

Nothing to Disclose: MLK, LI, TTS, MR, SA, LA, DC, DD, IF, BL, WR, SS, PS, ST, RR

P3-703**Retinol Binding Protein 4 Is Associated with Markers of Adiposity-Related Co-Morbidities in Children.**

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In adults, levels of Retinol Binding Protein 4 (RBP4) have been associated with biochemical markers of adiposity related co-morbidities, including increased insulin resistance, triglycerides, LDL cholesterol, waist-to-hip ratio, body mass index (BMI) and systolic blood pressure, as well as decreased HDL cholesterol. Studies in the pediatric population have been less consistent with respect to such relationships.

This study examined the relationship between circulating levels of RBP4 and risk factors for co-morbidities of adiposity in a population of children in early adolescence.

We analyzed anthropometric (BMI, % fat, waist circumference), metabolic (lipids, glucose, adiponectin), and inflammatory (TNF- α , Interleukin-6, C-reactive protein) markers for adiposity-related co-morbidities, as well as serum alanine aminotransferase (ALT, a risk marker for non-alcoholic fatty liver disease) in 40 school children (20 males, 20 females) 11-15 yrs of age (mean 13.0 \pm 1 yrs) as part of the ROAD (Reduce Obesity and Diabetes) Project. Insulin sensitivity was assessed by quantitative insulin sensitivity check index (QUICKI). Insulin secretory capacity was measured as acute insulin response [AIR (mean rise in insulin 3 and 5 minutes after 0.5gm/kg of IV dextrose)] and glucose disposal index [$\log_{10}(\text{AIR} \times [\text{fasting glucose}]/[\text{fasting insulin}])$].

Serum RBP4 was significantly correlated directly to BMI, waist circumference, and ALT, and inversely to HDL, adiponectin, and QUICKI (all P<0.05). Correlations with ALT, HDL, BMI, and QUICKI remained significant when corrected for % body fat.

Table 1. Correlations of RBP4 With Co-morbid Indices

| Variable | Mean(SEM) | r | Semi-partial r adjusted for % fat |
|--------------------------|------------|--------|-----------------------------------|
| BMI (kg/m ²) | 22.6(0.8) | 0.45* | NS |
| Waist (cm) | 78.3(2.3) | 0.35* | NS |
| HDL (mg/dl) | 51(2) | -0.34* | -0.32* |
| ALT(U/L) | 12.6(0.7) | 0.42* | 0.38* |
| Adiponectin (ug/ml) | 13.0(0.7) | -0.36* | -0.34* |
| QUICKI | 0.35(0.01) | -0.41* | -0.35* |

* P<0.05; NS: not significant

In early adolescent boys and girls, circulating concentrations of RBP4 are positively correlated with multiple risk factors for adiposity-related co-morbidities (increased central and total body fatness, insulin resistance and ALT, as well as decreased HDL cholesterol and adiponectin). The observation that many associations persisted when corrected for % body fat suggests that RBP4 can be viewed as an independent marker of adiposity-related co-morbidity risk in children.

Sources of Research Support: Sponsored through AMDeC.

Nothing to Disclose: RMC, IF, AAH, AMJ, DD, KP, ES, SC, LI, RR, DEC, CB-B, PWS, FJJ, LM, SA, RG, SS, ST, WR, MR

P3-704**Retinol Binding Protein 4 (RBP-4) Correlates with Triglycerides (TG) and Subcutaneous Adipose Tissue (SAT) but Not Insulin Resistance (IR) in Prepubertal (PP) Children with Premature Adrenarche (PA) and Controls.**

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Background: RBP-4, an adipokine secreted by adipose tissue and liver, has been shown to correlate with obesity, IR and cardiovascular(CV) risk factors including elevated systolic blood pressure(BP), TG and decreased HDL in pubertal children and adolescents. Adipose derived RBP-4 is thought to signal the presence of IR and may represent a link that could explain the connection between IR and obesity. RBP-4 may serve as an easily obtained marker for comorbidities in children with PA, a condition associated with increased risk for IR and CV disease.

Objectives: To determine if PP children with PA have higher RBP-4 than controls and if RBP-4 correlates with comorbidities in this group.

Design/Methods: 38 PP children(5-9 yr);18 PA(m:5, f:13, BMIz: 1.0±1.1), 20 controls (m:13, f:7, BMIz:1.8±1.0) had fasting blood for glucose(G), insulin(I), RBP-4, lipids and androgens. G and I levels were obtained at 30,60,90,120 min after a 1.75 g/kg(max 75 g) OGTT. FGIR, WBIS, SiM, G_{AUC}, I_{AUC}(IR measures)were calculated. Ht and wt were measured and BMIz calculated. A subset of subjects (PA:4,C:5) had body composition (BC) testing including whole body DXA for % body fat (%BF) and trunk fat (TF)(GE Lunar), ¹H NMR of the right tibialis anterior muscle for intramyocellular lipid and contiguous axial T1 weighted MRI of the trunk for visceral adipose tissue and SAT. Relationships between RBP4 and metabolic markers and BC data were evaluated by Pearson correlations and adjusted for BMIz. Group differences were evaluated by ANOVA .

Results: The PA group was more IR than controls with higher I_{AUC}(P=0.007), lower SiM(P=0.002), FGIR(P=0.003) and trend for lower WBIS(P=0.06). RBP4 and lipid levels were not different between PA and controls. In the subgroup with BC testing (n=9) PA had greater SAT(P=0.046) and trended towards greater TF(P=0.06). In the entire group(n=38) RBP-4 correlated with TG (r=0.48; p =0.003) and trended to correlate with HDL(r=(-)0.31; P=0.07). In the subgroup with BC testing(n=9) RBP-4 trended to correlate with SAT(r=0.778, P=0.068) and with % BF(r=0.738; P=0.094).

Conclusions: In this group of PP children PA subjects were more IR, had greater SAT and TF. RBP-4 correlated with TG and trended to correlate with HDL(-), SAT and % BF; however, in this group, RBP-4 did not correlate with IR. The lack of correlation of RBP-4 with IR may be due to the very early stage of metabolic abnormalities in this young population.

Nothing to Disclose: ABS, EL, EAF, JR, DG, JH, DS, AH, DJM, WSB, SEO

P3-705

Cord Plasma Levels of Proinsulin, Leptin, and Adiponectin in Term Newborns from Diabetic and Non-Diabetic Hispanic/Latino Mothers: A Pilot Study.

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Diabetes and obesity, which are tightly associated, currently threaten the health, well-being and economic welfare worldwide. In the US, statistics show that Hispanic/Latino Americans have substantially high rates of diabetes and obesity. Leptin and adiponectin are two adipocyte-derived hormones with profound metabolic effects related to obesity and insulin sensitivity. The presence of leptin and adiponectin in umbilical cord blood suggests their potential roles in fetal development, which may be associated with diabetes in later life. However, whether diabetic pregnancy affects fetal growth and cord levels of leptin and adiponectin and how these factors are mechanistically related remain to be explored, especially in the Hispanic/Latino population. In this pilot study, we aimed to determine the relationships among multiple factors, including leptin, adiponectin, and proinsulin levels in cord blood, weight and adiposity of newborns from diabetic and non-diabetic Hispanic/Latino mothers.

Methods: Cord blood samples from 36 singleton term newborns (17 from healthy and 19 from diabetic Hispanic/Latino mothers) were collected. Concentrations of leptin, adiponectin, and proinsulin in cord plasma were measured by ELISA. Body Mass Index (BMI) and Ponderal Index (PI) were calculated to estimate the body fat mass in mothers and newborns, respectively. Comparisons and correlations among the variables were evaluated statistically.

Results: Mean concentrations of leptin, adiponectin, and proinsulin in cord plasma were 19 ng/mL, 30 ug/mL, and 33 pM, respectively. Cord proinsulin level in neonates from diabetic mothers was higher than that in healthy group (45 vs. 19 pM, $P=0.02$). Cord levels of leptin and adiponectin were not significantly different between the two groups. Diabetic group has higher adiposity than healthy group (maternal BMI: 36.2 vs. 31.4, $P=0.01$; infant PI: 3.1 vs. 2.7, $P=0.01$). For all subjects, cord leptin level was positively correlated with proinsulin, maternal BMI, and infant weight ($P<0.05$). Within the healthy group, cord leptin level was positively correlated with infant weight, and adiponectin was positively correlated with gestational age ($P<0.05$).

In conclusion, cord plasma concentration of proinsulin and adiposity of infants and mothers in diabetic group are higher than those in healthy group. Cord leptin level shows moderate correlations with other parameters of interest. These interesting findings warrant prospective, larger-scale studies.

Sources of Research Support: St. Joseph's Foundation (SJF) Pilot/Feasibility Grant and partially by a Science Foundation in Arizona (SFAz) Award (to Y.H.).

Nothing to Disclose: YG, RB, KW, LC, JB, YH

P3-706

Fulminant Type 1 Diabetes in Korean Children with Type 1 Diabetes.

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Purpose ; Recently fulminant type 1 diabetes has been identified as a new subtype of idiopathic type 1 diabetes. To date most of the patients have been reported in Japanese population, and more than 90% of the patients were adults. The aim of this study was to investigate the prevalence of fulminant type 1 diabetes and the clinical characteristics of the disease among newly diagnosed type 1 diabetic patients under the age of 18 years.

Methods ; Using data retrieved from 3 university hospitals, we identified all patients newly diagnosed with type 1 diabetes from 1 January 2000 to 31 December 2008. Information on clinical manifestations and laboratory data were obtained by reviewing medical records. All variables were expressed as mean +/- SD, and a *p* value of <0.05 was considered statistically significant.

Results ; We identified 144 patients newly diagnosed with type 1 diabetes. Among these patients, 92 patients (63.9%) were positive, and 52 (36.9%) were negative for a minimum of one type of islet autoantibodies. Two patients (1.4%) fulfilled the criteria for fulminant type 1 diabetes, and they were negative for islet autoantibodies. Children with this subtype had a higher age of onset (median 14.4 vs 9.8 or 10.7 years, *p*<0.05), lower HbA1c (median 6.4 vs 12.3 or 12.8%, *p*<0.01) than children with autoimmune or antibody negative type 1 diabetes. They also showed significantly increased serum aspartate aminotransferase and amylase levels, and decreased fasting serum C-peptide level.

Conclusion ; In Korean children under the 18 years of age, the prevalence of fulminant type 1 diabetes was 1.4% among all patients newly diagnosed with type 1 diabetes. Although this type of diabetes is most likely an adult-onset disease, it is possible that fulminant type 1 diabetes has not been fully recognized in children, and may be more common than initially thought.

Nothing to Disclose: MSK, CJK, CWK, DYL

P3-707

Outcome of a 2-Year Search for Neonatal Diabetes in Singapore.

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Background: Neonatal Diabetes Mellitus (NDM) (1 in 100,000-300,000 live births), in either its permanent or transient form, has not been previously reported in Singapore, an island state with approximately 40,000 live births per year.

Methods: This was a combined prospective and retrospective study to identify NDM among children in Singapore. In the prospective arm, we determined the molecular etiology of all babies who presented to our centre with very early onset diabetes from January 2008 to December 2009. In the retrospective arm, we reviewed the type 1 diabetes database in our centre to identify and study the genotype of older children who had presented with very early onset diabetes.

Results: A total of 4 cases were identified, three of whom [CLA (June 2008), JMB (November 2008) and TBC (July 2009)] presented prospectively with one [LZQ(1994)] identified from the database review. Of the prospectively identified children, one had PND [CLA (R201H of the *KCNJ11* gene)] and has since been converted from insulin injections to oral Glibenclamide. Two children had TND, one with a common molecular cause [TBC (paternal duplication of Chromosome 6q24)] and the other with a novel mutation [JMB (F1182S in exon 28 of the *ABCC8* gene)]. Both these children with TND are no longer on insulin and have normal blood glucose levels. In the retrospective arm, 372 patients were registered with type 1 diabetes in our database and one child (now 16 years) with onset of diabetes at 6 months of age was identified. She was found to have a novel mutation [LZQ (C109Y in exon 3 of the *INS* gene), which is consistent with a phenotype of PND.

Conclusion: Our 2-year study suggests that the incidence of TND in Singapore is at least 1 in 40,000 and may be more common than currently reported. Based on the 15 year interval between the two cases diagnosed with PND, the incidence of PND is estimated to be 1 in 300,000 live-births, less common than TND but consistent with international estimates. We emphasize the need for medical practitioners to consider molecular testing for all patients who present with diabetes below 7 months of age as this will facilitate accurate diagnosis and appropriate therapy.

Nothing to Disclose: RFV, FKPY, SHL, JXS, SE

P3-708

Seasonality of Birth and Diagnosis of Type 1 Diabetes Mellitus (T1DM) in Children (0-19 Years) from Southeastern WI, USA.

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Context: Studies supporting a relationship with increasing weight and younger age at diagnosis in T1DM are numerous. The seasonality of diagnosis and birth of T1DM have also been well documented, although both with conflicting results.

Objectives: To describe the seasonality of T1DM as it relates to increased weight at diagnosis and younger age at diagnosis.

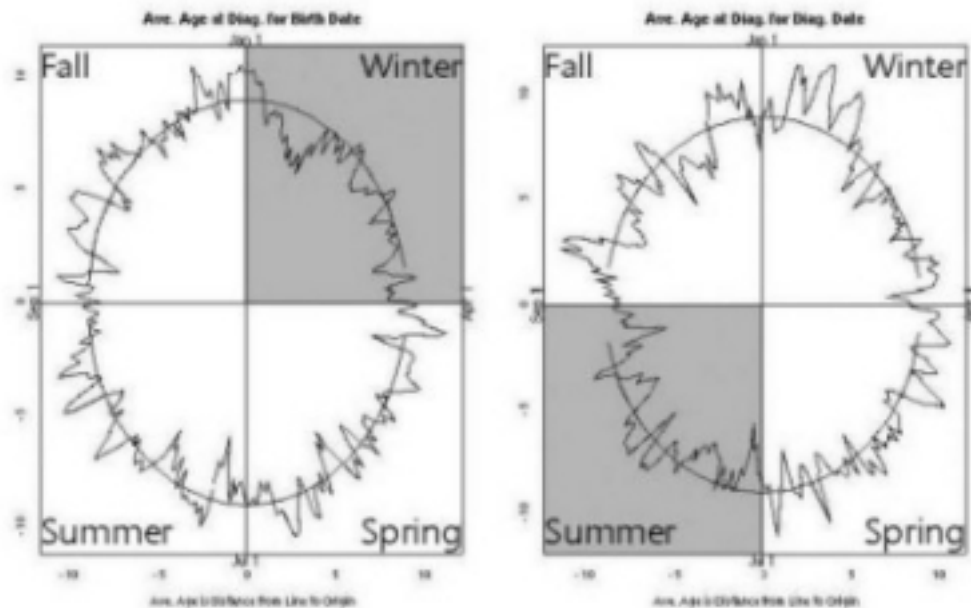
Design: Retrospective chart review of physician-diagnosed T1DM

Setting: Children's Hospital inpatient and outpatient records

Participants: 1596 children aged 0-19 with newly diagnosed T1DM from January 1995-December 2004.

Results: 52.1% male; 80.1% white, 10.8% black. Overall there was no difference in the season of birth ($p = 0.26$), the season of diagnosis was approaching significance ($p = 0.02$). A greater percent of children were diagnosed in the Fall and Winter months (F: 26.6, W: 27.0) as compared to the Spring and Summer months (Sp: 23.9, Su: 22.4). Although children diagnosed in the summer had younger age at onset then those diagnosed in winter and spring ($p_{W,Sm} \leq 0.01$; $p_{Sp,Sm} \leq 0.01$). Children born in winter were more likely to have a younger age at diagnosis then those born in Fall and Spring ($p_{W,F} \leq 0.01$; $p_{W,Sp} \leq 0.01$). Also those born in Summer were more likely to be diagnosed in Winter and Fall ($p \leq 0.01$). There was no difference between increasing BMI at diagnosis as compared to birth or diagnosis season. A p-value of ≤ 0.01 was considered significant based on Holm adjustment.

Figure 1. Seasonal Relationship with Age at Diagnosis based on birth date and diagnosis date.



Conclusions: Our findings regarding seasonality of diagnosis and birth are consistent with published literature with seasonal peaks in cooler months for diagnosis, predominately in the youngest age group. Environmental factors related to seasonality are currently being investigated as possible contributors to increasing prevalence; viral transfer from mother to child leading to β -cells damage; vitamin D interactions, and nutritional factors.

Nothing to Disclose: JME, RA

P3-709

Post-Operative Surveillance Improves Detection of Post-Prandial Hypoglycemia after Fundoplication.

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Late dumping syndrome, particularly post-prandial hypoglycemia (PPH), is a frequent, but often undetected, complication in children after fundoplication surgery for severe gastroesophageal reflux. The prevalence of dumping syndrome after fundoplication was reported to be 30% in a previous study¹. As of July 1, 2007, we established a post-operative surveillance plan in the The Children's Hospital of Philadelphia (CHOP) Neonatal Intensive Care Unit (NICU) to monitor post-prandial blood glucose (BG) levels in all children after fundoplication, while other hospital units continued routine care. We performed a retrospective chart review to measure the prevalence of PPH since the surveillance plan was instituted. Our primary goal was to estimate the prevalence of PPH after fundoplication and to describe the frequency of symptoms associated with PPH. We hypothesize that universal screening will detect cases of PPH that otherwise would have been undiagnosed if relying on symptoms alone. From July 1, 2007 to July 1, 2009, 302 children (newborn to 18 yrs) underwent a fundoplication in all units at CHOP. Of these, 286 charts (NICU=64; other units=222) were available for review and analyzed for rate of screening, evidence of PPH (post-prandial BG \leq 60 mg/dL), evidence of hyperglycemia (post-prandial BG \geq 140 mg/dL) preceding PPH, timing of PPH presentation, and symptoms of PPH. Within the NICU, 95.3% of children were screened, compared to 30.6% in all other hospital units. Of those screened in all units, 31.8% were identified to have PPH (n=41/129), as compared to 7.0% of children (n=11/157) who were identified with PPH by symptoms alone without routine monitoring. Within the NICU, the majority of children with PPH showed evidence of PPH within 1 week (90%) after establishment of full feedings. Only 70% of affected children were found to have hyperglycemia preceding PPH and only 50% of children with PPH had symptoms of dumping syndrome. These results suggest that the implementation of a post-operative surveillance plan can better identify patients at risk for PPH, many of whom have co-morbidities that may make symptoms less reliable. Earlier identification of PPH could lead to earlier treatment and minimize the effects of unidentified hypoglycemic events, such as seizures, developmental delays, and permanent brain damage. An ongoing prospective cohort study will help confirm the findings of this retrospective review.

(1) Samuk I et al., Journal of Pediatric Gastroenterology and Nutrition 1996; 23(3):235-240

Nothing to Disclose: ACC, PRG, RS, TAB, DDDL

P3-710

Disturbed Glucose Homeostasis and Markers of Neurological Injury in Critically Ill Children.

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Introduction:

Targeting glycemia to age-adjusted normal fasting blood glucose levels with intensive insulin therapy (IIT) improved short-term outcome of critically ill children as compared with conventional insulin therapy (CIT), but concomitantly increased the incidence of hypoglycemia [1]. Both hyperglycemia and hypoglycemia may adversely affect the developing brain of young children. We therefore studied the impact of targeting normoglycemia with IIT on circulating markers of brain injury in these patients.

Methods:

This was a pre-planned analysis of all 700 pediatric critically ill patients included in a prospective, randomized, controlled study on IIT [1]. Patients were randomly assigned to the target of normal for age fasting blood glucose levels (2.8-4.4 mmol/L for age <1 year and 3.9-5.6 mmol/L for age ≥1 year) with insulin infusion throughout intensive care unit (ICU) stay (IIT), or to insulin infusion only to prevent excessive hyperglycemia (CIT). Serum S100B and neuron-specific enolase (NSE), markers of astrocytic and neuronal damage, were measured by ELISA on fixed days (n=700) and before and after hypoglycemia in a "nested case-control" design (n=126).

Results:

Admission levels of S100B and NSE differed according to diagnosis and severity of illness. Patients admitted to the ICU after cardiac surgery had higher admission S100B and NSE levels than patients in the other diagnostic categories (p<0.0001), with the highest levels observed for patients with the highest Risk Adjustment for Congenital Heart Surgery (RACHS) score (both p=0.002). Admission levels of S100B and NSE were higher in patients needing intensive care for at least 3 days versus short-stay patients (both p<0.0001) and in ICU non-survivors versus survivors (p=0.002 and p=0.0002).

IIT did not affect the levels of S100B or NSE as compared with CIT. Patients experiencing hypoglycemia at any time during ICU stay revealed higher S100B and NSE levels on admission (p<0.0001 and p=0.0007) than those without hypoglycemia. In the nested case-control study, S100B and NSE decreased after hypoglycemia (p=0.001 and p=0.009) in the "cases", unlike in the "controls" on matched days.

Conclusions:

IIT in pediatric ICU did not evoke neurological damage, as evaluated by S100B and NSE, despite an increased incidence of brief hypoglycemia. Elevated markers in patients with hypoglycemia were not caused by hypoglycemia but explained by an increased risk of hypoglycemia in the most severely ill.

(1) Vlasselaers D et al., Lancet 2009; 373:547

Sources of Research Support: Research Foundation - Flanders (MG); K.U.Leuven Research Council (IV); Clinical Research Fund of the University Hospitals Leuven (DM); GVdB via the K.U.Leuven, receives structural research financing via the Methusalem program, funded by the Flemish government.

Nothing to Disclose: MG, IV, MB, PJW, DM, GVdB

P3-711

Benefits of Supplemented Preterm Formulas after NICU Discharge on Insulin Sensitivity and Body Composition.

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Nowadays, an increased number of premature infants survive. The medical challenge is to reduce their postnatal morbidities with a special focus towards an increase in metabolic risks due to an interaction of in utero and postnatal growth. Nutrition is a key factor determining early growth trajectory. The aim of this study was to evaluate body composition (DEXA) and fasting insulin (RIA) as a surrogate of insulin sensitivity and proinsulin (RIA) in premies receiving longer periods of preterm formulas (higher protein +docosahexaenoic acid) compared to a cohort who received these formulas for shorter periods.

Subjects: 560 VLBW born infants were included in the intervention (I) and 529 in the baseline (B) group. A subset of 95 subjects (I) and 87 (B) with similar birth weight (1230 ± 300 grs.), length (37.6 ± 3.2 mm), gestational age (27 ± 2.4) and % born SGA (40%) to the complete group, were included in this arm of the study. Bone mineral density, content and lean mass were not different at 1 and 2 yrs. However, total fat mass (%) was lower in the I group by the 2nd yr (19.3 ± 5.3 vs. 21.7 ± 4.2 %, $p < 0.01$). Trunk fat was already lower at first year in the I group (14.7 ± 5.0 vs. 16.9 ± 4.9 %, vs $p < 0.005$) and at the 2nd yr (14.1 ± 5.7 vs. 17.2 ± 4.7 %, $p < 0.001$). Fasting insulin was lower by 1 yr (13.2 ± 7.1 vs. 17.2 ± 13.6 mIU/l, $p = 0.06$) and 2nd yr (13.6 ± 6.1 vs. 26.4 ± 14.2 , $p < 0.001$) in the I group. There were no differences in proinsulin at 1 or 2 yr of evaluation. The results of this study unique in evaluating the impact of a nutritional intervention with the prolonged use of an enriched formula in body composition and fasting insulin during the important period of weight and length recovery in preterms infants, so called catch up growth. We have shown important effects on body composition and fasting insulin which may have important consequences in long term risk. However, the follow up of these children will confirm whether the benefits of differences in the first year diet on body composition and fasting insulin, will be permanent or overcome by the exposure to similar environmental exposures after the second year of life. Until results of this and other trials are not available we should be cautious on making recommendations .The cost-effectiveness of this type of intervention and the potential unintended consequences need to be assessed in order to select best use of limited resources.

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Nothing to Disclose: EP, PV, VM

P3-712

The Metabolic Syndrome and Body Composition in Childhood Cancer Survivors.

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Improved treatment modalities including surgery, chemotherapy and radiation therapy, as well as hematopoietic stem cell transplantation have dramatically increased childhood cancer survival. As for this reason, post-treatment complications and quality of life issues over the long term have become important.

The metabolic syndrome is a cluster of features including: dyslipidemia, hyperglycemia, hypertension and central obesity and a risk factor for the subsequent development of type 2 diabetes and cardiovascular disease. Several studies reported that long-term survivors of childhood cancer were the risk group of the metabolic syndrome, particularly for patients with growth hormone deficiency. The purpose of this study was to investigate the frequency of the metabolic syndrome and associated factors in childhood cancer survivors at a single center in Korea.

All surviving 98 subjects that had been diagnosed and treated for childhood cancer less than 18 years of age at the department of pediatrics, Samsung medical center, Seoul, Korea, between 1996 and 2007 were enrolled in this study. We analyzed the frequency of metabolic syndrome and differences of obesity and laboratory findings according to irradiation treatment, irradiation dose and site and growth hormone deficiency.

A total of 19 (19.4%) patients had the metabolic syndrome and this is about twice the frequency in the general population of children. Seventeen (17.3%) patients were overweight or obese and the body mass index and waist circumference percentiles were correlated with the cranial irradiation dose. The median body fat percent was 31.5% and it tended to be higher in patients that had cranial irradiation with higher doses. In total, 61 (62.2%) patients had at least one abnormal lipid value. The percent body fat was positively correlated with the Triglyceride ($r = 0.26$, $P = 0.03$) and negatively correlated with the HDL-C ($r = -0.26$, $P = 0.03$).

In childhood cancer survivors, the frequency of the metabolic syndrome appeared to be increased and this findings were associated with cranial irradiation. Of note, was the finding that the body composition of childhood cancer survivors was metabolically unfavorable even if they were not obese. Therefore, childhood cancer survivors should have thorough metabolic evaluation including measurement of the percent body fat even if their BMI is in the normal range for improving health outcomes and reducing the incidence of cardiovascular disease in adults.

Nothing to Disclose: SHK, YBS, SWP, SJK, DKJ

P3-713

AACE/ACE 2009 Guidance for Glycemic Control: An Assessment of the Implications for Initial Combination Therapy.

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¹AstraZeneca, LP Wilmington, DE.

A recent algorithm developed by a panel of expert clinicians details treatment pathways for newly diagnosed patients with T2DM based on initial HbA1c levels. Unlike earlier recommendations for step therapy, this new algorithm suggests initial combination therapy for patients with HbA1c values between 7.6% and 9.0%, and insulin or triple combination therapy for those with HbA1c >9.0%. [1] For this study we examined the distribution of HbA1c values among patients newly diagnosed with T2DM.

Patients were identified through a retrospective analysis of administrative claims and laboratory data (2006-2009) from a large U.S. managed care plan. Inclusion criteria were a first diagnosis for T2DM (ICD-9 250.X0, 250.X2) during the study period with follow-up diagnosis within 6 months, no prior treatment (insulin or oral anti-diabetic [OAD]) treatment, age ≥18 at diagnosis, 6 months plan eligibility prior to diagnosis, and an HbA1c value recorded within 30 days of diagnosis. The cohort was stratified by those receiving treatment early (0-180 days), treatment late (181-365 days), and no treatment during the year following diagnosis.

A total of 10,364 patients met the study criteria. The average age was 50.9 years (SD 9.6 years) and 57.7% were male. The mean follow-up time was 628 days and 88.4% initiated treatment within 6 months of diagnosis. The average HbA1c value at diagnosis was 8.57% among all newly diagnosed patients. Over half of the newly diagnosed patients (54.6%) had baseline HbA1c values ≥7.6%, with 36.9% of baseline HbA1c values ≥9%. Patients with higher baseline values had a shorter time to treatment initiation (table). Among those not receiving OAD or insulin therapy (n=639), a considerable proportion (16%) have HbA1c values for which dual therapy or insulin treatment would be recommended according to the new guidelines.

| | Total | Early Treatment | Late Treatment | No Treatment |
|-------------|---------------|-----------------|----------------|---------------|
| HbA1c Value | % of Patients | % of Patients | % of Patients | % of Patients |
| Mean (sd) | 8.57 (2.34) | 8.78 (2.37) | 7.10 (1.36) | 6.95 (1.32) |
| <7.6% | 45.4% | 40.8% | 76.4% | 84% |
| 7.6-9.0% | 18.5% | 19.4% | 14.6% | 10.2% |
| >9.0% | 36.0% | 39.8% | 9.1% | 5.8% |

A substantial proportion of newly diagnosed T2DM patients have high baseline HbA1c values placing them in the group to receive initial treatment with either dual OAD therapy or insulin based on recent expert recommendations. Many may currently be undertreated.

(1) Rodbard, et al. Statement by an American Association of Clinical Endocrinologists/American College of Endocrinology consensus panel on type 2 diabetes mellitus: an algorithm for glycemic control. *Endocr Pract.* 2009 Sep-Oct;15(6):540-59.

Sources of Research Support: AstraZeneca, LP.

Nothing to Disclose: MFP, LNH, SAW, CH

P3-714

Validity of the Diagnosis of Diabetes in Veterans in the Southeastern United States.

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There is great interest in using electronic medical records to improve care. Since costs are lower with undiagnosed compared to diagnosed type 2 diabetes, strategies aimed at prevention and management could help to reduce costs and improve outcomes. One strategy would be to focus early in the natural history, when preventive management would be expected to slow the progression of disease. However, targeting newly diagnosed diabetes could be appropriate only if the diagnosis were valid. We assessed the validity of the primary care diagnosis of diabetes; if accurate, initial outpatient use of the 250.xx diabetes ICD-9 code could be used to prompt appropriate management. Glycemia and diabetes drug use were evaluated in veterans with continuity of care (follow-up ≥ 2 yr before, ≥ 3 yr after diagnosis). Using a database including SC, GA, and AL, we compared initial primary care diagnosis pts (initial use of 250.xx at a primary care visit) to controls (matched for age, gender, race, VA facility; without code or diabetes drug), and to pts meeting VA Diabetes Epidemiology Cohort criteria (DEpiC - any use of 250.xx twice, or diabetes drug). 2,980 primary care diagnosis, 13,397 control, and 2,456 DEpiC pts were largely male, with mean age 63 yr and BMI 30 kg/m². Primary care diagnosis was based on American Diabetes Association criteria in only 3.7% of cases; 78.2% used nonfasting morning plasma glucose ≥ 126 mg/dl or A1c $\geq 6.5\%$ - criteria that were not standard at the time. However, antecedent "diagnostic accuracy" criteria (≥ 2 of morning plasma glucose ≥ 126 , random glucose ≥ 200 , or A1c $\geq 6.5\%$) were met in 73% primary care diagnosis pts vs. 0% controls ($p < 0.001$), and 76% DEpiCs ($p = 0.7$ vs. primary care diagnosis). Subsequent "predictive accuracy" criteria (A1c $\geq 6.5\%$ or use of diabetes drug within 4 yr) were met in 88% of primary care diagnosis pts vs. 12% controls ($p < 0.001$) and 93% DEpiCs ($p = 0.08$). Delay from antecedent hyperglycemia to diagnosis averaged 12.5 months in primary care diagnosis pts, but 20.1 months in DEpiCs ($p < 0.001$). **Conclusions:** Initial primary care diagnosis of diabetes in the VA is often based on nonstandard criteria, but the diagnosis is valid in both diagnostic and predictive accuracy. Initial primary care diagnosis also identifies patients earlier in their natural history than DEpiC criteria, although both are delayed. Thus, initial outpatient use of the 250.xx ICD-9 code could be used to trigger electronic reminders aimed to facilitate management.

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Nothing to Disclose: JGT, QL, MZ, PFWF, KMVN, LSP

P3-715

Disease Management Intervention for High Risk Patients with Diabetes in Medicare Advantage Plan Reduces Hospitalizations and Healthcare Costs.

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Background: Diabetes is responsible for significant morbidity and financial cost. The direct and indirect cost of diabetes in the US in 2007 is estimated at \$174 billion.¹ The American Diabetes Association reports that 56% of all diabetes-related health care expenditures are for those ages 65 years and older with much of this financial burden covered by Medicare.¹ Much of the suffering and cost associated with diabetes could be mitigated with appropriate care. However, fewer than 45.4% of persons with diabetes receive recommended care.² This care gap is due to many factors such as inconsistent quality care from healthcare providers, lack of patient education and lack of support for self-management such as meal planning, weight control, exercise, glucose monitoring, smoking cessation, and medication adherence. The goal of disease management is to support healthcare providers and patients to close these care gaps.

Aim: This study evaluated the effectiveness of a telephonic diabetes disease management intervention in a Medicare Advantage population with comorbid diabetes and coronary artery disease (CAD).

Methods: This was a prospective controlled repeated measures study of 526 members of a Medicare Advantage segment of a large health plan. High-risk and high-cost patients identified as having diabetes and CAD were randomized into an Intervention Group or a usual-care Control Group. Wilcoxon signed-ranks tests were used to compare the groups on all-cause hospital admissions, diabetes-related hospital admissions, all-cause and diabetes-related ER visits and all-cause medical costs. Self-reported clinical outcomes were also measured in the Intervention Group.

Results: A telephonically-based disease management intervention involving high-risk patients with diabetes and CAD significantly decreased all-cause hospital admissions and diabetes-related hospital admissions ($p \leq .05$). The Intervention Group decreased their all-cause total medical costs by \$984,870 per thousand members per year (PTMPY), compared to a \$4,547,065 PTMPY increase in the Control Group ($p \leq .05$). All clinical quality measures significantly improved from baseline ($p \leq .05$) including A1C, LDL, and microalbumin testing, retinal exams, foot exams ACE or ARB use, and aspirin use.

Conclusion: A telephonically-based disease management program for high risk patients with diabetes and CAD is effective in reducing hospital inpatient admission and total costs in a Medicare Advantage population.

(1) American Diabetes Association. Economic Costs of Diabetes in the U.S. in 2007. *Diabetes Care* 2008 March;31(3):596-615.

(2) Elizabeth A. McGlynn, Steven M. Asch, John Adams, Joan Keesey, Jennifer Hicks, et al. The Quality of Health Care Delivered to Adults in the US. *New England Journal of Medicine* 2003;348(26):2635-45.

Disclosures: JLR: Advisory Group Member, Alere Medical. MST: Researcher, Alere.

P3-716

Assessing Knowledge of Nurses Regarding In-Patient Hypoglycemia and Development of a Web-Based Educational Tool.

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Background

Occurrence of hypoglycemia is a major barrier for achieving optimal glycemic control in patients during hospital admission. For inpatients, nurses often make the initial assessment, decide the management of hypoglycemia and also alter the dose of insulin before meals. There is lack of data regarding the knowledge and practices of nurses in regard to management of acute hypoglycemia in the inpatient setting.

Objective

To assess the knowledge of nurses regarding management and prevention of hypoglycemia in the inpatient setting, and to study the effect of a web based teaching module.

Methods

We developed a 9-question survey: definition of hypoglycemia (1), simple management decisions (4), complex management decisions leading to prevention of hypoglycemia (3), and time devoted to clinical duties (1). Over a 3-week period, the survey was offered online to all the registered nurses involved in adult patient care in a large academic center as a pretest, followed by an online teaching module. The same survey was again administered as a post-test. The teaching module was designed based on current ADA guidelines for the recognition and management of hypoglycemia.

Results

Of the 1543 registered nurses in our academic center, 732 completed the pretest survey and 758 the post-test survey. More than 75% of the nurses spent a majority of their time in direct patient care. In the pretest only 41.42% nurses correctly identified blood glucose level cut-off for hypoglycemia (45.7% did not reply). This improved to 81.77% correct post test responses (15.3% did not reply) ($p<0.001$). For the questions about simple management decisions, correct pretest responses were 51.9%, 21.5%, 68.9%, and 39.48%. These improved to 76.5%, 75.12%, 79.55% and 83.11% respectively after the teaching module ($p<0.05$). For complex management decisions correct responses for the pretest were 22.26%, 41.5% and 28.41%; post-test were 25.2%, 47.23% and 77.18% respectively ($p=0.03$ for the last response). The 2 questions in this category that did not show improvement were not addressed in the teaching module. Analysis of the answers indicated that hypoglycemia is over-treated and the prevention strategies currently practiced by nurses may lead to hyperglycemia.

Conclusion

This study shows that the fear of hypoglycemia likely leading to its overtreatment in the inpatient setting is common in the nursing staff. A targeted teaching module was effective in improving knowledge in this regard.

Nothing to Disclose: TS, RP, AK, HD, AS

P3-717

Resident Education Improves Follow up of Newly Diagnosed Hyperglycemia in Hospitalized Patients.

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Aim: To determine how resident education affects management of newly diagnosed hyperglycemia in hospitalized patients.

Background: Hyperglycemia in hospitalized patients is common(1). Failure to follow up newly diagnosed hyperglycemia is a missed opportunity for early diagnosis and management of diabetes. A previous study in 2007 at a tertiary care teaching hospital found that only 77.1% of patients with newly diagnosed hyperglycemia had repeat FPG ordered, 5.8% had A1C measured, 12.9% had insulin ordered and 7.9% had post discharge instructions(2).

Methods: Internal Medicine residents were given diabetes education over one month via lectures and emails.

Recommendations were made to order FPG if admission glucose ≥ 100 mg/dl, A1C if FPG > 125 mg/dl, diabetic teaching and appropriate discharge planning when FPG > 125 on two occasions and A1C > 6 . In cases where appropriate tests were not ordered by residents, the study investigators obtained patient consent and ordered the tests. Patients ≥ 21 years with no prior history of diabetes admitted to teaching services from 10/01/09 to 11/01/09 were included in the study. These charts were reviewed for residents' orders and follow up planning. Exclusion criteria were: previous history of diabetes, DKA as primary diagnosis, and ICU admissions. We compared our findings with the 2007 study (2) conducted in the same hospital without physician education.

Results: 350 patients with glucose ≥ 100 on admission were included for review; 60 met study requirements. Residents ordered FPGs in all cases. Repeat FPG was > 125 mg/dl in 48 patients. FPGs > 125 were followed with A1C in 33% (16/48) of cases. Insulin was appropriately ordered in 22.9% (11/48) of patients. Of the patients with FPG > 125 , 13 had A1C > 6 , 46.1% (6/13) were given appropriate discharge instructions. However, only 2 patients received nutrition and lifestyle modification teaching by a dietitian.

Conclusion: We found that specific education targeted towards residents regarding newly diagnosed hyperglycemia improves management. However, there is need for further improvement. While a protocol for management of newly diagnosed hyperglycemia including A1C, nutrition and lifestyle counseling, and follow up with primary care physician is important, further educational strategies need to be developed.

(1)Clement S, Braithwaite SS, Magee MF, et al. Management of diabetes and hyperglycemia in hospitals. *Diabetes Care*. 2004;27:553-591.

(2)Chiniwala N, Fitzpatrick R, Woolf PD. Follow up of newly diagnosed hyperglycemia among hospitalized patients. Poster. The Endocrine Society 90th Annual Meeting 2008, San Francisco, CA

Nothing to Disclose: SS, LL, RAF

P3-718

Knowledge, Attitude and Practices of Diabetic Patients in a Rural Community: Phase I of the Community-Based Diabetes Self-Management Education (DSME) in San Juan, Batangas, Philippines.

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Introduction. Diabetes self-management education (DSME) has been recognized as fundamental component of diabetes care. The present study on knowledge, attitude and practices of diabetic patients in San Juan, Batangas comprises the Phase I of community-based DSME program. Data derived from this study will be incorporated to the educational material.

Method. This is a cross-sectional analytic study. Participants were adult diabetic residents from a rural community in the Philippines who were selected using stratified cluster sampling. Their knowledge and attitude were assessed using investigator-administered questionnaires adapted from American Association of Clinical Endocrinologists (AACE) Knowledge Evaluation Form and Diabetes Attitude Scale 3, respectively. Both questionnaires were translated in Filipino and validated in local setting. Practices were ascertained using focused group discussions (FGD).

Result. One hundred fifty-six diabetic residents were recruited. Mean age was 56 ±10 years. Majority were female (67%), did not finish high school education (63%) and have not attended diabetes education classes (87%). The mean score for diabetes knowledge is 42.7%. The highest and the lowest mean percentage scores among the knowledge subscales were 61.15% and 23.93% for treatment and self-monitoring, respectively. They correlated significantly with age ($p=0.026$) and highest educational attainment ($p=0.046$). On diabetes-related attitudes, only 38% believed on patient's autonomy. Fewer still believed the value of tight glucose control (10%) and the seriousness of their condition (1.28%). Thirty-five patients were included on FGD on diabetes practices. Majority of them consult private doctors (72%) but only 43% consult regularly. Regular HbA1c monitoring is done by 23% of respondents and 25% persisted smoking despite advice to quit by their doctors.

Conclusion. There is a need to increase knowledge among diabetic residents in this rural community to influence diabetes-related attitudes and practices. The present study highlights the importance of evaluating knowledge, attitudes and practices as crucial means to understand observed behaviors and to guide behavioral change.

Nothing to Disclose: PCP, GJRA, EPP, CAJ, FLL-A, EP, NRJ

P3-719

Diabetes Awareness among the Asian Subgroups in California.

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Diabetes Mellitus is the leading cause of blindness for Americans aged 21-65 years. The prevalence of diabetes in Asian-Americans is 60% higher than non-Hispanic whites(1). Asian-Americans, while comprising 4% of the US population, comprise 30-50% of the population in parts of California(2). For any Body Mass Index, Asians are more at risk for diabetes non-Hispanic whites(1).

Purpose: Use of a questionnaire (QN) to assess knowledge of diabetes in the Chinese (Ch), South Asian (SA) and Korean (K) subgroups as compared to non-Hispanic whites (W).

Methods: QN about diabetes, risk factors and treatment was distributed at Asian churches, temples, food markets, and schools. QN was translated into the respective Asian languages.

Results: 464 QN: 121 Ch, 100 SA, 143 K, 100 W. The results of the W QN vs. Ch QN showed no significant differences (p=ns).

| Questions | Have you seen a doctor in the past 1-2 years | Diabetes can lead to blindness | Diabetes can cause bleeding in the eye | If you have a parent with diabetes, you are at risk for getting diabetes |
|-----------|--|--------------------------------|--|--|
| Ch vs. SA | * (Ch 88% vs. SA 75%) | * (Ch 90% vs. SA 74%) | * (Ch 69% vs. SA 49%) | * (Ch 98% vs. SA 75%) |
| SA vs. K | ns | * (SA 74% vs. K 85%) | * (SA 49% vs. K 74%) | * (SA 75% vs. K 92%) |
| Ch vs. K | * (Ch 88% vs. K 79%) | ns | ns | * (Ch 98% vs. K 92%) |
| W vs. Ch | ns | ns | ns | ns |
| W vs. SA | * (W 90% vs. SA 75%) | * (W 92% vs. SA 74%) | ns | * (W 94% vs. SA 75%) |
| W vs. K | * (W 90% vs. K 79%) | ns | * (W 61% vs. K 74%) | ns |

Table1. Percentages indicate yes responses. * = p ≤ 0.05, ns = not significant.

Conclusion: Chinese and non-Hispanic whites share similar levels of health literacy. South Asians and Koreans vs. Chinese are less aware of diabetic risk factors such as the role of family history and eye disease. Thus, the differential lack of knowledge about diabetes among the Asian subgroups suggests that future targeted public education initiatives may be useful.

- McNeely, M. J. and E. J. Boyko (2004). "Type 2 Diabetes Prevalence in Asian Americans." *Diabetes Care* 27(1): 66-69.
- U. S. Census Bureau Population Estimates Program, Profiles of General Demographic Characteristics: National summary: 2000 census of population and housing, 2001.
http://www2.census.gov/census_2000/datasets/demographic_profile/0_National_Summary/2khus.pdf. Accessed 7 January 2009.

Nothing to Disclose: JS, BH, GW, JT

P3-720

Diabetes in the Desert: What Do Patients Know about the Heat?.

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Living with diabetes in hot climates poses unique care challenges (1). Increasing awareness about the interaction between heat and diabetes should be a priority as more patients are living in regions with high temperatures. Data is sparse on what diabetes patients understand concerning heat or what precautions they should take under extreme heat conditions. We conducted a survey of patients attending a Southwestern U.S. diabetes clinic that gauged types of personal protective measures taken against the heat, examined knowledge of safe temperatures and exposure times, assessed comprehension of weather data, and determined sources of weather information. From 30 November to 31 December 2009, 169 patient questionnaires were completed. Mean patient age was 66 years, diabetes duration 15 years, 52% were men, 85% had type 2 diabetes, 62% were non-Hispanic white, 67% were on insulin injections, and 6% on insulin pumps. Mean hemoglobin A1c was 7.9%, 38% had a hemoglobin A1c value $\geq 8.0\%$, and nearly 40% had values $\geq 8.0\%$ during the hottest summer months (July and August). Patients employed a variety of personal protective measures, and 68% limited heat exposure to <1 hour. While respondents typically took steps to protect their diabetes equipment and medication (e.g., carrying items in a cooler), 36% simply left medications or supplies at home. Although 72% of respondents indicated they had received information regarding the effects of heat on insulin, only 40% acknowledged having received information about the effect of heat on oral medications, 41% on glucose monitors, and just 38% on glucose monitoring strips. There was considerable variability in temperatures at which patients would consider taking protective measures. Even though 82% knew the correct definition of humidity, only 55% knew the definition of the heat index. Overall, television was the primary source for weather information (89%). Results indicate many patients in this population have suboptimal glycemic control which may place them at risk for dehydration during the hottest months, and use a medication (insulin) particularly susceptible to heat damage. Most respondents had awareness as to the importance of heat in relation to their diabetes, although gaps in knowledge were evident. Increased public awareness of this important topic is needed, and diabetes education programs should include information about the heat where regionally appropriate. The views expressed are those of the authors and do not necessarily represent those of the National Weather Service.

1. Westphal SA et. al. Endocrine Practice, submitted

Nothing to Disclose: AAN, RDC, MEB, KAJ, MF, MJH, CBC

P3-721

A Multifactorial Approach To Prevent Adiposity and Improve Fitness in Predominantly Migrant Preschool Children: Cluster-Randomized Controlled Trial (The Ballabeina Study).

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Objective : To assess the effectiveness of a multifactorial lifestyle intervention program on adiposity and fitness in preschool children

Design : Cluster-randomized trial conducted from August 2008 to June 2009. Persons performing outcomes measurements, but not participants were blinded to group assignment.

Setting : Forty preschool classes from areas with a high migrant population were randomly selected and randomized into an intervention (n=20) and a control (n=20) arm by the use of opaque envelopes after stratification for language region (French vs German part of Switzerland).

Participants : 655 preschool children (mean age at baseline 5.1 ±0.7 yrs) entered the trial , 73% with at least one migrant parent

Intervention : Increased physical activity, promotion of healthy nutritional behavior and sleep and reduction in media use were implemented through lessons, infrastructural changes and involvement of parents and teachers.

Outcome measures: Primary outcomes included BMI and aerobic fitness (20 m shuttle run test). Secondary outcomes included body fat (sum of 4 skinfolds), % body fat (bioelectrical impedance), waist circumference, overall fitness (obstacle course), physical activity (accelerometry), nutritional behavior, media use and sleep (the last four by questionnaires).

Results : Compared with controls, children in the intervention group had no difference in BMI, but a more favorable improved performance in aerobic and overall fitness (adjusted differences (95% CI): 0.32 stages (0.07 to 0.57), p=0.01 and -0.53 sec (-0.93 to -0.13), p=0.009) and significant relative decreases in body fat, % body fat and waist circumference (adjusted differences: -3.6 mm (-6 to -1.2), p=0.003; -1.1 % (-2.0 to -0.2), p=0.017 and -1.0 cm (-1.6 to -0.4), p=0.001). There were significant differences in reported, but not in measured physical activity, in media use and some aspects of nutritional behavior, but not in sleep duration.

Conclusion: Our multifactorial approach reduced body fat and improved fitness in preschoolers.

Sources of Research Support: Principally Swiss National Science Foundation (Grant # 3200B0-116837) and Health Promotion Switzerland (Project # 2104).

Nothing to Disclose: JJP, PM-V, LZ, IN, FB, VE, TH, UM, CS, AN, SK

P3-722

Medical Resource Use and Costs in Children with Central Precocious Puberty.

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Introduction: The objective of this study was to assess the burden of disease by examining healthcare resource utilization and costs among children with central precocious puberty (CPP) versus an age-gender matched cohort of children without CPP.

Methods: A propensity matched cohort study of 905 children less than 15 years of age from a geographically-diverse administrative claims database (Thomson Medstat) was conducted to assess the burden of CPP. The CPP cohort included commercially insured U.S. patients newly diagnosed with precocious sexual development and puberty (ICD-9-CM code 259.1x) between January 1, 2004 and June 30, 2006 who were dispensed gonadotropin-releasing hormone agonists during the 12-month post-index period. Each CPP patient was matched to 4 non-CPP controls based on age, gender, region, and health plan type. The date of the initial diagnosis was designated as the "index date" for CPP cohort, whereas the index date for control patients was set at the end of first year of continuous enrollment. Overall and CPP-related healthcare resource utilization and costs were assessed in the 12 months prior to and following the index date for study patients.

Results: A total of 181 CPP patients and 724 control patients were identified after matching. Seventy-two percent of all patients were under 10 years of age (mean: 8.5 years) and 83% were female. CPP patients had significantly higher use of inpatient and outpatient services compared with the control patients in both the pre- and post-index periods. On average, CPP patients had higher annual healthcare costs than controls during the pre-index period (\$11,373 vs. \$971, $p < 0.05$) as well as the post-index period (\$21,715 vs. \$923, $p < 0.05$). For CPP patients, the overall annual healthcare costs increased by \$10,342 following diagnosis. Monthly total healthcare costs for CPP patients increased sharply in the first month following diagnosis and remained high throughout the post-index period.

Conclusion: This is the first study to assess the burden of CPP. Healthcare resource utilization and costs among CPP patients appeared to be substantially higher prior to and following the initial CPP diagnosis.

Sources of Research Support: Research grant provided by Abbott Laboratories.

Nothing to Disclose: SC, MJF, LB, KC, PMR, SEM

P3-723

Development of Comprehensive Care Centers for Management of Patients with Congenital Adrenal Hyperplasia.

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Patients with congenital adrenal hyperplasia (CAH) face many challenges from birth to adulthood. Despite major progress in the diagnosis and treatment of children with CAH, the delivery of multidisciplinary care coordinated among subspecialists and across the lifespan of these patients remains challenging. To address these deficiencies, the Congenital Adrenal Hyperplasia Research, Education, and Support (CARES) Foundation convened a conference in September 2009 to develop guidelines to establish Comprehensive Care Centers (CCCs) for patients with CAH. This group of physicians, surgeons, psychologists, and nurses with expertise in CAH met with the sponsors, consultants, and consumers to identify needs, draft minimal criteria for a CCC, and establish metrics for evaluation of the CCCs. Consumers assisted in the development of the guidelines, assured that neglected areas of concern to patients and their parents were addressed, and shared their experiences. Important components of a CCC include communication, educational resources, support for families, geographic centralization, mechanisms for access, training for healthcare providers, infrastructure for collaborative research, and formal transitioning procedures. Major impediments to the development of CCCs were identified, including limited expertise in internal medicine endocrinology and OB/GYN in the management of patients with CAH; a similar lack of pediatric endocrinologists, pediatric urologists, and behavioral health specialists skilled in the care of patients with sex steroid imbalance, virilizing CAH, or gender dysphoria; and the acquisition of institutional and financial support. The importance of establishing a registry amongst the CCCs to facilitate research, share expertise, and develop common protocols and educational aids was given high priority as an integral component of the CCCs. The CCCs will integrate research, coordinated care, and patient advocacy to improve the quality of life for CAH patients and the training of healthcare providers. Moving forward, the implementation of CCCs should involve a few initial sites, which are accredited after inspection by a panel of experts and consumer advocates. Centers will be periodically evaluated and re-accredited, and data collected from the registry will document improvements in the quality metrics. The net result will be improved health and quality of life for patients with CAH with new data to guide treatment and health care policy.

Sources of Research Support: New York-Mid-Atlantic Consortium for Genetics and Newborn Screening Services; National Newborn Screening and Genetics Resource Center; US Department of Health and Human Services, Health Resources and Services Administration; Intramural Research Program of the National Institutes of Health.

Disclosures: EAE: Study Investigator, Abbott Laboratories; Medical Advisory Board Member, ENDO Pharmaceutical. MEG: Principal Investigator, Pfizer, Inc.; Poster Reviewer, Pfizer, Inc.; Scientific Board Member, Pfizer, Inc.

Nothing to Disclose: RJA, SW, PCW, LAB, AB, SAB, KJL, DM, TN, DPP, FGR, RR, SAR, DES, TS, RNS, CV, MGV, JEA, TB, PAL, WLM, CAQ, ES, RA, SF, KL, GAL, DS, DMM, FXS, ALK, KP, MAL-P, DB, BK, KBH, BLT

OR28-1

The Role of Oocyte *trkB* Receptors on Follicular Development and Fertility.

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Previous results, using conventional *trkB*-null mice, identified TrkB, the high-affinity receptor for neurotrophin-4/5 and brain-derived neurotrophic factor, as being required for follicular assembly, early follicular growth, and oocyte survival. Alternative splicing generates both a full length receptor (TrkB-FL) and truncated isoforms (TrkB-T1 and TrkB-T2), which lack the intracellular domain. TrkB-T1 is predominantly expressed in oocytes of infantile mice. To determine if oocyte-specific deletion of all TrkB receptor isoforms affects ovarian function we used a conditional *trkB* KO mouse. Mice expressing Cre recombinase under the control of the *Gdf9* promoter were bred to mice with a floxed *trkB* allele and to reporter mice (*mT/mG*). In the reporter mice, a loxP-flanked sequence encoding red fluorescent protein (RFP) is located upstream from a cDNA encoding green fluorescent protein (GFP), so that Cre-mediated deletion of RFP allows expression of GFP. *Gdf9-Cre/mT/mG* mice showed GFP expression only in oocytes, indicated that *Gdf9* promoter-directed Cre recombination is oocyte-specific. Oocytes from wild-type 12-day-old mice only express *TrkB-T1* mRNA, the levels of which are markedly reduced in *Gdf9-Cre/TrkB^{fl/fl}* mice. Immunohistofluorescence detection of the TrkB-T1 protein showed that Cre-mediated recombination had occurred in most, but not all oocytes. Antral follicle formation was delayed in *Gdf9-Cre/TrkB^{fl/fl}* mice on postnatal day 12, but much less on day 28, indicating that the lack of TrkB-T1 in oocytes only transiently delays antral follicle development. *Gdf9-Cre/TrkB^{fl/fl}* mice had a normal age at puberty, but became infertile after having only one or two litters. Reproductive failure was associated with widespread oocyte death and a striking loss of follicular organization, suggesting that oocytes devoid of TrkB receptors cannot survive after the initiation of estrous cyclicity. This survival signal is not provided by TrkB-T1 receptors, because oocytes die only after the ovary is exposed to preovulatory levels of gonadotropins, despite the early loss of TrkB-T1. Since oocytes exposed to elevated gonadotropin levels are rich in TrkB-FL receptors (1), our results are consistent with the interpretation that TrkB-FL receptor expression is induced in oocytes by the preovulatory LH surge, and that TrkB-FL-mediated signaling is essential for oocytes to survive at the onset of adult reproductive cyclicity.

(1) Kawamura et al, Proc Natl Acad Sci USA 2005; 102:9206

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Nothing to Disclose: MD, CG-R, BX, GAD, SRO

OR28-2

Loss of ER β Activity Results in Dysregulation of Follicular Extracellular Matrix Genes in Response to Follicle Stimulating Hormone.

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Within the ovary, ER β is localized to the granulosa cells of growing follicles. Adult ER β -null females are subfertile and possess ovaries with reduced numbers of growing follicles. Estrogen via ER β augments the actions of FSH in granulosa cells and this action is required for optimal granulosa cell differentiation and formation of a preovulatory follicle. Using microarray and biochemical analysis, we show that loss of ER β causes dysregulation of follicular extracellular matrix (ECM) composition and cell adhesion in ER β -null ovaries. We find that expression of many ECM and cell adhesion genes is dysregulated in ER β -null granulosa cells, and have begun to characterize these genes in WT and ER β -null ovaries. We are currently focussed on the ECM proteins, cartilage oligomeric matrix protein (Comp), Nidogen 2 (Nid2), and Collagen 11a1 (Col11a1). Comp is a secreted glycoprotein which mediates cell-cell and cell-matrix adhesion, and has been well-characterized in cartilage but not in the ovary. Interestingly, women with Comp mutations present with prolonged menstrual cycles, suggesting a role for Comp in normal reproductive function. We find that Comp expression increases 300-fold in granulosa cells from PMSG-treated WT mice compared to vehicle-treated mice, but only 3-fold in ER β -null cells, indicating a major role for ER β in regulation of Comp expression in response to FSH. We have localized Comp protein to the ECM surrounding granulosa cells in antral follicles, but not smaller follicles. In vitro, Comp mRNA increases in FSH-treated WT primary granulosa cells but not in ER β -null cells, supporting a role for ER β in regulation of Comp expression. In contrast, both Nid2 and Col11a1 are expressed up to 7-fold higher in ER β -null granulosa cells than in WT cells isolated from PMSG-treated mice, suggesting that ER β negatively regulates their expression. In addition, both Nid2 and Col11a1 levels increase in FSH-treated WT primary granulosa cells, suggesting that FSH acts directly on these cells to regulate their Nid2 and Col11a1 expression. In agreement with the disrupted ECM expression in ER β -null granulosa cells, primary ER β -null granulosa cells are unable to form aggregates when treated with FSH, in contrast to WT cells, which aggregate when exposed to FSH, suggesting a role for ER β in granulosa cell adhesion in response to FSH. These are the first data to indicate a role for ER β in the regulation of cell adhesion and ECM composition during mouse folliculogenesis.

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Nothing to Disclose: BJD, SR, AZ

OR28-3

FOXL2 Mutations, Associated with Premature Ovarian Failure (POF), Likely Dimerize with Wild Type FOXL2, Leading to Altered Regulation of Genes Associated with Granulosa Cell Differentiation.

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Objective: Premature ovarian failure is present in the autosomal dominant disorder, Blepharophimosis-Ptosis-Epicanthus Inversus (BPES) type I. Mutations in the gene encoding Forkhead L2 (FOXL2), have been found in patients with BPES type I producing putative truncated proteins. We have previously demonstrated that FOXL2 acts as a transcriptional repressor of the Steroidogenic Acute Regulatory (StAR) gene, P450SCC, aromatase (CYP19) and cyclin D2, all markers of ovarian follicle proliferation and differentiation (1, 2) Further, we have found that mutations of FOXL2 may regulate wild type FOXL2 leading to loss of repressor activity of select genes, StAR and CYP19. Thus, we set out to determine the potential regulatory mechanisms associated with mutations of FOXL2 that leads to the POF phenotype.

Methods: CHO cells were transfected with pcDNA3-FOXL2 and mutant FOXL2 expression constructs with/without various lengths of CYP 19 promoter-luciferase vector. For immunofluorescence, CHO cells were grown on coverslips and transfected with wild type FOXL2 and/or mutant FOXL2, followed by incubation with 1^o and 2^o antibodies.

Immunoprecipitation of CHO cells transfected with FOXL2, wild type and/or mutant, was performed to identify wild type and mutant interactions. Electrophoretic mobility shift assays were performed to evaluate DNA binding.

Results: Wildtype and mutant FOXL2 are localized in the nucleus and increasing concentrations of mutant FOXL2 do not displace wild type FOXL2 from the nucleus. Further, immunoprecipitation demonstrates that wild type and mutant FOXL2 bind together and to each other, suggesting dimerization. Although repressor activity of FOXL2 at the CYP19 promoter is lost in the presence of mutant FOXL2, this effect is not observed in the -57 bp CYP19 promoter, which contains only 1 putative FOXL2 consensus site. Further, wild type FOXL2 binds to the -57 bp CYP19 promoter, whereas mutant FOXL2 does not.

Conclusion: Mutations of FOXL2 produce truncated proteins that are localized to the nucleus and bind to wild type FOXL2, leading to loss of transcriptional repressor activity. However, in the presence of a single FOXL2 consensus site, aberrant effects of mutant FOXL2 are lost, possibly due to abnormal binding. Thus, wild type and mutant FOXL2 may exist as homo- or hetero-dimers and aberrant transcriptional activity of mutant FOXL2 occurs through its interaction with wild type FOXL2 and not interference with DNA binding.

(1) Pisarska MD et al. *Endocrinology* 2004;145:3424

(2) Bentsi-Barnes, et al. *Fertil Steril* 2009 Nov 14. [Epub ahead of print]

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Nothing to Disclose: IB-B, GB, FTK

OR28-4

FZD1 May Transduce the WNT4/RSP01/CTNNB1 Ovarian Developmental Signal.

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Canonical WNT signal transduction occurs via Frizzled (*Fzd*) receptors and results in the stabilization of CTNNB1 (β -catenin), which then enters the cell nucleus to modulate the transcriptional activity of target genes. During ovarian development, a canonical WNT signal is initiated by WNT4 and modulated by RSP01, the absence of which results in developmental defects including partial sex-reversal. In the adult ovary, WNT4 regulates follicle development and modulates the expression of a variety of ovarian genes, including several involved in steroid hormone biosynthesis. The FZD receptor(s) involved in transducing the WNT4 signal have not been identified, although *Fzd1* is expressed in the embryonic reproductive tract and in the granulosa cells of periovulatory follicles. A gene knockout approach was therefore used to determine the potential roles of *Fzd1* in the ovary. *Fzd1*^{-/-} mice were viable and were found to be devoid of any morphological or physiological defects of their non-reproductive systems. Mating experiments showed that 73% (n=8 of 11) of female *Fzd1*^{-/-} mice were fertile. Whereas mice featuring a granulosa cell-specific inactivation of *Wnt4* have previously been shown to produce small litters due to small ovaries bearing reduced numbers of antral follicles, fertile *Fzd1*^{-/-} mice produced litters at a rate and of a size comparable to controls up to 1y of age ($P > 0.05$), and their ovaries contained normal numbers of follicles at 42d and 6m of age. It therefore seems unlikely that FZD1 functions non-redundantly as a WNT4 receptor in the adult ovary. Conversely, 27% of *Fzd1*^{-/-} mice (n=3 of 11) were sterile. The ovaries of 6 month-old sterile *Fzd1*^{-/-} females showed a complete depletion of the follicular reserve and the presence of tubulostromal hyperplasia. Moreover, tubular structures lined by cells resembling Sertoli cells were found in the ovaries of these mice, and structures resembling efferent ducts were identified in proximity to the ovaries. Sterile *Fzd1*^{-/-} mice also had severe uterine fibrosis and few uterine glands. A subset of 3 week-old *Fzd1*^{-/-} mice had very small ovaries (< 1mg) containing very few preantral follicles, which failed to develop in response to exogenous gonadotropin stimulation (eCG, 5IU, ip). As these phenotypic anomalies closely mimic those observed in *Wnt4*- and *Rspo1*-null mice, we conclude that FZD1 may function as a physiological WNT4 receptor during ovarian development.

Nothing to Disclose: EL, MP, AB, JSR, DB

OR28-5

Receptor Activity Modifying Protein 2 (RAMP2) Haploinsufficiency Causes Hyperprolactinemia and Endocrine Dysfunction in Mice.

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Receptor activity-modifying protein-2 (RAMP2) is a single transmembrane protein that can regulate the trafficking, ligand binding and signaling of several G protein-coupled receptors (GPCRs) *in vitro*, including parathyroid hormone receptor (PTH1R), calcitonin receptor (CTR) and calcium sensing receptor (CaSR). The most well-characterized role of RAMP2 is in the regulation of adrenomedullin (AM) binding to calcitonin receptor-like receptor (CLR) and our previous *in vivo* studies using knockout mouse models support this canonical signaling paradigm. For example, *Ramp2*^{-/-} mice die at midgestation with a precise phenocopy of the *AM*^{-/-} and *Calcr1*^{-/-} mice. In contrast, *Ramp2*^{+/-} mice are viable and exhibit an expanded variety of phenotypes that are distinct from those of *AM*^{+/-} and *Calcr1*^{+/-} mice, suggesting that RAMP2 may have physiological functions beyond AM/CLR signaling. *Ramp2*^{+/-} female mice bred to *Ramp2*^{+/-} or WT males have severe reproductive defects resulting in fetal growth restriction, fetal demise and postnatal lethality. Fetal loss is independent of the fetuses' genotype and gender suggesting that complete dosage of maternal *Ramp2* is required for normal pregnancy. Of the litters that survive to birth, 24% of the litters die by postnatal day 2 and 40% of the litters lose at least 1 pup before wean, owing to the lack of milk in their stomach. *Ramp2*^{+/-} female mice have mild hyperprolactinemia at basal conditions as well as during pregnancy. *Ramp2*^{+/-} female mice also exhibit hyperplastic anterior pituitary gland, accelerated mammary gland development, and decreased bone mineral density and content; phenotypes that are commonly associated with hyperprolactinemia. Taken together, these data demonstrate that RAMP2 is required for maintaining endocrine homeostasis in female mice. Since RAMP2 has been shown to associate with numerous GPCRs, it is likely that signaling of one or more of these GPCRs is compromised in *Ramp2*^{+/-} mice, yet the precise identification of these receptors remains to be elucidated. Taken together, this work reveals an essential role for RAMP2 in endocrine physiology and provides the first *in vivo* evidence for a physiological role of RAMP2 beyond that of AM/CLR signaling.

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Nothing to Disclose: MK, KLF-S, ML, KMC

OR28-6

Identification of a Novel Estrogen Responsive Estrogen Receptor α (ESR1) Variant in the Pregnant Uterus.

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The role of estrogen and estrogen receptors in the onset of human labor is not clearly defined. It has been postulated that increased ER action at term may inhibit uterine PR transactivation. These events would lead to a functional withdrawal of PR action and thus the onset of labor. We hypothesize that alternate ER transcripts in the pregnant uterus may play a critical role in governing PR responsiveness at term. There is mounting evidence that potential alternative ER proteins encoded by mRNA splice variants such as the hER α 46 variant may play a role in the mediation of uterine estrogen responsiveness. This is evidenced by the fact that cell lines unable to express the classical ER and ER knockout mice retain partial uterine estrogenic responsiveness.

Utilizing multiple ER antibodies we identified by western blot analysis that hER α 46 is the major ER isoform found in the myocyte nuclear fraction of human pregnant myometrial tissues, explant cultures and in our telomerase immortalized myometrial cell line (hTERT). Whereas hER α 66 was confined to the cytoplasmic fraction in all of the samples examined. To confirm the presence of the hER α 46 variant transcript, we undertook 5' RACE and Northern blot analysis of hTERT cell line as well as myometrial tissues isolated from term and pre-term patients. The 5' RACE analysis identified an alternate ER α transcript which when translated would give rise to a 46kDa ER α protein. These data also identified that the uterine ER α 46 utilized a novel promoter in intron 1 as its proximal promoter.

We also determined that estrogen responsiveness of hER α 46 in primary human myometrial explant cultures differ significantly between myometrium isolated from non-laboring pre-term (n=4) and term patients (n=4). Increased levels of nuclear hER α 46 result upon exposure to estrogen in term patients whereas in pre-term myometrium the 46kDa isoform level remains unresponsive to estrogen.

In conclusion we have identified a novel ER α 46 protein and transcript that utilizes a novel promoter in the uterine myocyte. We have identified that ER α 46 is preferentially isolated in the nuclear fraction, whereas the classical ER α 66 is restricted to cytoplasmic fraction. Furthermore we speculate that this estrogen responsive ER α 46 may as term approaches and estrogen levels rise have the capacity to act as the major uterine nuclear ER isoform playing a role in PR functional withdrawal.

Sources of Research Support: NIH OPRU U01 supplement (HD047905).

Nothing to Disclose: CRM, SNC, JCC, PJ

OR29-1

Loss of the C-Terminus of Melanocortin Receptor 2 (MC2R) Results in Impaired Cell Surface Expression and ACTH Insensitivity.

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Familial glucocorticoid deficiency (FGD) is a syndrome which usually manifests with hyperpigmentation due to grossly elevated ACTH and low cortisol, deficiency of adrenal C19 steroids but normal levels of mineralocorticoids. Several mutations have been described for the melanocortin 2 receptor (MC2R) gene and the MC2R accessory protein (MRAP) gene in roughly 50% of the patients presenting with FGD. MC2R binds ACTH at highest affinity and is expressed throughout the adrenal cortex. MRAP is involved in trafficking of MC2R from the endoplasmic reticulum (ER) to the cell surface and is involved in MC2R signaling. At present, little is known about the interaction of MRAP with MC2R. We found a novel homozygote MC2R mutation (K289fs) in a patient presenting with typical FGD. The patient from Gambia had consanguineous parents and presented at the age of six weeks with severe hypoglycemia; hormonal analysis revealed non-detectable cortisol (< 1 µg/dl), markedly elevated ACTH (> 1250 pg/ml) but normal electrolytes. K289fs is the first nonsense mutation identified in the C-terminus of the MC2R gene that is located in the intracellular part of the protein. K289fs causes a change in the last 8 amino acids when compared to wild-type protein and encodes for a longer protein. We performed detailed in vitro studies of this mutation. We created MC2R expression vectors for the K289fs mutant, a M290X deletion mutant as well as alanine-substituted constructs of each of the 8 C-terminal amino acids. The functional impact of K289fs, M290X and all 8 alanine-substituted mutation constructs was studied in a OS3-cell based reporter assay system that has an intact downstream cAMP signal transduction pathway but fails to express the endogenous MC2R. Transfection of OS cells with wild type (WT) or mutant MC2Rs together with a cAMP-linked luciferase reporter revealed a dose-response to 100nM (1-24)-ACTH for WT and alanine-substituted constructs but a total loss of function for K289fs and M290X. Cell surface assays and localization studies showed that the K289fs as well as the M290X mutants were not found at the cell surface indicating that their transport from the ER to the cell membrane is disrupted. Coimmunoprecipitation experiments showed no alteration in the interaction of mutant MC2R with MRAP. These findings resemble those found for the majority of naturally occurring missense MC2R mutations and indicate the extreme sensitivity of this receptor to structural disruption.

Sources of Research Support: ESPE Visiting Scholarship 2009 to Andrea Hirsch.

Nothing to Disclose: AH, LA, EM, AJLC, CEF

OR29-2

Dissection of the MC2R-MRAP Complex in Live Cells Using Bioluminescence Resonance Energy Transfer.

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The pituitary hormone Adrenocorticotropin (ACTH) acts via the melanocortin 2 receptor (MC2R) to stimulate glucocorticoid production in the adrenal cortex. The Melanocortin 2 Receptor Accessory Protein (MRAP) is essential for cell surface expression and function of this receptor. Elegant studies from Sebag & Hinkle (2007) suggested that MRAP functions as a unique antiparallel homodimer. Using both co-immunoprecipitation (Co-IP) of epitope-tagged MC2R and Bioluminescence Resonance Energy Transfer (BRET) we have demonstrated that MC2R exists as a constitutive homodimer in transfected HEK293 cells at both the endoplasmic reticulum and at the cell surface. MRAP homodimers generated a BRET signal only for MRAP labelled to detect the antiparallel homodimer and no BRET signal was generated in cells labelled to detect parallel MRAP dimers. However the parallel and antiparallel dimer-induced BRET signal increased significantly in the presence of MC2R, which may be attributed to the homodimerisation of MC2R bringing parallel MRAP molecules from two antiparallel dimers (one associated with each MC2R molecule) into proximity. The MC2R-MRAP interaction also generated a BRET signal. ACTH had a significant enhancing effect on this signal. Co-IP did not show that ACTH increased the physical interaction between the MC2R and MRAP, implying that a conformational change in the MC2R-MRAP interaction was probably induced by agonist stimulation. Real-time analysis revealed two distinct phases of this ACTH-dependent BRET increase. The competitive antagonist ACTH 11-24 only showed the first rapid phase whilst unstimulated cells and cells with ACTH 1-13 (non binding peptide) did not show either. We propose that the first phase may be due to ligand binding to the receptor complex leading to a transient conformational change, whilst the second phase of BRET increase may be a consequence of ACTH-generated signal transduction. Consistent with this view, the ACTH-induced BRET signal was significantly reduced in the presence of the PKA inhibitor KT5720 suggesting that the receptor phosphorylation may play a significant role in the rearrangement of the pre-existing receptor-MRAP complex. These data suggest that the MC2R and MRAP exist in a hexameric complex comprised of a MC2R homodimer and two antiparallel MRAP homodimers. Ligand-induced BRET increase is dependent on the conformational change of the activated receptor rather than increased dimerisation of the proteins.

Sebag JA, Hinkle PM., Proc Natl Acad Sci USA 2007; Dec 18; 104(51) :20244-9

Nothing to Disclose: SNC, TTC, LS, AJLC

OR29-3

MET Receptors Induce Increased Cell Migration Via an ERK1/2 and Sam68-Dependent Mechanism.

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The hepatocyte growth factor (HGF)/Met receptor signaling pathway is deregulated in many human malignancies and plays a central role in oncogenesis, tumor progression and invasive cancer cell growth. For example, Met receptors are frequently overexpressed in advanced breast cancers, and are thought to contribute to the metastatic behavior of these tumors. In addition, deregulated expression and splicing of the cell adhesion marker CD44 has been identified in several types of cancers. In particular, the expression of CD44 isoforms containing variable exon-5 (v5) correlates with enhanced invasiveness and metastatic phenotypes of some tumors, including breast cancer. Little is known about the regulation of CD44v5 in response to membrane-initiated signaling events in normal and neoplastic cell models. We sought to first understand how CD44v5 expression is regulated in normal cell contexts. Our model system, the immortalized non-tumorigenic keratinocyte (HaCaT) cell line, abundantly expresses both the tyrosine kinase growth factor receptor, c-Met, and the transmembrane glycoprotein CD44v5. Here we show that HGF (the c-Met ligand) stimulates increased HaCaT cell migration and CD44v5 protein expression. We also demonstrate that HGF-dependent CD44v5 up-regulation requires Sam68, a protein involved in RNA processing, splicing and v5 inclusion in a Ras-ERK MAPK dependent manner. Knock-down of Sam68 or treatment of cells with the MEK/MAPK inhibitor (U0126) blocked CD44v5 expression and cell migration in response to HGF as measured by Boyden Chamber assays. We hypothesized that HGF/Met signaling might also regulate breast cancer cell migration and invasion via a similar mechanism. Similar to HaCaT cells, highly migratory MDA-MB-231 breast cancer cells also required ERK1/2 signaling and Sam68 expression for HGF-induced migration. Surprisingly, however, in these cells, HGF-induced migration occurred independently of CD44v5 or v6 expression, suggesting a role for Sam68-dependent splicing of alternate gene targets. Future experiments will be aimed at elucidation of Sam68 regulated splice variants of genes involved in HGF-induced migration in breast cancer cells. Blockade of Sam68, a mediator of alternate splicing and cell migration, may provide a new avenue for therapeutic inhibition of metastatic cancers.

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Nothing to Disclose: AL, NEC, CAL

OR29-4

Gpr-54 Signals Via a Feedback Loop Involving ERK-P90rsk-GSK3 β and FAK To Affect *In Vitro* Cell Migration.

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Kisspeptin is an inhibitor of cell migration and malignant cell metastasis. Kisspeptin has also been implicated in trophoblast cell invasion during placental development and affects the function of hypothalamic neurons. We investigated intracellular pathways activated by kisspeptin/gpr-54 relevant to migration using a CHO cell line stably-expressing human gpr-54. Kisspeptin-10 (kp-10) dose dependently inhibited the migration of these cells in an *in vitro* scratch assay monitored over 22 hours. Kp-10 activated the MAPK pathway via ERK1/2 phosphorylation. Activation of ERK1/2 correlated with p90rsk-dependent inhibition of GSK3 β by phosphorylation at Ser⁹ and this promoted release of β -catenin to the cytoplasm. GSK3 β inhibition was amplified by a feedback loop involving FAK phosphorylation at Tyr⁹²⁵. Phosphorylation at this site causes FAK to bind the Grb-SOS complex and activate Ras, promoting subsequent phosphorylation of ERK1/2. The ERK1/2 and GSK3 β feedback loop is critical for inhibition of CHO cell migration in the scratch assay. Mouse GnRH neuron-like GT₁₋₇ cells were much less motile compared to CHO cells. This difference in migration capacity correlated with differences in the signaling response following activation of gpr-54 stably expressed in GT₁₋₇ cells. Although ERK1/2 and GSK3 β were phosphorylated in GT₁₋₇ cells, there was no elevation of β -catenin or p-FAK. The results suggest that kisspeptin can activate complex pathways to affect the migration of motile cells and these pathways are differentially active in different cell types.

Nothing to Disclose: AKR, KM, RPM

OR29-5

Dissecting the Glycoprotein Hormones - Receptors Interactions Using Receptor Antibodies.

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Earlier studies from the laboratory (1) showed that the Hinge regions of the extracellular domains (ECD) of glycoprotein hormone receptors (TSHR, LH/CGR, FSHR) play important role in signaling and are not mere scaffolds between the leucine rich repeats (LRR) of ECD and the transmembrane domain (TMD)(2). In the present study, polyclonal and monoclonal antibodies to different regions of TSHR and LHR have been used to elucidate the mechanisms of hormone binding and signal transduction. In case of TSHR, an antibody against amino acids 21-200 (LRR1-6) was able to inhibit hTSH binding and consequently response, whereas the antibody against the hinge region (261-413) completely inhibited hormone stimulated response without affecting its binding. This antibody inhibited partially the response to the stimulatory Graves' autoantibodies. A hinge region specific monoclonal antibody (MAb 311/62) also stimulated the full length TSHR (aa 1-764) as well as a truncated TSHR with deletion of the entire LRR (aa 261-764). The MAb had no effect on the constitutively active S281I TSHR mutant, but stimulated another TSHR hinge region mutant D410N that exhibits low basal activity and is responsive to the hormone. Phage display analysis revealed that this MAb recognizes a discontinuous epitope around amino acids S278 to S281. Interestingly, this antibody increased affinity of the receptor for the hormone similar to that shown by S281I mutation (3). These results suggest that in case of both TSHR and FSHR, the region around cysteine box 2 acts as tethered inverse agonist of the TMD and the antibodies or activating mutation can overcome this inverse agonism.

In contrast, antibodies to LRR 1-6 and the hinge region of LHR inhibited both hormone binding and response clearly indicating differences in the interactions of LH and FSH/TSH with their receptors. The antibodies against LRRs 4-6 were most effective in inhibiting hormone binding as well as response suggesting that the LRRs 4-6 are the critical contact points between the LH/hCG and LHR which is in complete agreement with the crystal structure of FSH-FSHR complex (4) and the previous data from the laboratory demonstrating interactions between the seat belt portion of the β subunit and LRRs 4-6 of LHR (5).

- (1) Agrawal G et al., J Biol Chem 2009; 284:2636.
- (2) Mueller S et al., R. Trends Endocrinol Metab; 2009.
- (3) Kopp P et al., J Clin Invest 1997; 100:1634
- (4) Fan QR et al., Nature 2005; 433:269.
- (5) Gadkari RA et al., Mol Cell Endocrinol 2007; 23:260-262

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Nothing to Disclose: RM, RSR, SR, RRD

OR29-6

Deletion of Follistatin-Like 3 (FSTL3) Results in Development of Osteoarthritis.

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Osteoarthritis (OA) is a crippling disease, characterised by articular cartilage (AC) lesions, osteophyte formation and loss of joint structural integrity, which is affecting more people as lifespan increases. The causes and mechanisms of OA are complex and new therapeutic targets need to be identified. Transforming growth factor β (TGF β) signalling is important for cartilage growth and maintenance, yet the role of FSTL3 (an inhibitor of activin and related TGF β ligands) in cartilage, remains to be defined. To address whether FSTL3 plays a role in AC integrity, we examined FSTL3 knockout (KO) mouse joints. Multiple sections at 100 μ m intervals across entire joints from 18-month old male mice were scored for AC lesions (0 (normal) to 6 (exposed bone)) and osteophyte maturity and abnormal pathological features were noted. WT mice showed some evidence of low grade AC lesions (maximum grade = 1.75; mean grade = 0.81) and no other tissues were affected. In contrast, FSTL3 KO mice showed very severe AC lesions (maximum grade = 5.33; mean grade = 4.2) and partial closure of the growth plate along with cellular synovial infiltration and fibrosis. Meniscal structure was also severely affected with localised osteophyte formation. Most FSTL3 KO mice exhibited chondrogenesis in meniscal ligaments and some ossification. Similar chondrogenesis as well as cell hypertrophy and clustering were seen in cruciate ligaments. Pronounced osteophytes were found throughout all knee joint compartments. Heterozygous FSTL3 (HET) mice showed less severe, but obvious, AC lesions (maximum grade = 3; mean grade = 1.88). They also showed less marked evidence of growth plate closure and synovial infiltration and fibrosis. Early osteophytes were observed with a more restricted distribution than in FSTL3 KO mice. Our findings show that a deficiency of FSTL3 results in the development of severe OA. Unlike other previously described FSTL3 KO mice phenotypes, this OA shows clear evidence of gene dose-dependency. This suggests a unique sensitivity to FSTL3 function in the maintenance of synovial joint integrity. We are currently investigating whether the OA phenotype is age, gender and/or mechanically dependent. Studies to elucidate the mechanisms by which FSTL3 protects the joint from OA development are also underway. Our findings are the first to describe a vital role for FSTL3 in joint health and could identify this as a new target for therapeutic intervention in OA.

Nothing to Disclose: BP, JM, AP, AM

OR30-1

Novel Role of Catestatin (Chromogranin A352-372) in Lipid Mobilization and Insulin Sensitization.

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Chromogranin A (CHGA/Chga), a 48 kDa acidic secretory pro-protein, is ubiquitously expressed in neuroendocrine tissues (1-3). It is proteolytically processed to several biologically active peptides, including the dysglycemic pancreastatin (4), vasorelaxant vasostatin (5), catecholamine release inhibitory and antihypertensive catestatin (CST) (6). To gain a better insight into the functions of full-length CHGA and its derivative peptides, we have developed Chga knockout (Chga-KO) mice, which are hypertensive, hyperadrenergic and sensitive to insulin (7, 8). In addition, Chga-KO mice possessed increased abdominal adipose tissue mass, which contributed up to 4% of increase in body weight (8). CST administration (5 µg/g, IP; once daily for 10 days) to Chga-KO mice markedly reduced visceral adiposity, comparable to that observed in wild-type mice. Gene expression studies suggest that F4/80, CD68 and IL-6 levels in liver and epididymal white adipose tissue are comparable between Chga-KO and wild-type littermates. Although CST administration caused decrease in plasma triglyceride content, it had no noticeable effect on triglyceride and NEFA content in liver and adipose tissue of Chga-KO mice, suggesting that depletion of adipose tissue mass was not due to inflammation. Our data indicate that increased phosphorylation of AMPK by CST prevented lipogenesis and stimulated lipid disposal through increased fatty acid oxidation. Complete β-oxidation of fatty acid leads to production of CO₂ along with shorter chain acid soluble metabolites (ASM). A part of ASM gets further oxidized to produce CO₂ and the rest gets into circulation as incomplete oxidation products and ketone bodies, suggesting CO₂ and ASM levels as good indicators of fatty acid oxidation. CST treatment profoundly increased oxidation of fatty acids in liver and adipose tissue as judged by a dramatic increase in ASM. Clamp studies revealed a 50% increase in suppression of hepatic glucose production after CST treatment, indicating increased hepatic sensitivity to insulin in response to CST. Combined with our previous studies where we demonstrated that CST normalizes hypertension (7) and improves baroreflex sensitivity (9), current results on lipid mobilization and increased glucose disposal in HFD diet-induced insulin resistant mice suggest that CST could be a novel drug for amelioration of metabolic syndrome.

1. Winkler H, Fischer-Colbrie R 1992 The chromogranins A and B: the first 25 years and future perspectives. *Neuroscience* 49:497-528
2. Taupenot L, Harper KL, O'Connor DT 2003 Mechanisms of disease: The chromogranin-secretogranin family. *New Engl J Med* 348:1134-1149
3. Montero-Hadjadje M, Vaingankar S, Elias S, Tostivint H, Mahata SK, Anouar Y 2008 Chromogranins A and B and secretogranin II: evolutionary and functional aspects. *Acta Physiol (Oxf)* 192:309-324
4. Tatemoto K, Efendic S, Mutt V, Makk G, Feistner GJ, Barchas JD 1986 Pancreastatin, a novel pancreatic peptide that inhibits insulin secretion. *Nature* 324:476-478
5. Aardal S, Helle KB, Elsayed S, Reed RK, Serck-Hanssen G 1993 Vasostatins, comprising the N-terminal domain of chromogranin A, suppress tension in isolated human blood vessel segments. *J Neuroendocrinol* 5:405-412
6. Mahata SK, O'Connor DT, Mahata M, Yoo SH, Taupenot L, Wu H, Gill BM, Parmer RJ 1997 Novel autocrine feedback control of catecholamine release. A discrete chromogranin A fragment is a noncompetitive nicotinic cholinergic antagonist. *J Clin Invest* 100:1623-1633
7. Mahapatra NR, O'Connor DT, Vaingankar SM, Sinha Hikim AP, Mahata M, Ray S, Staite E, Wu H, Gu Y, Dalton N, Kennedy BP, Ziegler MG, Ross J, Jr., Mahata SK 2005 Hypertension from targeted ablation of chromogranin A can be rescued by the human ortholog. *J Clin Invest* 115:1942-1952
8. Gayen JR, Saberi M, Schenk S, Biswas N, Vaingankar SM, Cheung WW, Najjar SM, O'Connor DT, Bandyopadhyay G, Mahata SK 2009 A novel pathway of insulin sensitivity in chromogranin a null mice: A crucial role for pancreastatin in glucose homeostasis. *J Biol Chem* 284:28498-28509
9. Gayen JR, Gu Y, O'Connor DT, Mahata SK 2009 Global disturbances in autonomic function yield cardiovascular instability and hypertension in the chromogranin A null mouse. *Endocrinology* 150:5027-5035

Nothing to Disclose: SKM, JRG, ST, GB, DTO

OR30-2

Maternal Gestational Glucose Concentration Is Associated with Offspring Insulin Sensitivity and β -Cell Function in Children Aged 5-10 Years.

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Background: Evidence suggests that intrauterine exposure to elevated glucose concentrations may be a mediating factor in prenatal programming of offspring disease risk. However, studies examining the effects of maternal glucose concentration on robust measures of insulin sensitivity and β -cell response in prepubertal children are limited. Therefore, the objective of this study was to determine the associations of maternal glucose concentration with robust and physiologic measures of insulin sensitivity and β -cell response. **Methods:** Participants were 21 children aged 5-10 years. Children's insulin sensitivity index (S_I) and measures of basal, static, dynamic, and total β -cell response were determined by mathematical modeling using insulin, glucose, and c-peptide values following a liquid meal tolerance test. Dual-energy X-ray absorptiometry was used for the determination of children's percent total body fat (%BF). Maternal glucose concentration was determined following a 50-g, 1-hr oral glucose challenge test at 24-28 weeks' gestation and ranged from 75-229 mg/dl. Independent associations of maternal glucose with S_I and β -cell response indices were determined by multiple linear regression analyses. **Results:** Maternal glucose concentration was significantly, inversely associated with S_I , independent of %BF (Parameter Estimate \pm SE: -0.88 ± 0.27 , $P < 0.01$). A significant, positive association was observed for maternal glucose concentration with static β -cell response, independent of %BF and S_I (Parameter Estimate \pm SE: 1.12 ± 0.41 , $P < 0.05$). **Conclusions:** Maternal glucose concentration significantly impacts insulin sensitivity and β -cell response, independent of adiposity, in offspring at 5-10 years of age. These results suggest that fetal programming occurs both at the pancreas and at the level of insulin target tissues such as skeletal muscle and liver.

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Nothing to Disclose: NCB, PCC-L, WMG, DJR, BAG

OR30-3

Short-Term Administration of Olanzapine Induces Insulin Resistance in Healthy Subjects, Independent of Weight Gain.

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The atypical antipsychotic, olanzapine is associated with an increased incidence of obesity, diabetes and cardiovascular disease. The mechanism underlying these metabolic defects are not understood, although it has been speculated that the initiating pathophysiology is weight gain, secondary to centrally mediated increases in appetite. To determine if short-term olanzapine administration impairs glucose and lipid metabolism independent of weight gain or psychiatric disease, we examined the effect of 9 days of drug administration in nine healthy normal weight drug-naive subjects during a 12-day inpatient stay. We utilized a physiologically-relevant mixed nutrient meal challenge (600 kcal) which was given prior to and following olanzapine administration. The meal was labeled with [1-¹³C] glucose to monitor glucose appearance and 6,6-[²H₂]-glucose was infused to measure glucose disposal and endogenous glucose production. Inpatient activity was maintained for each subject at a level matched to their free living activity. Weight remained stable for the 12-days. (69.8±2.6, pre:70.1±2.9, post-olanzapine). Following 9 days of olanzapine administration, the meal challenge revealed large increases in post-prandial insulin, resulting from decreased glucose disposal. Olanzapine induced increases in post-prandial glucose (area under the curve: pre, 4436±3993 vs post, 6705±1903 mg/dl/360 min, P<0.03), insulin (6883±4400 vs 13431±6651 uU/ml/360 min, P<0.002) and triglyceride (1962±3995 vs. 4200±3938 mg/dl/360 min, P<0.01) levels as well as significant decreases in glucose disposal (P<0.01) relative to pre-treatment responses. Increases in plasma glucagon-like peptide and decreases in free fatty acids were also observed post-olanzapine. In conclusion, short-term administration of the atypical antipsychotic olanzapine to normal subjects induces a profound state of insulin resistance, with impairment of both glucose and lipid metabolism in the post-prandial state.

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Nothing to Disclose: KLT, MRR, JG, KR

OR30-4

Obesity Does Not Cause Insulin Resistance in Mice Lacking Lymphocytes.

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The mechanisms linking diet-induced obesity (DIO) to insulin resistance are unclear. Recent studies have shown that immune cell infiltration into adipose tissue of obese animals may play a causal or permissive role in this link. The present study was designed to test whether DIO would lead to insulin resistance in NOD/SCID IL2 receptor gamma chain knockout (NOG) mice, which lack lymphocytes and NK cells.

Litters of NOG mice were randomized to obese or control groups. Obese litters were culled from 5-7 mice down to 2 female mice at day of life (DOL) #5, in order to increase milk consumption and weight gain prior to weaning (DOL #28), at which time they were put on a high-fat diet (60% calories from fat). Control litters were kept at 5-7 mice/litter until weaning, and then the females put on a control diet (10% calories from fat). At 12-14 wks of age, mice underwent either glucose (IPGTT, n=10 obese/13 control) or insulin (ITT) tolerance tests (9 obese/11 control). At sacrifice, fat pads were tested for the presence of macrophages using rtPCR for the macrophage marker F4/80.

Obese mice were heavier at the time of weaning (15.8 ± 2.3 vs. 13.9 ± 2.2 g, $p=0.014$), and remained heavier throughout the study (at 14 wks: 30.5 ± 4.6 vs. 22.5 ± 1.4 , $p<0.001$). Fasting blood glucose was not statistically different between groups (125 ± 24 vs. 117 ± 18 mg/dL, $p=0.40$), nor was glucose tolerance (glucose AUC during IPGTT: 31.4 ± 7.1 vs. 30.1 ± 7.3 mg*min/dLx10³, $p=0.67$). Surprisingly, obese mice were slightly more insulin sensitive than controls during the ITT as measured by glucose AUC (6.1 ± 1.2 vs. 8.7 ± 1.5 mg*min/dLx10³, $p=0.001$), net decrease in glucose (112 ± 17 vs. 84 ± 21 mg/dL, $p=0.004$), and glucose suppression (74 ± 8 vs. $63 \pm 13\%$, $p=0.03$). So far, we have confirmed the presence of macrophages in the parametrial and omental fat pads of two obese mice (1.4 - 4.6% of β -actin). The expression levels were similar to those of an obese male C57Bl/6 mouse (1.5 - 2.3% of β -actin), an accepted model of obesity-induced insulin resistance.

Thus, selective culling of litters coupled with a high fat diet leads to obesity in mice lacking lymphocytes. However, while these mice exhibit adipose tissue macrophage infiltration, they do not develop impaired glucose tolerance or insulin resistance. These results demonstrate that lymphocytes are not required for the obesity-induced recruitment of macrophages into adipose tissue, but may be necessary for the associated insulin resistance.

Sources of Research Support: NCI Grant CA139060; NICHD K12 awarded to SDM.

Nothing to Disclose: JWB, EAE, XS, RP, YMK, YH, SDM

OR30-5

Insulin Sensitivity of Adipocytes Varies between Different Depots in High Fat Diet-Fed Mice.

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AIMS: Different fat depots in mice are thought to have different biological/metabolic function. However, often perigonadal fat depots are analyzed only when evaluating metabolic properties of white adipose tissue. Thus, the aim of the present study was to metabolically characterize adipocytes isolated from different fat depots (perigonadal, inguinal, mesenteric) of mice fed a regular chow or high fat diet (HFD) for 8 weeks.

METHODS: C57BL/6J mice were put on chow or high fat diet for 8 weeks. Adipocytes from different fat pads were isolated by collagenase digestion. Viability, basal and insulin-stimulated D-[U-¹⁴C]-glucose incorporation and lipolysis (basal as well and insulin- and isoproterenol stimulated) were determined.

RESULTS: High fat feeding led to an increase in both fat pad weight as well as adipocyte diameter. In regular chow-fed mice, the fold increase in insulin-stimulated glucose incorporation was highest in mesenteric adipocytes. However, this difference completely vanished in HFD-fed mice in response to insulin. Basal glycerol release per cell was significantly higher in perigonadal and inguinal adipocytes isolated from HFD-fed compared chow-fed mice, but interestingly there was no difference in mesenteric adipocytes. Remarkably, the ability of insulin to inhibit lipolysis was blunted under HFD only in adipocytes isolated from perigonadal fat pads but was maintained in adipocytes from the other two depots.

CONCLUSIONS: In HFD mice, insulin sensitivity of adipocytes is altered depot-specifically regarding lipolysis but not regarding glucose incorporation.

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Nothing to Disclose: SW, EJS, DK

OR30-6

Salicylate-Induced Insulin Sensitisation in Obesity Is Mediated through Downregulation of Omental Adipose 11 β -Hydroxysteroid Dehydrogenase Type 1.

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Anti-inflammatory salicylates improve insulin sensitivity, but their mechanism of action remains unclear. Pro-inflammatory cytokines upregulate expression of the glucocorticoid-regenerating enzyme 11 β -hydroxysteroid dehydrogenase type 1 (11 β HSD1) in adipocyte cell lines and we have observed reduced 11 β HSD1 expression in cultured murine and human adipocytes with salicylate. We hypothesised that the insulin sensitising mechanism of salicylate involves downregulation of adipose 11 β HSD1 and have explored the effects of salicylate in diet-induced obese mice with and without targeted disruption of 11 β HSD1.

Male C57Bl/6 wild-type (WT) mice (8 weeks) were studied after 10 week high-fat diet (58kcal% fat with sucrose). Male homozygous 11 β HSD1 knockout mice (HSD1KO) were also studied on high-fat diet, weight-matched with WT mice. Groups received either sodium salicylate (120mg/kg/day, 4 weeks, subcutaneously (SC) by minipump) or vehicle (N=8/treatment). Glucose tolerance tests (ip GTT) were performed after 3 weeks and tissues obtained 1 week later. Plasma biochemical indices of insulin sensitivity were quantified by spectrophotometry and gene transcripts by qPCR (% change vs WT vehicle). Data are mean \pm SEM; vehicle vs salicylate, *P<0.05.

Salicylate treatment in WT mice reduced 11 β HSD1 mRNA levels in omental adipose (-67.4 \pm 4.6%*), but not in SC adipose or liver. Salicylate also improved glucose tolerance (30 \pm 5.6%* reduction in area under curve for glucose during GTT), reduced fasting plasma non-esterified fatty acid (NEFA) levels (0.76 \pm 0.05 vs 0.55 \pm 0.04mM*), enhanced post-prandial NEFA suppression (20.9 \pm 3.5% vs 43.3 \pm 3.8%*), and reduced omental adipose mRNA levels of TNF α (-61.6 \pm 9.6%*) and MCP-1 (-71.4 \pm 5.1%*), as well as increasing mRNA levels of adiponectin (179.9 \pm 57.9%*). In HSD1KO mice at the same weight as WT, glucose tolerance and plasma NEFA levels were unresponsive to salicylate. Whilst HSD1KO mice had reduced omental adipose mRNA levels of TNF α (-65.2 \pm 4.0%*) and MCP-1 (-45.9 \pm 10.1%*) and increased levels of adiponectin (563.5 \pm 212.9%*) compared to WT mice, salicylate did not induce further changes in transcript levels.

These data show that salicylates reduce adipose 11 β HSD1 in vivo in accordance with our recent findings in humans. Since both salicylate-induced insulin sensitisation and downregulation of pro-inflammatory cytokines in adipose were absent in HSD1KO mice, we conclude that 11 β HSD1 is a key mediator of the metabolically favourable action of salicylate.

Nothing to Disclose: MN, DEL, RA, BRW

OR31-1

The Hypothalamic-Pituitary-Testicular Axis and Physical Frailty in Middle Aged and Older European Men: Results from the European Male Aging Study.

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Introduction: Frailty is associated with risk of adverse outcomes and functional decline. This condition may arise from interrelated dysregulation of multiple key physiological systems. The decline in testosterone (T) levels with age may contribute to this process, but the evidence is inconsistent. While frailty is believed to relate to impairment in physiological systems, most studies have focused on T alone without regard to the feed-forward and feedback relationships that regulate the hypothalamic-pituitary-testicular (HPT) axis. This study aimed to evaluate the relationships between the hormones of the HPT axis and frailty in men.

Methods: 2700 community-dwelling men mean age 59.6 (SD=11), range 40-79 yr from the European Male Aging Study (EMAS) were included in this analyses. Using an adapted version of Fried's Cardiovascular Health Study criteria frailty was defined as the presence of ≥ 3 , pre-frail as 1 - 2 and robust as 0 out of 5 specified criteria; slowness, weakness, exhaustion, low activity and sarcopenia. Relationships between frailty and hormones were evaluated using multinomial logistic regression with adjustment for pertinent confounders.

Results: 2.5% of men were frail and 29% prefrail. Compared to the robust, frail and pre-frail men had lower levels of free T and higher levels of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), Sex Hormone Binding Globulin (SHBG) and estradiol (E). Variations in the same hormones were significantly associated with overall frailty (combining frail and prefrail) and pre-frailty after adjustments for cofounders. However, only free T (Standardised Relative Risk Ratio (95%CI)) 1.43 (1.05 to 1.94) and SHBG 1.40 (1.1 to 1.79) were related to frailty (≥ 3 criteria). Amongst the individual frailty criteria, free T and FSH were associated with sarcopenia while LH, FSH, SHBG and E and were related to slowness or exhaustion.

Discussion: Lower free T was associated with frailty and sarcopenia. Higher LH, FSH, and SHBG were consistently related to different levels of frailty and physical 'exhaustion'. While these data suggest that dysregulation in the HPT axis in men may be related to frailty as currently defined, the underlying mechanisms are likely to be complex and not all components that constitute the condition can be attributed simply to androgen deficiency.

Nothing to Disclose: MDLO, AT, SAR, TWO, AJS, JDF, GB, SB, FFC, GF, AG, TSH, ITH, KK, MEJL, NP, MP, DV, FCWW

OR31-2

Interrogating the Human GnRH Neuron *In Vivo*: Effects of Exogenous Kisspeptin on GnRH Neuroendocrine Dynamics.

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Background: The neuropeptide kisspeptin is a potent stimulus for GnRH-induced LH secretion across mammalian species. We administered kisspeptin to healthy men to define the effects of kisspeptin on the pulsatile pattern of GnRH release.

Methods: Healthy male volunteers (n=13) underwent q10 min blood sampling for 12 h. Kisspeptin 112-121 0.24 nmol/kg (0.31 mcg/kg) was administered IV at the 6h time point.

Results: Kisspeptin elicited an immediate LH response in all subjects, regardless of the timing of the previous endogenous GnRH-induced LH pulse (Fig 1). Using conditional analysis, in which asynchronous endogenous pulses average out to an essentially flat baseline, kisspeptin was found to stimulate a single, large GnRH-induced LH pulse (Fig 1B).

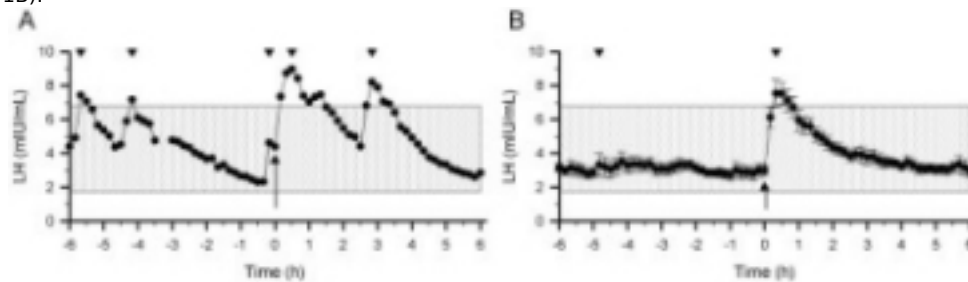


Figure 1. A. Representative LH pulse profile. B. Conditioned data, all subjects. Arrowheads, LH pulses. Arrows, time of kisspeptin administration. Shaded area, reference range (1).

The kisspeptin-induced pulses were bigger than the largest individual endogenous pulses (amplitude 4.8 ± 0.8 mIU/mL vs. 2.7 ± 0.5 mIU/mL, $P=0.02$). The kisspeptin-induced pulses were also more rounded than endogenous pulses (Fig 1A), with a longer time from nadir to peak (28 ± 2 min vs. 23 ± 1 min, $P=0.01$) and a greater width at 75% maximal pulse amplitude (36 ± 4 min vs. 23 ± 2 min, $P<0.01$). There was no correlation between the amplitudes of the kisspeptin-induced pulses and testosterone concentrations prior to kisspeptin administration ($P=0.8$). When the kisspeptin-induced pulse was excluded from analysis, LH pulse amplitude and maximal LH were no different before and after kisspeptin administration. There was no significant change in the number of LH pulses after kisspeptin (before 2.5 ± 0.3 , after 2.9 ± 0.3 , $P=0.2$).

Conclusions: Exogenous kisspeptin induces a single GnRH-induced LH pulse with a modified morphology compared to endogenous pulses. Kisspeptin does not appear to “reset” the GnRH pulse generator, as conditional analysis revealed only one kisspeptin-induced pulse. These findings refine our fundamental understanding of how kisspeptin acts on GnRH neurons and lay the groundwork for the development of kisspeptin as a clinical tool.

(1) Spratt DI et al., Am J Physiol 1988; 254:E658

Sources of Research Support: NIH R01 HD43341.

Nothing to Disclose: Y-MC, NP, SBS

OR31-3

Kisspeptin 10 Rapidly and Potently Stimulates LH Secretion in Men.

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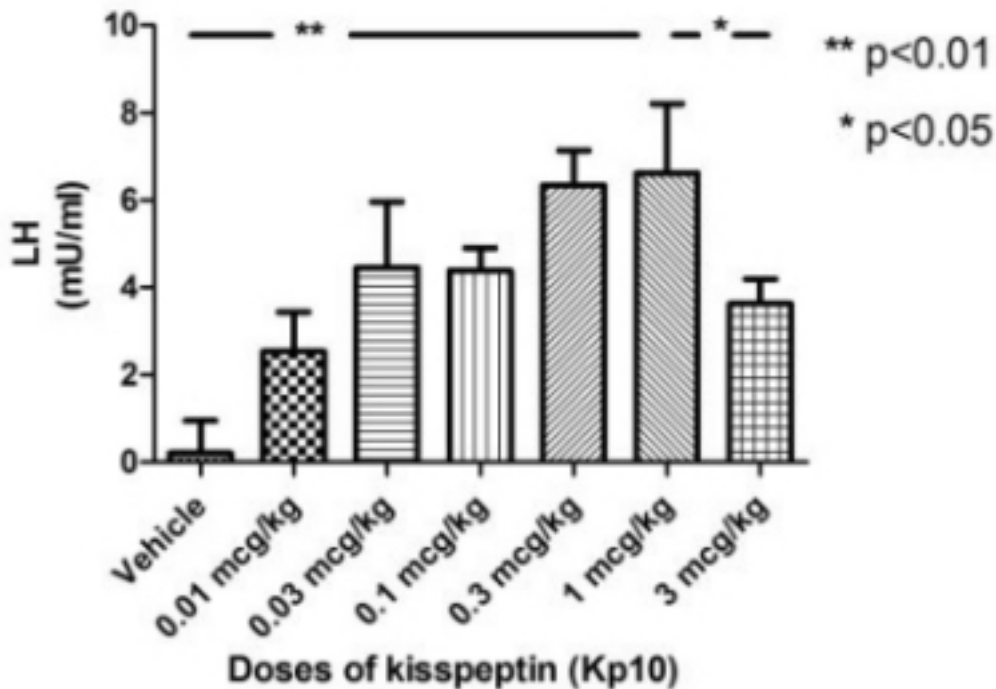
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Exogenous kisspeptin (Kp) stimulates Gonadotropin Releasing Hormone (GnRH) and gonadotropin release in a number of species. The 54 amino acid (aa) peptide (Kp54) is cleaved in vivo to 13, 12 and 10 (Kp10) aa peptides sharing the same C terminal sequence. Gonadotropin responses to Kp54 have been studied in men and women. Kp10 has similar intrinsic activity to Kp54 in both in-vitro and in-vivo animal studies, but human studies of Kp10 have not been reported. Moreover, recently described Kp antagonist and agonist analogues with therapeutic potential are modifications of the Kp10 decapeptide sequence.

To assess the effect of Kp10 on Luteinising Hormone (LH) secretion in men and to determine dose-response relationships, 6 healthy adult men were administered 0.01-3.0 mcg/kg iv GMP grade human Kp10 or vehicle with appropriate ethical and regulatory approvals. Blood samples were collected frequently for 60 min before (baseline) and 90 minutes after (stimulated). All men received at least 3 doses. ANOVA with Tukey's multiple comparison test was used to determine statistical significance of stimulated LH AUC.

Doses of Kp10 of 0.03mcg/kg and above resulted in a rapid increase in LH with maximal levels seen by 45 min post Kp10 whilst vehicle elicited no change. 60 min AUC, calculated by trapezoid integration, showed a clear dose-response relationship with a maximal response at 1.0mcg/kg ($p < 0.01$). The response to 3.0mcg/kg was significantly reduced compared to 1.0mcg/kg ($p < 0.05$). Stimulated LH concentrations were significantly higher than baseline for all doses studied ($P < 0.05$, Student's t test). No adverse events were reported. Blood pressure, heart rate, liver function, renal function, electrolytes and full blood count were not affected by Kp10.

Area Under Curve of LH Post Stimulation



Peak LH responses to 100mcg GnRH were markedly greater than the maximal responses to Kp10 in all the subjects studied.

In conclusion, iv Kp10 elicits rapid and potent LH secretion in normal men with doses as low as 0.03mcg/kg and there is evidence for desensitisation at 3 mcg/kg.

Sources of Research Support: Medical Research Council (UK).

Nothing to Disclose: JTG, RAA, SBS, RPM

OR31-4

Human AR Q-Tract Length Determines In Vivo Androgen Sensitivity in Mice.

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The hypothalamic-pituitary-testicular axis is regulated by negative androgen feedback mediated via the androgen receptor (AR) which governs serum LH and testosterone levels. The variable length CAG triplet repeat polymorphism encoding a polyglutamine stretch (Q-tract) in exon 1 of the human AR is thought to determine human AR (hAR) sensitivity, based on an inverse relationship with in vitro AR transcriptional activity as well as some but not all epidemiological studies, but direct in vivo proof is lacking. To overcome the limitations of artificial in vitro experiments and observational studies, we used knock-in mouse lines containing hAR alleles ("humanized" AR) with short (12Q), median (21Q) or long (48Q) Q-tract variations (1). In mature male mice 14 days after orchidectomy with subdermal depot DHT implants for the last 5 days, suppression of castrate serum LH levels demonstrated a significant rank order of suppression according to Q tract length [12Q (94%) > 21Q (74%) > 48Q (52%) (p<0.005 test for trend)] despite similar levels of serum DHT and its 3 α and 3 β Diol metabolites (by LC tandem MS). Similar significant rank order trends according to Q-tract length were evident for weights of all prostate lobes (AP, DLP, VP) as well as seminal vesicles (SV) (full, emptied, secretions) in orchidectomized mice with or without DHT treatment.

Table 1: Serum LH and FSH, prostate and SV weights in DHT-treated castrate males (shown as % of intact 12Q).

| Q-tract | Gonadotrophins (% of intact 12Q) | | Prostate (% of intact 12Q) | | | Seminal vesicles (% of intact 12Q) | | |
|-----------|-------------------------------------|--------|-------------------------------|---------|--------|---------------------------------------|--------|-----------|
| | LH | FSH | VP | DLP | AP | Full | Empty | Secretion |
| 12Q | 30 ± 3 | 40 ± 6 | 91 ± 7 | 119 ± 8 | 95 ± 7 | 50 ± 2 | 89 ± 4 | 22 ± 8 |
| 21Q | 115 ± 34 | 20 ± 2 | 83 ± 6 | 105 ± 9 | 87 ± 6 | 50 ± 2 | 81 ± 2 | 27 ± 12 |
| 48Q | 165 ± 41 | 44 ± 9 | 60 ± 4 | 83 ± 5 | 48 ± 2 | 35 ± 1 | 59 ± 2 | 16 ± 3 |
| p [trend] | 0.005 | 0.65 | 0.003 | 0.005 | <0.001 | <0.001 | <0.001 | 0.17 |

These findings demonstrate significant effects of AR Q-tract length mediating DHT responsiveness of post-castration serum LH levels and androgen-dependent organ weights. These experiments in this paradigm of castrate DHT-replaced mice provide the first direct proof that hAR Q-tract length inversely determines in vivo androgen sensitivity, and suggest a mechanism by which AR Q-tract length differences impact multiple aspects of human health and disease.

(1) Albertelli MA, Scheller A, Brogley M, Robins DM (2006) Mol Endocrinol. 20(6):1248-60.

Nothing to Disclose: US, DD, MB, R-YG, MJ, DTH, DJH, DMR

OR31-5

Aromatase Blockade Is Associated with Increased Mortality in Acute Illness.

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Aromatase mRNA expression and activity increase during acute illness in humans leading to elevated serum estrogen levels (1). In rodent models, estrogens appear to promote cardiac, hepatic, and immune function during trauma and sepsis (2). We have previously reported that in mice aromatase expression increases in cardiovascular as well as in adipose tissue during acute illness. We hypothesized that blocking aromatase action using letrozole would lead to increased mortality from acute illness. Methods: Seven to eight week old male black 6 mice were used. Illness was induced with cecal ligation and puncture (CLP) with sham surgery (SS) serving as a control procedure. The gauge of the needle and the number of punctures were adjusted to produce a 50% mortality rate over 2-5 days without letrozole administration. Letrozole (10 mcg daily) or vehicle was administered for 7-14 days prior to procedure and continued afterwards. Fifty mice underwent CLP with 30 receiving letrozole and 20 receiving vehicle. Thirty mice underwent SS with 20 receiving letrozole and 10 receiving vehicle. Survival analysis was performed with mice censored if alive at day 5. The log rank test was used to assess statistical significance of differences in survival curves. Results: No mice died in the SS with vehicle or with letrozole groups. Mortality in mice following CLP with or without letrozole are listed in Table 1.

Cumulative Deaths by Days After CLP or SS

| Group | Day 1 | Day 2 | Day 3 | Day 4 |
|--------------|--------|---------|---------|---------|
| SS/veh n=10 | 0 | 0 | 0 | 0 |
| SS/let n=20 | 0 | 0 | 0 | 0 |
| CLP/veh n=20 | 1 (5%) | 5 (25) | 7 (35) | 11 (55) |
| CLP/let n=30 | 4 (13) | 14 (47) | 23 (77) | 25 (83) |

The mortality rate following CLP was greater in mice receiving letrozole than in those receiving vehicle ($P<0.01$). Conclusions: Increased aromatase expression is seen not only in adipose tissue but in cardiovascular tissue as a response to acute illness suggesting that both systemic and local estrogen responses may enhance cardiovascular function during illness. Increased mortality with blockade of aromatase activity suggests that the increased aromatase expression and consequent increased estrogen production may confer a survival advantage in acute illness.

(1) Spratt DI et al, Am J Physiol Endocrinol Metab 2006; 291:E631

(2) Yu HP and Chaudry IH, Shock 2009; 31:227

Sources of Research Support: Maine Medical Center Research Strategic Plan.

Nothing to Disclose: JJC, JEP, DAP, FLL, DIS

OR31-6

Testosterone Reduces Non-Alcoholic Fatty Liver Disease (NAFLD) of Hypogonadism.

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Purpose: NAFLD is the leading cause of hepatic steatosis and is associated with obesity, diabetes and Metabolic Syndrome (Met S). It is also known that androgen deficiency is a risk factor for developing diabetes and Met S, but the role of androgen deficiency in hepatic dysfunction has not been well studied. We established a rat model of hepatic steatosis to investigate the effects of testosterone (T) on pathogenesis of NAFLD.

Methods: Male rats were randomly placed into four groups: castrated rats on high-fat diet (HFD), castrated rats with T replacement on HFD, intact rats on HFD, and intact rats on regular chow diet (RCD). The HFD provided 71% energy from fat; RCD provided 16% of energy from fat. The rats were fed ad libitum for 15 weeks. DEXA scan was performed to evaluate body fat composition and weight. Serum and tissues were analyzed.

Results: Serum T level was not detectable in castrated rats, and T replacement led to higher serum T levels than in intact rats. Castrated rats on HFD gained less body weight but their % body fat was higher than T-treated rats on HFD. Liver enzymes were also higher in the T-deficient rats as compared to rats of the other groups. There was no significant difference in serum glucose or insulin levels between the treatment groups. Liver histopathology revealed a severe micro- and macrovesicular accumulation of fat in hepatocytes with multiple inflammatory foci of castrated rats fed HFD. However, hepatocytes of the T-treated and intact rats on HFD, demonstrated only a mild to moderate microvesicular steatosis.

Conclusion: The degree of hepatic micro- and macrovesicular steatosis as well as hepatic inflammation were considerably greater in rats with undetectable serum T than in the other three groups. T replacement led to significant reduction of hepatic steatosis. This study demonstrates that T plays a protective role in fat accumulation and NAFLD development and that androgen deficiency contributes to the severity of hepatic steatosis.

Sources of Research Support: General Clinical Research Center at Harbor-UCLA Medical Center (MO1 RR00425).

Disclosures: RS: Consultant, Clarus.

Nothing to Disclose: LN, MD-A, SL, CW, JY, YJ, YL, SF

OR32-1

Parathyroid Hormone Related Protein (PTHrP) Enhances Human β -Cell Proliferation and Function with Simultaneous Induction of Cyclin-Dependent Kinase 2 (cdk2) and Cyclin E Expression.

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A major goal in the treatment of diabetes is to induce β -cell growth while maintaining its function. Although numerous factors enhance rodent β -cell proliferation and function, there are relatively few studies in human islets. PTHrP and its receptor, parathyroid hormone-1 receptor (PTH1R), are both expressed in rodent β -cells. Furthermore, PTHrP enhances rodent β -cell growth and function both in vitro and in vivo. PTHrP is also made in human islets and its expression is increased in insulinomas. However, there is virtually nothing known regarding the effect of PTHrP in human β -cells. Based on the rodent studies, we hypothesized that PTH1R is expressed in human β -cells, and that PTHrP has the potential to enhance human β -cell proliferation and/or function.

This study shows that human islets indeed express PTH1R mRNA and protein, and that the receptor is made in human β -cells as shown by co-staining with insulin and PTH1R antibodies. Importantly, overexpression of PTHrP by adenoviral transduction caused a significant 3-fold increase in human β -cell proliferation. Furthermore, treatment with only the amino-terminus 1-36 peptide of PTHrP for 24 hours was sufficient to induce human β -cell replication, without causing significant changes in the expression of differentiation markers. Not only did PTHrP(1-36) peptide enhance proliferation, but significantly augmented glucose-stimulated insulin secretion, both at physiological (5 mM) and pathophysiological (22 mM) glucose concentrations by 36% and 67% respectively.

Analysis of cell cycle molecules regulated by PTHrP(1-36) in human islets showed a significant and specific increase in the expression of the late G1/S cell cycle activator proteins cyclinE and cdk2. We further examined whether these cell cycle molecules by themselves could enhance human beta cell proliferation. Overexpression of cyclinE alone, but not cdk2, induced a 5-fold increase in human β -cell proliferation; and when both molecules were expressed simultaneously there is a further marked synergistic increase of 16-fold in replication.

Thus, PTHrP(1-36) peptide enhances human β -cell proliferation as well as function, with associated upregulation of two specific cell cycle activators, which together can induce human β -cell proliferation several-fold. The future therapeutic potential of PTHrP(1-36) for the treatment of diabetes is especially relevant given the complementary therapeutic efficacy of PTHrP(1-36) in post-menopausal osteoporosis.

Sources of Research Support: Grants from the National Institutes of Health (DK078060 and DK072264 to RCV; DK055023 and DK07052-32 to AFS) and JDRF Research Awards (1-2008-46 to RCV; 1-2008-39 and 34-2008-630 to AFS). We are grateful to Drs. Irene Cozar-Castellano and Nathalie Taesch for sharing their expertise on the cell cycle; to Darinka Sipula, Taylor Rosa, Jeffrey Kleinberger and Fatimah Salim for superb technical assistance. We acknowledge the ICR and JDRF Basic Science Islet Distribution Programs for supplying human islets.

Disclosures: AS: Member, Osteotrophin LLC.

Nothing to Disclose: NGK, SJG, GH, KW, XZ, KKT, PZ, DS, AG-O, RCV

OR32-2

Proliferating Intra-Islet Endothelial Cells Affect Beta Cell Mass and Regeneration.

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Pancreatic islet cells secrete vascular endothelial growth factor-A (VEGF-A) and this recruits endothelial cells leading to highly vascularized islets. To test the hypothesis that increased VEGF-A expression would increase endothelial cell number and promote islet function, differentiation, and proliferation, we increased beta cell production of VEGF-A using a "tet-on" inducible bi-transgenic (Bi-Tg) system (rat insulin promoter-reverse tetracycline activator and tet-operon-VEGF-A). In this system, VEGF-A overexpression by beta cells of adult mice induces a dramatic expansion and proliferation of intra-islet endothelial cells but this is accompanied by beta cell loss. To investigate how endothelial cell proliferation affects adjacent beta cells, we induced VEGF-A using doxycycline (Dox; added to drinking water) for 1 week and collected pancreatic tissues at 1, 2, 3, 4, and 6 weeks after Dox withdrawal. In Bi-Tg mice without Dox treatment, endothelial cells and beta cells occupied $28 \pm 2\%$ and $73 \pm 2\%$ of area per islet, respectively and only $5.1 \pm 0.5\%$ of beta cells were BrdU-positive (1 week BrdU pulse). However, after 1 week of VEGF-A induction, the endothelial cell area increased to $81 \pm 1\%$ while the beta cell area decreased to $20 \pm 1\%$ and BrdU-positive beta cells were reduced to $1.3 \pm 0.4\%$ without a change in alpha cell number. After Dox withdrawal, the islets were repopulated by beta cells. This process was marked by a decline in endothelial cell number and an increased rate of beta cell proliferation at 1 week ($25 \pm 5\%$), 2 weeks ($39 \pm 5\%$), and 3 weeks ($9 \pm 1\%$) after Dox withdrawal. Six weeks after Dox withdrawal, regenerated Bi-Tg islets resembled the morphology and function of control Bi-Tg islets as assessed by immunocytochemistry and glucose-stimulated insulin secretion by isolated islets in a perfusion system. These data suggest that proliferation of intra-islet endothelial cells induced by VEGF-A overexpression inhibits beta cell proliferation and leads to beta cell loss. Removal of the VEGF-A stimulus reduces intra-islet endothelial cell proliferation and number and this allows residual beta cells to proliferate and regenerate with restoration of islet architecture and function. These findings suggest that intra-islet endothelial cell number and proliferation can influence beta cell proliferation and ultimately beta cell mass.

Nothing to Disclose: JYH, MB, QC, AS, ACP

OR32-3

The HGF/cMet Signaling Pathway Is Essential for Pancreatic Beta-Cell Regeneration.

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Hepatocyte growth factor (HGF) and its receptor, cMet, are present in the pancreatic beta-cell. Pancreas-specific cMet knockout (PancMetKO) mice exhibit normal blood glucose and plasma insulin, glucose tolerance, beta-cell proliferation, and beta cell mass. This indicates that HGF/cMet signaling is not required for basal glucose homeostasis and beta-cell growth. However, whether HGF/cMet signaling is required for beta-cell regeneration in situations of diminished beta-cell mass is completely unknown. Therefore, we analyzed beta-cell proliferation in PancMetKO and normal littermates following beta-cell mass reduction by multiple low-dose streptozotocin (MLDS, 40mg/kg BW/day for 5 days) administration and partial (50-60%) pancreatectomy (Ppx).

In the MLDS mouse model, a surrogate model of Type I diabetes with high blood glucose, islet infiltration, and decreased beta-cell mass, normal mice display increased beta-cell replication rates two weeks after the last streptozotocin injection ($0.17\pm 0.05\%$ untreated normal mice vs $0.59\pm 0.05\%$ MLDS-normal mice, $p < 0.05$). In contrast, PancMetKO littermates displayed blunted beta-cell proliferation following the same treatment ($0.15\pm 0.04\%$ untreated PancMetKO vs $0.21\pm 0.05\%$ PancMetKO-MLDS). We next analyzed beta-cell replication in PancMetKO mice and control littermates 7 days after Ppx. Normal mice exhibited a 5-fold increase in beta-cell proliferation ($0.13\pm 0.04\%$ normal-sham vs $0.68\pm 0.1\%$ normal-Ppx, $p < 0.05$). In striking contrast, beta-cell proliferation was significantly diminished in PancMetKO mice ($0.10\pm 0.03\%$ PancMetKO-sham vs $0.28\pm 0.1\%$ PancMetKO-Ppx, $p < 0.05$ vs normal-Ppx). Importantly, this reduced beta-cell proliferation resulted in decreased beta-cell mass in PancMetKO mice 28 days after Ppx compared to normal mice ($2.56\pm 0.77\text{mg}$ normal-Ppx vs $1.45\pm 0.28\text{mg}$ PancMetKO-Ppx). Immunoblot analysis revealed a striking decrease (~50%) in Cyclin D1, D2, and D3 protein levels in islets from PancMetKO mice compared to islets from normal littermates 7 days after Ppx. Impaired up-regulation of G1/S cell cycle activators in the absence of HGF/cMet signaling might explain the limited induction of beta-cell proliferation and the inadequate beta-cell regeneration after partial pancreatectomy. Taken together, these results indicate that HGF/cMet signaling is required for beta cell regeneration in situations of diminished beta-cell mass.

Nothing to Disclose: SJE, CD, JMM-G, TCR, SRV, SAV-G, TAT, AG-O

OR32-4

Signalling Pathways Involved in Regulation of β -Cell Proliferation in Pregnancy.

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β -cell mass increases during pregnancy to accommodate for insulin resistance. This increase is mainly due to β -cell proliferation, a process that requires intact prolactin receptor (Prlr) signalling. We have found that mice with a heterozygous deletion of the Prlr (Prlr^{+/-}) have impaired glucose tolerance, decreased β -cell number and reduced insulin secretion during pregnancy¹. Signalling molecules that are known to regulate β -cell proliferation include members of the insulin signalling pathway (i.e. Akt), the tumor suppressor menin, and the cell cycle proteins. However, whether dysregulation of Akt, menin, or cell cycle pathways in the pregnant Prlr^{+/-} mice are responsible for the reduction in prolactin-mediated β -cell proliferation during pregnancy is unknown. Using the Prlr^{+/-} mice, our objective was to delineate whether these signaling molecules participate in prolactin-mediated β -cell proliferation during pregnancy. Pancreatic islets isolated from Prlr^{+/+} and Prlr^{+/-} mice on days 0 and 15 of pregnancy were processed for determination of protein expression by Western immunoblotting. We found that in the wild type mice (Prlr^{+/+}), the expression of Akt in pancreatic islets increased by 2.1 fold during pregnancy, while in the mutant Prlr^{+/-} mice, the increase in Akt expression was attenuated at 1.7 fold. We did not detect a difference in Akt expression levels between the non-pregnant Prlr^{+/+} and Prlr^{+/-} mice. Menin expression was reduced by 50% between days 0 and 15 of pregnancy in the Prlr^{+/+} mice, while in the Prlr^{+/-} mice, menin expression was reduced by only 20%. Again, there was no significant difference in menin expression levels in non-pregnant mice. Interestingly, between days 0 and 15 of pregnancy, expression of the cyclin inhibitory protein p21^{cip} increased by 1.8 fold in the Prlr^{+/+} mice, but this increase was not observed at all in the Prlr^{+/-} mice. Lastly, we did not find any difference in the expression levels of cyclin D1 or D2 between the pregnant Prlr^{+/+} and Prlr^{+/-} mice. Therefore, we conclude that during pregnancy, placental hormones act through Prlr to increase β -cell mass by upregulating β -cell proliferation and engage Akt, menin, and p21. Future studies will determine the relative contribution of these molecules in maintaining normal glucose homeostasis during pregnancy.

(1) Huang C et al, Endocrinology 2009; 150:1618-26.

Sources of Research Support: Alberta Children's Hospital Foundation; Canadian Diabetes Association.

Nothing to Disclose: CEH, CTLH

OR32-5

Systemic Signals Promote Enhanced β -Cell Mitosis in Insulin Resistant Mice.

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Rodents manifesting insulin resistance and compensatory islet hyperplasia do not develop overt diabetes, a feature that also likely occurs in humans. Here, we investigate the mechanistic basis for this protection from diabetic progression. Using complementary in vivo mouse models, we demonstrate that mice rendered insulin resistant through tissue-specific deletion of insulin receptors in hepatocytes (LIRKO mice) produce a circulating factor that enhances β -cell mitosis. Intriguingly, parabiosis experiments in which control mice were surgically joined to LIRKO animals, to create a common, anastomosed circulatory system, demonstrated that exposure to blood-borne factors from LIRKO mice for only 12 weeks increases proliferation in control β -cells by \sim 4-fold ($p < 0.05$; mitoses expressed as %bromodeoxyuridine (BrdU)+/insulin+ cells after 6 h in vivo exposure), and nearly matched the high levels of BrdU incorporation in individual LIRKO mice and in parabiotic LIRKO mice joined to either control or LIRKO partners. Neither fasting blood glucose nor insulin levels, after 12 weeks of parabiosis, differed significantly between partners in heterotypic (LIRKO joined to control) parabiosis ($p = \text{NS}$; $n = 6$), and β -cell apoptosis was not different. These data suggest that circulatory signals act either directly or indirectly (via neural mechanisms) to promote β -cell proliferation in normoglycemic controls, while, conversely, the circulatory environment of control mice fails to suppress β -cell replication in LIRKO partners. Thus, we next used a transplantation approach to rule out potential neural effects. Islets isolated from LIRKO or control mice were transplanted under the kidney capsule of control and LIRKO recipients. Six weeks later, control and LIRKO recipients showed equivalent random fed blood glucose levels ($P = \text{NS}$; $n = 5$); however, control grafts harvested from LIRKO hosts exhibited \sim 8-fold elevation in β -cell mitosis, which was similar to β -cell replication in LIRKO grafts harvested from LIRKO hosts (control 0.140 ± 0.030 vs LIRKO 0.173 ± 0.065 % BrdU+/insulin+ cells; $n = 4-5$). In contrast, control or LIRKO grafts harvested from control hosts showed an equivalent, low level of replication (control, 0.018 ± 0.018 vs LIRKO 0.059 ± 0.033 ; $n = 3$). In summary, these data support the existence of a systemic non-cell-autonomous, non-neural factor(s) that promotes β -cell growth in insulin resistant LIRKO mice.

Nothing to Disclose: DK, CWL, JH, AJK, JLS, HK, WQ, AJW, RNK

OR32-6

CRFR1 and CRFR2 Receptors Are Expressed in Pancreatic Islets, Promote Beta Cell Proliferation and Potentiate Insulin Secretion in a Glucose-Dependent Manner.

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Corticotropin-releasing factor (CRF), originally characterized as the principal neuroregulator of the hypothalamus-pituitary-adrenal axis, has broad central and peripheral distribution and actions. Using a transgenic reporter mouse, we demonstrate the presence of CRF receptor type 1 (CRFR1) on primary beta cells and show that activation of pancreatic CRFR1 promotes insulin secretion, thus contributing to restoring the normoglycemic equilibrium. Stimulation of pancreatic CRFR1 initiates a cAMP response that promotes insulin secretion and leads to the phosphorylation of CREB and the induction of the expression of several immediate-early genes. Furthermore, we demonstrate in vivo that the insulinotropic actions of pancreatic CRFR1 oppose the activation of CRFR1 on anterior pituitary corticotropes that lead to the release of glucocorticoids. Interestingly, glucocorticoids inhibit the transcription of CRFR1 and incretin receptor in beta cells in vitro and in vivo in a glucocorticoid receptor-dependent manner. In contrast, the islet expression of CRF receptor type 2 (CRFR2), the cognate receptor for beta cell-derived Urocortin 3 (Ucn 3), is robustly increased in response to glucocorticoids. This suggests that glucocorticoids not only functionally oppose the actions of insulin, which are aimed at normalizing hyperglycemia, but can differentially modulate the sensitivity of the endocrine pancreas to inputs from incretins as well as CRF family peptides, with possibly effects on insulin output.

Furthermore, stimulation of primary rat islets or the MIN6 insulinoma line with CRF also activates the MAPK signaling cascade leading to rapid phosphorylation of Erk1/2 in response to CRFR1-selective ligands, which induce proliferation in primary rat neonatal beta cells. Importantly, CRFR1 stimulates insulin secretion only during conditions of intermediate to high ambient glucose and the CRFR1-dependent phosphorylation of Erk1/2 is greater with elevated glucose concentrations. This is reminiscent of the actions of incretins, whose full insulinotropic actions require elevated ambient glucose conditions. The presence of CRFR1 and CRFR2 within the endocrine pancreas adds a novel layer of complexity to the intricate network of paracrine and autocrine factors and their cognate receptors whose coordinated efforts can dictate islet hormone output and regulate beta cell proliferation.

Sources of Research Support: Juvenile Diabetes Research Foundation, the Adler Foundation, the Leona M. & Harry B. Helmsley Charitable Trust, the Clayton Medical Research Foundation, and NIDDK, Grant P01 DK26741 from the National Institutes of Health. Human islets were obtained through the ICR Basic Science Islet Distribution Program.

Nothing to Disclose: MOH, TvdM, APP, MM, JMV, CJD, HP, NJJ, NB, WWV

OR33-1

Targeted Ablation of Growth Hormone Receptors in the Skeletal Muscle Improves Metabolism and Protects Against Diet-Induced Obesity in Mice.

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Growth hormone (GH) has a positive influence on skeletal muscle mass and function. It is believed that these effects are exerted via its stimulation of insulin-like growth factor -1 (IGF-1) production, either from the liver or locally in the skeletal muscle. However, recent data suggest that GH may have IGF-1-independent effects on skeletal muscle physiology¹. Moreover, analyses of the skeletal muscles in currently available whole-body GH -transgenic and -deficient mouse models are confounded by the systemic changes that arise due to the manipulation. Thus, in order to determine the role of GH in the skeletal muscle alone, we have used the Cre-loxP system to specifically inactivate the growth hormone receptor (GHR) in the post-natal muscle by crossing the GHR-floxed mice with Mck-Cre mice that express Cre recombinase driven by the muscle creatine kinase (MCK or Ckm) promoter [GHR-MKO mice]. Acute stimulation with GH shows >90% reduction in phosphorylation of STAT5, the predominant downstream effector of the GH signal, in the GHR-MKO quadriceps when compared to the control (GHR-floxed) mice. Moreover, male GHR-MKO mice show reduced body weight starting at 8 weeks of age owing to a decrease in absolute lean body mass, suggesting that GH is important for maintenance of muscle mass in adulthood. Interestingly, the male GHR-MKO mice also tend to possess reduced absolute fat mass starting at 8 weeks of age when compared to the control mice. At 16 weeks of age, the GHR-MKO mice display a significant decrease in the weight of the gonadal and subcutaneous fat pads, as well as circulating free fatty acid (FFA) levels. Moreover, at this age, the GHR-MKO mice show lower fed blood glucose levels and a slight improvement in glucose tolerance, while the plasma insulin levels remain unchanged. Furthermore, when challenged with a high-fat diet (60% calories from fat), the male GHR-MKO mice show reduced body weight, while their weight gain is comparable to the control mice on the high fat diet. In addition, the GHR-MKO mice have decreased fat mass, both in terms of absolute weight and relative to body weight, when compared to control mice. Thus, our study suggests that GH is involved in skeletal muscle mass maintenance, and indicates that it has a role in metabolic homeostasis in the skeletal muscle, possibly via regulation of lipid profiles.

¹ A. Sotiropoulos, M. Ohanna, C. Kedzia, et al., Growth Hormone Promotes Skeletal Muscle Cell Fusion Independent of Insulin-Like Growth Factor 1 up-Regulation, Proc Natl Acad Sci U S A, 103 (2006), 7315-20.

Nothing to Disclose: AV, YW, HS, CL, SY, DL

OR33-2

Profound Fatty Liver in Mice with Hepatocyte-Specific Deletion of JAK2 Is Completely Rescued by Abrogation of Growth Hormone Secretion.

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Fatty liver disease is common and not well understood. Mice with hepatocyte-specific deletion of either signal transduction and transcription factor 5 (STAT5) or growth hormone receptor (GHR) develop fatty liver (FL). Interestingly, global disruption of neither GHR nor STAT5 leads to FL. There is contradictory evidence as to the role of GH in FL. However, FL has not been described in any mouse models of GH excess or deficiency. Somatic mutations of the Janus kinase 2 (JAK2) have been described in association with hematologic malignancies, and many JAK2 inhibitors are in clinical development. With reports of FL in mice with liver-specific deletion of GHR and STAT5, understanding the physiological effects of JAK2 inhibition in the liver is an important goal. Here, we report mice with hepatocyte-specific deletion of JAK2 (JAK2L). Expression of genes encoding *igf1* and *igf1als* was reduced to 0.1 and 12%, respectively in JAK2L livers, and serum IGF-1 was reduced to 4% of control (26 ± 2.7 vs 593 ± 45.4 ng/ml, $P < 0.001$). Serum GH was increased (60 ± 0.44 vs 26.3 ± 9.7 ng/ml, $P < 0.01$). JAK2L Livers were enlarged and and yellow ($10.9 \pm .55$ vs $4.9 \pm .42$ %body weight, $P < 0.001$). Oil-red-O staining revealed near-total replacement of the liver with lipid. TLC analysis of liver extracts showed a marked increase in triglyceride (TG), and measurements revealed a 20- and 5-fold increase in liver TG and total cholesterol, respectively (198 vs 11.4 mg/g and 11.2 vs 2.5 mg/g, $P = 0.001$ and 0.01). There was no defect in insulin or glucose sensitivity. The mice were lean and there was an increase in plasma free fatty acids (FFA, $1.28 \pm .11$ vs $0.79 \pm .07$, $P = 0.01$). There was no increase in FA synthesis in liver as measured *in vivo*, nor was there a defect in TG secretion after injection of Triton WR-1339. Given the decrease in adiposity and increase in FFA, and because of the known role of GH in promoting lipolysis, we asked whether dysregulated GH secretion might cause FL through increasing plasma FFA levels. We crossed JAK2L mice with growth hormone deficient *little* mice and found that abrogation of GH secretion completely rescued the FL phenotype. Expression of CD36 was dramatically increased in the livers of JAK2L mice and might augment uptake of plasma FFA. Overall, this work has important implications for the safety of JAK2 directed therapies and suggests multiple lines of future research aimed at understanding the effect of GH signaling on lipolysis and on lipid flux in hepatocytes.

Sources of Research Support: In part by a Pilot/Feasibility grant from the UCSF Liver Center (E.J.W.) (P30 DK026743) and by NIH Grant CA117930 (K.U.W.).

Disclosures: MB: Employee, Genentech, Inc. PC: Employee, Genentech, Inc.

Nothing to Disclose: BCS, CH, SMN, JLT, KUW, EJW

OR33-3

Targeted Deletion of Growth Hormone (GH) Receptor in Macrophage Reveals Novel Effects of GH on Adiposity and Glucose Homeostasis.

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The GH receptor (GHR) is expressed on macrophages, but the precise role of GH on the macrophage is unclear. Our previous studies using *in vitro* cell culture models (Endocrinology 2010) had indicated that GH's action on the macrophage increases adipocyte differentiation in a paracrine manner. To investigate the potential effects of GH action on macrophage *in vivo*, we created a macrophage-specific GHR knockout mouse line (MacGHR KO) via Cre recombination technology using the LyzMCre mice. MacGHR KO and their control littermates were fed either a normal (10% fat) or high fat diet (HFD, 45% fat) and these mice were then studied via anthropometric measurements, body composition analysis, glucose and insulin tolerance (ITT) tests. Specific deletion of GHR in macrophage did not alter the growth profile of the mice. On a normal diet, MacGHR KO and their controls exhibited similar responses to intraperitoneal glucose challenge and similar insulin sensitivity profiles on ITT. However, body composition measured via NMR indicated that MacGHR KO mice had significantly less total body fat ($13.4 \pm 1.07\%$ vs $9.3 \pm 1.9\%$; $p < 0.05$, cntr vs KO) with concomitant increase in lean tissue ($73.4 \pm 1.06\%$ vs $77.5 \pm 1.69\%$; $p < 0.05$, cntr vs KO). When these mice were challenged with HFD, both MacGHR KO mice and their controls exhibited similar weight gains. Compared to the mice on normal diet, impaired glucose tolerance was observed in both MacGHR KO and control groups fed HFD. However, HFD fed MacGHR KO mice had higher fasting glucose levels compared to the control group (115 ± 7.9 mg/dL vs 168 ± 2.9 mg/dL in male mice; 117 ± 8.4 mg/dL vs 160 ± 6.1 mg/dL in female; $p < 0.05$, cntr vs KO), indicating that GH action on macrophage is important for maintaining post-absorptive glucose homeostasis. Preliminary analysis also indicates alterations in the M1 vs M2 phenotype of adipose tissue macrophage (ATM) in the MacGHR KO mice fed HFD. Additionally in HFD fed mice the number of crown-like structures (CLS) was different between MacGHR KO and their controls. We conclude that GH's actions on the macrophage *in vivo* regulate body composition and modulate glucose homeostasis.

Sources of Research Support: NIH (DK49845 [RKM] and P60DK-20572 [Michigan Diabetes Research and Training Center]).

Nothing to Disclose: CL, AKP, RR, CL, YF, MAS, RKM

OR33-4

Prolactin and Vasoinhibins Exert Opposite Effects on Chondrocyte Survival.

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Chondrocyte apoptosis plays an important role in endochondral bone formation and in cartilage degradation during aging and inflammation. Prolactin (PRL) receptors have been identified in chondrocytes and the treatment with PRL in vivo protects chondrocytes against the proapoptotic effect of inflammatory cytokines. Moreover, inflammatory cytokines stimulate the production of matrix metalloproteases (MMP) able to proteolytically process PRL to vasoinhibins, a family of peptides that inhibit angiogenesis and vascular function. Here, we used primary cultures of articular chondrocytes to investigate whether treatment with PRL modifies the proapoptotic effect of a mixture of the inflammatory cytokines (cyt), interleukin-1 β , interferon- γ , and tumor necrosis factor- α , and whether vasoinhibins affect chondrocyte survival. PRL, at all doses tested, inhibited the apoptosis of chondrocytes induced by a 24-hour incubation with the cyt as determined by ELISA. In contrast, vasoinhibins elicited a dose-dependent stimulation of chondrocyte apoptosis. The cyt stimulated nitric oxide (NO) production in chondrocytes and blockage of NO production with L-NAME prevented cyt-induced chondrocyte apoptosis. However, neither PRL nor vasoinhibins modified basal or cyt-induced NO production, indicating that their opposing effects on chondrocyte apoptosis are independent of NO signaling. Incubation of PRL with chondrocyte lysates resulted in the processing of PRL to vasoinhibins, a conversion that was abolished by the MMP inhibitor, GM-6001. Thus, the PRL molecule has the potential to exert either of two dichotomous effects on chondrocyte survival during inflammation; which effect prevails depends on the activity of MMP produced by chondrocytes. Understanding these mechanisms may be of preventive and therapeutic value in inflammatory arthropathies characterized by cartilage degradation.

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Nothing to Disclose: IM, NA, AQ-S, GMdIE, CC

OR33-5

Growth Hormone Regulation of Lipid Metabolism in Adipose Tissue: A Microarray Analysis in Hypopituitary Men.

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Adipose tissue is a major target of GH action. GH stimulates lipolysis and reduces fat mass. The molecular mechanism underlying cellular and metabolic effects of GH in adipose tissue is not well understood. The aim of this study is to identify GH-responsive genes that regulate lipid metabolism in adipose tissue.

Eight men with GH deficiency underwent metabolic studies and subcutaneous fat biopsies before and after one month of GH treatment (0.5mg/day). Lipolysis was estimated by measurement of plasma free fatty acid (FFA) level and whole body lipid oxidation was quantified by indirect calorimetry. Gene expression profiling was performed using Agilent 44K G4112F arrays utilising a two-colour design. Differentially expressed genes were identified using an empirical Bayes, moderated t-test, using a false discovery rate of < 5% to indicate statistical significances. Genes involved in GH receptor signalling, lipolysis, triglyceride biosynthesis, adipogenesis, as well as those encoding components of extracellular matrix (ECM) were analysed. Target genes were validated by quantitative RT-PCR using TaqMan gene expression assays.

GH significantly increased circulating IGF-I, FFA and stimulated fat oxidation. A total of 246 genes were differentially expressed, of which 135 were up-regulated and 111 down-regulated. GH treatment enhanced adipose tissue expression of IGF-I and SOCS3. It did not change expression of key enzymes governing lipolysis, but differentially regulated genes promoting diacylglycerol syntheses. GH repressed hydroxysteroid (11-beta) dehydrogenase 1, which activates local cortisol synthesis, and CIDEA, a novel gene with roles in lipolysis and lipid droplet formation. GH significantly suppressed genes encoding components of the ECM, including integrins, thrombospondin and adhesion proteins. ECM serves important roles in cell migration, proliferation, differentiation and inflammation.

In conclusion, GH induced concordant changes in circulating IGF-I and expression in adipose tissue. GH stimulation of lipolysis is mediated at a translational and/or post-translational level. GH suppressed genes encoding local factors regulating adipocyte differentiation, size and inflammation.

Nothing to Disclose: JTZ, MJC, PL, VB, WK, KKYH

OR33-6

Role of Prolactin Receptor in Growth Hormone Signaling in Human Breast Cancer Cells.

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Recent studies suggest that growth hormone (GH) may be important in breast cancer pathophysiology and that GH activates acute JAK2/STAT5 signaling in the human breast cancer cell line, T47D. hGH can signal via GH receptor (GHR) or prolactin receptor (PRLR), which has structural similarity to GHR. In contrast, human PRL signals through PRLR, but not GHR. To further investigate the role of PRLR in GH signaling in human breast cancer cells, we compared T47D cells in which PRLR was knocked down by stable expression of a shRNA against PRLR (T47D-shPRLR) to T47D cells expressing a scrambled shRNA (T47D-scr). Although PRLR abundance was dramatically reduced in T47D-shPRLR cells, JAK2 and STAT5 levels were not changed. Notably, however, GHR abundance was significantly increased in T47D-shPRLR compared to T47D-scr. GHR mRNA levels did not differ between the two cell lines, but GHR protein half-life was increased in T47D-shPRLR, possibly accounting for its enhanced GHR abundance. Effects of PRLR knockdown on GH signaling depended on the duration of GH exposure. Acutely (≤ 1 h of GH exposure), T47D-shPRLR cells exhibited markedly increased GH-induced GHR and JAK2 tyrosine phosphorylation and enhanced GH sensitivity for STAT5 activation (EC_{50} ~ 45 ng/ml GH for T47D-shPRLR vs. ~ 210 ng/ml GH for T47D-scr). Notably, while acute GH-induced STAT5 activation in T47D-scr was not affected by pure GHR antagonists (B2036 or our inhibitory monoclonal anti-GHR ext-mAb), both inhibitors blocked GH-induced STAT5 activation in T47D-shPRLR, suggesting that absence of PRLR enhanced GHR abundance and GH's utilization of GHR for signaling. In contrast to acute signaling, longer GH treatment (1-24 h) yielded reduced STAT5 activation and cyclin D1 protein expression in T47D-shPRLR compared to T47D-scr cells. This disparate effect of PRLR knockdown on sustained vs. acute GH signaling may be explained by a more rapid GH-induced GHR downregulation in T47D-shPRLR vs. T47D-scr cells, as assessed by anti-GHR blotting. We conclude that the presence of PRLR in human T47D cancer cells allows GH-induced PRLR-mediated GH signaling and may negatively regulate GH-induced GHR-mediated signaling. Knockdown of PRLR enhances early GH-induced GHR signaling, but may decrease net long-term GH effects by virtue of diminished PRLR response and rapid GHR downregulation. We hypothesize that relative PRLR:GHR expression in human cancer cells may be a key factor in determining the GH responsiveness of those cells.

Sources of Research Support: NIH R01 DK 58259.

Nothing to Disclose: JX, JJ, PEL, SYF, SJF

OR34-1

A Circadian Clock Entrained by Melatonin Is Ticking in the Rat Fetal Adrenal.

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The fetal adrenal gland is an important endocrine organ that orchestrates maturational processes important for the transition to newborn. In non-human primates circadian clock genes are present in several fetal tissues, including a prominent expression in the fetal adrenal gland (1). Here we explored *in vivo* and *in vitro* whether the rat fetal adrenal is a peripheral circadian clock entrained by melatonin. We measured expression of clock genes *Per2* and *Bmal1*, and their relationship with *StAR* and *Mt1* melatonin receptor expression. Additionally, we explored the effect of a melatonin pulse in the expression of clock genes, *StAR* and *Mt1*.

Methods: Fetal adrenal glands were dissected from fetuses obtained from pregnant dams euthanized (thiopental overdose) at 18 days of pregnancy. **In vivo studies:** four dams were euthanized every 2h around the clock. The collected fetal adrenal glands (about 96 adrenals per clock time) were preserved in trizol until RNA extraction. **In vitro studies:** in three separate experiments, twelve dams were euthanized at 20-24 hrs, and about 100 fetal adrenals were collected and pooled in 15 ml DMEM-F12, pre-incubated by 6 hrs at 37°C and then aliquoted in culture dishes (8 adrenals/ well) in triplicate. An additional set of adrenals were treated with a pulse of 100 nM melatonin given at 04-08-h. Starting at 04h, fetal adrenals were collected every 4 hrs for 48 hrs. RNA was extracted using a commercial kit and the expression of the clock genes *Per2* and *Bmal1*, *Mt1* melatonin receptor and *StAR* were measured by real time-PCR.

Results: *In vivo* the fetal adrenal showed antiphasic oscillatory expression of *Bmal1* (acrophase at 23h), *Per2* (acrophase at 13h). Additionally, *StAR* and *Mt1* oscillate with acrophases at 06h and 080h respectively. *In vitro* the oscillatory expression of *Bmal1*, *Per2*, *StAR* and *Mt1* was maintained. However the delay in acrophases between *Per-2* and *Bmal-1* became closer, showing a 4 h delay (*Bmal1*-acrophase at 8h; *Per2*-acrophase at 12h). The 4 hours pulse of melatonin induced a phase delay in all the genes studied and restored the 12 hours phase delay between *Per-2* and *Bmal-1* observed *in vivo*.

Conclusions: Altogether, the present demonstrate that the rat fetal adrenal is a functional peripheral clock, active at 18 days of gestation. The presence of *MT1* receptor expression plus the effect of melatonin in culture suggests that *in vivo* the rat fetal adrenal rhythms are synchronized by maternal melatonin.

1.- Torres-Farfan et al., *Endocrinology* 2006; 147:4618

Sources of Research Support: Fondecyt-1080649.

Nothing to Disclose: CT-F, NM, NV, LA-C, MS-F

OR34-2

Identification of Novel Ligands Targeted to the Cholesterol Recognition/Interaction Amino Acid Consensus (CRAC) Domain of the Translocator Protein (TSPO).

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The translocator protein (TSPO) is an outer mitochondrial membrane protein critical for the synthesis of steroids in the adrenal cortex and gonads. TSPO, in concert with the mitochondrial-targeted steroidogenic acute regulatory protein (StAR), controls the principal regulatory step of steroidogenesis - delivery of cholesterol from extramitochondrial stores to the CYP11A1 enzyme in the mitochondrial matrix - in which cholesterol is metabolized to the precursor of all steroids, pregnenolone. TSPO binds cholesterol with high affinity. This cholesterol binding is mediated through a C-terminal cholesterol recognition / interaction amino acid consensus (CRAC) domain, which has been found necessary for steroidogenic cholesterol transport. To further our understanding of TSPO's, and the CRAC domain's, involvement in steroidogenesis, we have identified novel TSPO ligands targeted against its CRAC domain. A pharmacophore model of the TSPO CRAC motif was computationally constructed and small-molecule ligands identified via a high-throughput in silico screen. The biological activity of the identified molecules was subsequently tested in the TSPO-rich, hormone-responsive MA-10 mouse tumor Leydig cell line. A series of compounds was identified capable of inhibiting the hormone-stimulated progesterone production of these cells in a dose-dependent manner. Among these compounds, we noted a substituted sterol able to inhibit hCG-stimulated steroid synthesis by 90%. This inhibition was localized to the delivery of cholesterol to CYP11A1 in the mitochondrial matrix, as the cells retained the ability to synthesize steroids when supplied with 22R-hydroxycholesterol, a soluble cholesterol analog which bypasses the mitochondrial cholesterol-transfer step of steroidogenesis. Moreover, this drug had no effect on cellular viability. Finally, this new CRAC ligand inhibited StAR protein processing and import into mitochondria, evidenced by a decrease in the processed 30 kDa StAR protein with an increase in its initial 37 kDa form, recapturing previously observed effects of TSPO knockdown and inactivation. These results demonstrate the utility of the generated pharmacophore model as a tool for the study of the CRAC motif. Moreover, in addition to their advantage as tools to study TSPO function in steroidogenesis, identified CRAC ligands may prove effective lead compounds for the development of drug treatments for Cushing's disease and other maladies of steroid imbalance.

Sources of Research Support: Grants from NICHD and CIHR.

Nothing to Disclose: ASM, NA, LL, VP

OR34-3

Angiotensin II-Induced Activin A Switches Adrenocortical Steroidogenesis to Aldosterone by Suppressing CYP17A1 Expression.

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Objective:

The inhibin α -subunit (INHA) affects adrenocortical tumorigenesis in mice, whereas activin-related genes are differentially expressed in human adrenocortical tumors. To further study the (patho-)physiological roles of inhibin and activin we investigated the regulation of activin-related genes and their effects on steroidogenic enzyme expression in the human adrenal cortex.

Material & Methods:

Human adrenocortical carcinoma cells (H295R) and primary cultures of cells from normal, hyperplastic, adenomatous and carcinomatous human adrenal cortices were incubated with protein kinase A (PKA) or PKC agonists or activin. mRNA expression levels of activin subunits and receptors, the activin-binding protein follistatin and of steroidogenic enzymes were investigated using quantitative RT-PCR. Steroid and activin levels were measured in the culture supernatants.

Results:

INHA expression was upregulated by ACTH-mediated PKA signaling. The PKC stimulator PMA increased the expression of the β A-subunit of inhibin and activin (INHBA), both in H295R cells and primary cultures of normal adrenal cells. Incubation with angiotensin-II confirmed that INHBA expression and activin production were enhanced through the angiotensin-II/PKC signaling pathway. Both PMA and activin A suppressed CYP17A1 mRNA expression in H295R cells and primary cultures. Angiotensin II, PMA and activin A also dose-dependently reduced the androstenedione to progesterone ratio in the supernatant of H295R cells, reflecting decreased CYP17A1 activity. INHBA silencing partially counteracted the PMA-induced CYP17A1 downregulation. In primary cultures of adrenocortical carcinoma cells activin effects were smaller, whereas activin decreased CYP17A1 expression by 85% in a Conn adenoma. Expression levels of follistatin and activin receptors were decreased after PKA stimulation, whereas follistatin expression was increased after incubation with PMA or activin A.

Conclusion:

Since adrenal activin A is predominantly located in the zona glomerulosa it can be an auto- or paracrine intermediate in the PKC-induced downregulation of CYP17A1. Activin therefore appears to be an important factor in the functional differentiation of the adrenocortical cell layers by conserving aldosterone production in the zona glomerulosa while preventing the production of cortisol and adrenal androgens. INHA expression, regulated by ACTH, could serve to prevent local activin actions in the other adrenocortical zones.

Nothing to Disclose: JH, JS, LJH, PMvK, ME, FHvN, WWdH, RAF, FHdj

OR34-4

Differential Regulation of DHEA Sulfation by PAPS Synthase Isoforms.

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Androgen excess is a major feature of the polycystic ovary syndrome (PCOS). Androgen synthesis depends on the availability of the pro-hormone dehydroepiandrosterone (DHEA), the major product of the adrenal zona reticularis. DHEA is a crucial precursor of active androgen synthesis unless it is inactivated to its sulfate ester DHEAS, which is catalyzed by DHEA sulfotransferase (SULT2A1). DHEA sulfation requires provision of the universal sulfate donor 3'-phosphoadenosine-5'-phosphosulfate, PAPS. In humans, PAPS is generated by the PAPS synthase isoforms PAPSS1 and PAPSS2. Recently, inactivating *PAPSS2* mutations have been identified in a patient with androgen excess but undetectable DHEAS (1), implicating DHEA sulfation as the gatekeeper to human androgen synthesis. To examine why ubiquitously expressed PAPSS1 cannot compensate for loss of PAPSS2, we have investigated the differential impact of PAPSS1/2 on DHEA sulfation. Firstly, we determined the sub-cellular localisation of PAPSS1/2 by indirect immunofluorescence in NCIh295R, HepG2, and HEK293 cells, derived from adrenal, liver and kidney, respectively. We found that PAPSS1 invariably showed nuclear localisation whereas PAPSS2 was predominantly cytosolic in HEK293 cells. By contrast, in adrenal and liver cells, capable of DHEA sulfation by cytosolic SULT2A1, PAPSS2 was almost exclusively nuclear. We generated bicistronic constructs for concurrent overexpression of SULT2A1 with PAPSS1/2 in HEK293 cells that have negligible levels of SULT2A1 or PAPSS2 and only low endogenous PAPSS1. Realtime PCR demonstrated successful overexpression of all targets (Δ Cts 6-8). Enzymatic activity assays demonstrated that SULT2A1 overexpression provides HEK293 cells with some ability to sulfate DHEA. However, concurrent overexpression of SULT2A1 with PAPSS yielded a twofold (PAPSS1) and fivefold (PAPSS2) increase in DHEA sulfation rate. Differential siRNA knockdown of both PAPSS isoforms in the adrenal NCIh295-TR cell line demonstrated upregulation of PAPSS1 mRNA by PAPSS2 knockdown and vice versa. Additionally, siRNA knockdown of either PAPSS2 or SULT2A1 inhibited the sulfation of DHEA to 15-30 % of WT activity, whereas PAPSS1 knockdown yielded a slight increase (10% of WT). These findings demonstrate a tight co-regulation of PAPSS isoforms in support of sulfation and suggest that the activity and nuclear localisation of PAPSS2 is a precondition for efficient DHEA sulfation.

(1) Noordam et al., NEJM 2009; 360:2310

Nothing to Disclose: JCM, JI, EN, AW, VD, WA

OR34-5

Reversible Interconversion of Cortisol and Cortisone in Human Subcutaneous Adipose Tissue and Skeletal Muscle *In Vivo*.

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The only 11 β HSD isozyme expressed substantially in human adipose tissue and skeletal muscle is 11 β HSD1, which catalyses reductase (RED) regeneration of cortisol from cortisone and is a therapeutic target for inhibition in type 2 diabetes. Recent data suggest 11 β HSD1 can also catalyse dehydrogenase (DH) inactivation of cortisol, eg if NADPH generation by hexose-6-P-dehydrogenase (H6PDH) is impaired. We used stable isotope tracers to quantify RED and DH activities in subcutaneous adipose and forearm skeletal muscle *in vivo*, and test whether hyperinsulinaemia switches 11 β HSD1 directionality by increasing substrate supply to H6PDH.

Healthy males (age 42.3 \pm 3.6y, BMI 24.6 \pm 0.9kg/m²) received iv infusion of d2-cortisol (105.3 μ g/h) and d4-cortisol (40% in F, 1.74mg/h). Simultaneous blood samples were obtained from an arterialised hand vein, superficial epigastric vein, and deep forearm vein at steady state (180-210min) and after a hyperinsulinaemic-euglycaemic clamp (330-360min). Tracer/tracee ratios were quantified using LC-MS/MS and rates of appearance (Ra) calculated for cortisol, d3-cortisol (RED) and cortisone (DH), adjusting for blood flow measured by ¹³³Xe washout in adipose and forearm plethysmography in muscle.

See Table. Cortisol and d3-cortisol production indicated RED activity in adipose tissue (similar magnitude to previous report, but borderline statistical significance) and in skeletal muscle. Cortisone production indicated substantial DH activity in both tissues. Insulin increased whole body RED (p<0.001), but did not switch 11 β HSD1 direction in muscle or adipose.

| | Ra Cortisol | | Ra D3-cortisol | | Ra Cortisone | |
|----------------------------------|-----------------|----------------|-----------------|-----------------|------------------|----------------|
| | Baseline | + insulin | Baseline | + insulin | Baseline | + insulin |
| Whole body (nmol/min) | 62.6 \pm 8.4* | 63.0 \pm 6.5 | 24.9 \pm 1.0* | 31.6 \pm 1.6† | 65.1 \pm 6.9* | 60.2 \pm 5.6 |
| Adipose (pmol/L/100g tissue/min) | 29.3 \pm 21.1 | 20.8 \pm 4.9 | 11.5 \pm 7.9 | 12.4 \pm 2.6 | 38.7 \pm 13.0* | 3.8 \pm 12.6 |
| Muscle (pmol/L/100ml tissue/min) | 19.7 \pm 4.1* | 16.5 \pm 7.7 | 5.9 \pm 1.8* | 4.9 \pm 1.5 | 15.2 \pm 5.8* | 7.5 \pm 5.3 |

*p<0.05 for baseline v 0, †p<0.001 for baseline v insulin. Data = mean \pm SEM.

In conclusion, 11 β HSD1 is bidirectional in human subcutaneous adipose tissue and skeletal muscle *in vivo*. Insulin does not regulate the balance of activities. Dysregulation of 11 β HSD1 transcription, eg in obesity, may affect cortisol inactivation as well as regeneration. Inhibitors of 11 β HSD1 should preferentially target 11 β -reductase activity.

Sources of Research Support: Award from the Translational Med Res Collaboration.

Nothing to Disclose: KAH, KM, RA, FK, BRW

OR34-6

Hydroxysteroid (17beta) Dehydrogenase 12 Is Essential for Mouse Organogenesis and Embryonic Survival.

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Hydroxysteroid (17-beta) dehydrogenases (HSD17Bs) have a significant role in steroid metabolism by catalyzing the conversion between 17-keto and 17 β -hydroxysteroids. However, several studies in vitro have shown that some of these enzymes may also be involved in other metabolic pathways. Among these enzymes, HSD17B12 has been shown to be involved both in the biosynthesis of estradiol and in the elongation of the essential very long fatty acids in vitro and in vivo. To investigate the function of mammalian HSD17B12 in vivo, we have generated mice with a null mutation of the Hsd17b12 gene (HSD17B12KO mice) by using a gene-trap vector resulting in the expression of the lacZ gene of the trapped allele. The β -galactosidase staining of the heterozygous HSD17B12KO mice revealed that Hsd17b12 is expressed widely in the E7.5-E9.5 embryos, with the highest expression in the neural tissue. The HSD17B12KO mice die at E9.5 at latest, and present severe developmental defects. Analysis of the KO embryos revealed that the embryos initiate gastrulation, but organogenesis is severely disrupted. As a result, the E8.5-E9.5 embryos were void of all normal morphological structures. In addition, the inner cell mass of KO blastocysts showed decreased proliferation capacity in vitro, and the amount of arachidonic acid was significantly decreased in heterozygous HSD17B12 ES cells. The data suggest that in mouse, the HSD17B12 is involved in the fatty acid synthesis and in normal neuronal development during embryogenesis.

Nothing to Disclose: PR, HL, MK, JPS, LS, KS, PP, MP

OR35-1

Diminished Cell Survival and Altered Response to IGF-I in Children Born Small for Gestational Age without Post Natal Catch up Growth.

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Background: Infants born small for gestational age (SGA) usually show catch up growth within the first few years of life. However 10% of SGA children each year will fail to catch-up (in the UK 1500 and in the USA 10,000) and be eligible for GH treatment. In most cases no clearly defined endocrine cause will be found. In order to investigate growth failure in these patients we initiated an assessment of GH and IGF-I responses, including cell growth and cell signalling using fibroblast cell lines derived from SGA patients without post natal catch up growth.

Methods: Skin biopsies were obtained with informed consent and local ethics approval from healthy children (C) (n=4) and SGA children without post-natal catch up growth (n=4). Fibroblasts were serum starved and cultured with vehicle, GH (200ng/ml), IGF-I (100ng/ml) or both hormones for 5 days with proliferation being assessed by cell counting, and apoptosis monitored by TUNEL staining. IGF-I signalling was investigated by stimulating serum starved cells for 15 minutes and then analysing lysates by western blotting for both total and phospho isoforms of MAPK and Akt.

Results: The basal rate of proliferation in fibroblasts from SGA cells was similar to that of cells from C (1.4×10^5 v 1.5×10^5 respectively at day 5). The proliferation of both cell types was increased by treatment with either GH or IGF-I: For GH cell counts on day 5 were 1.7×10^5 in SGA cells v 1.6×10^5 for C, for IGF-I 2.4×10^5 v 4.1×10^5 cells; $p < 0.001$. However when cultured with GH and IGF-I, SGA cells grew at a similar rate to controls (4.3×10^5 cells v 4.7×10^5).

Apoptosis, induced by serum starvation, was significantly increased in SGA cells compared to controls (26.1% v 16.5 % after 5 days; $p < 0.001$). Both GH and IGF-I 'rescued' cells from apoptosis; by 5.7% for GH and 3.6% for IGF-I in SGA cells and 6.3% for GH and 5.2% for IGF-I in control cells. Overall apoptosis remained higher in SGA cells.

There was no difference between basal levels of Akt and MAPK expressed by SGA and control cells. IGF-I stimulated phosphorylation of MAPK was similar in the two cell types, while phosphorylated Akt 2 was detected in SGA but not in control cells.

Conclusion: Decreased IGF-I stimulated cell growth, increased rates of apoptosis and altered IGF-I signalling are associated with a non catch up SGA phenotype. Defining the roles of GH and IGF-I within these key pathways may have implications on the future treatment of these patients.

Nothing to Disclose: IB, PGM, AJW, MW, PEC

OR35-2

The Effect of Heterozygous *IGFALS* Gene Variants on Height and the IGF System in Normal (N), Idiopathic Short Stature (ISS) and GH Deficient (GHD) Children.

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Inactivation of the *IGFALS* gene results in ALS deficiency, characterized by low levels of IGF-I and IGFBP-3, that remain unchanged after GH treatment (1,2). Heterozygous carriers (HC) for *IGFALS* gene mutations are frequently shorter than their wild type (WT) relatives (3), suggesting that this gene could be involved in the etiology of short stature in a subset of ISS children. We characterized *IGFALS* gene mutations in N, ISS and GHD children, determining the impact of these mutations on height and the IGF system.

Levels of IGF-I, IGFBP-3, and ALS were measured in 196 N (5.0-16.3 y), 27 GHD (2.0-17.6 y) and 89 ISS children (1.8-16.9 y) and expressed as SDS in relation to controls. The *IGFALS* gene was sequenced, evaluating the effect of SNPs *in silico* by mean of PolyPhen analysis (classified as severe and non-severe).

Besides two common polymorphisms (D70D and Y462Y), the following genetic variants were found:

Genetic variants in the *IGFALS* gene

| | Severe nsSNPs | Non-severe nsSNPs | Synonymous SNPs |
|-----|--|---|--|
| N | N276S; R277H; R548W (2) 4/196=2.0% | G170S; V239M; G506R 3/196=1.5% | N236N; A267A; L271L (2); L487L (2); T522T (3) 9/196=4.6% |
| ISS | E35GfsX16; L97F; R277H; R548W (2) 5/89=5.6% | G83S; P287L; A330D; R493H; A546V 5/89=5.6% | P73P.L316L; P271L; T522T 3/89=3.4% |
| GHD | | R157H 1/27=3.7% | |

HC-ISS children show lower IGFBP-3 (-1.90±1.46 vs. -0.77±1.24 SDS; p=0.007) and ALS levels (-2.34±1.77 vs. -1.22±1.45 SDS; p=0.02) when compared to WT-ISS children.

The study was extended to 15 parents (7 HC) and 14 siblings (2 HC) of HC-ISS children.

Auxological, biochemical and genetic data in families of HC-ISS children

| SDS | Severe (n=8) | Non-severe (n=12) | WT (n=20) | One-way ANOVA |
|---------|---------------------------|-------------------------|------------|---------------|
| Height | -2.09±1.40 ^a | -2.26±0.83 ^a | -0.30±0.92 | p<0.0001 |
| IGF-I | -2.38±1.83 ^{a,b} | -0.99±1.21 | -0.46±1.08 | p=0.0024 |
| IGFBP-3 | -1.90±1.28 ^a | -1.14±1.24 ^c | 0.18±1.11 | p=0.0003 |
| ALS | -3.22±1.34 ^{a,d} | -1.07±1.19 | -0.66±1.04 | p<0.0001 |

^ap<0.001 vs. WT; ^bp<0.05 vs. non-severe; ^cp<0.05 vs. WT; ^dp<0.01 vs. non-severe

In families of ISS children the finding of lower levels of IGF-I, IGFBP-3 and ALS in HC for severe mutations suggests that both gene alleles are required to maintain the circulating IGF system and to fulfill growth potential. Functional characterization of mutant ALS-proteins is required to confirm the involvement of this gene in the etiology of short stature in a subset of ISS children.

(1) Domené HM et al., N Engl J Med 2004; 350:570

(2) Domené HM et al., Horm Res 2009; 72:129

(3) van Duyvenvoorde HA et al., Eur J Endocrinol 2008; 159:113

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Nothing to Disclose: HMD, PAS, ASM, ACK, VRP, SVB, LMK, MCG, MGR, MGB, EML, MAB, JJH, RAR, HGJ

OR35-3

Novel Mutations in the Growth Hormone Secretagogue Receptor Gene (*GHSR*) Associated with Constitutional Delay in Growth and Puberty (CDGP).

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Introduction: Ghrelin stimulates GH secretion by acting on *GHSR*. Recently, mutations in the *GHSR* gene were described in patients with IGHD and idiopathic short stature (ISS) (1, 2).

Objective: to study the *GHSR* gene in children with ISS.

Methods: The coding region of *GHSR* was directly sequenced in 96 patients with ISS, 39 of them with CDGP.

Results: Five different heterozygous mutations in *GHSR* were identified, all of them in patients with CDGP. One mutation is located in the 5'UTR, 6 bp prior to the initiation codon (c.-6 G>C). The other 4 mutations predicted amino acid changes in highly conserved residues in *GHSR*: p.Ser84Ile, p.Ala169Thr, p.Val182Ala and p.Ala358Thr. None of these alterations was found in 150 control individuals. Functional studies of the missense mutations showed that p.Ser84Ile and p.Val182Ala mutants have a decrease in both basal activity and in ligand-induced signaling. The p.Ser84Ile mutant also has a marked decrease in cell surface expression, whereas the p.Val182Ala mutant has a tendency toward decreased expression. The decrease in cell surface expression may in part account for the decrease in basal and ligand-induced signaling. In silico analysis demonstrated a slight decrease in the prediction of the use of the correct ATG initiation codon due to c.-6 G>C mutation (Score from 0.88 to 0.85).

The mutation p.Ser84Ile was identified in a female patient. At 13 yr, her height was 137.5 cm (-2.4 SD), BMI SDS of -2.6 and bone age of 11 yr. She had just started puberty and presented at Tanner stage 2 of puberty. She achieved a normal final height of 157.6 cm (-0.7 SD) and a BMI of 18.4 kg/m². Her brother (start of puberty unknown) and sister (menarche at 11 yr) of normal height and weight were also heterozygous for the same mutation. Her mother has a normal *GHSR* and her father was not available for genetic studies. The mutation p.Val182Ala was found in a female patient that started puberty at 13 yr (bone age 11 yr), with a height SDS of -2.5 and BMI SDS of 1.0. Her father who is of normal height and had normal sexual development carries the same mutation. At her last visit at 14.4 yr, her height was 148.7 cm (-1.9 SD).

Conclusion: This is the first report of functionally-significant *GHSR* mutations in patients with CDGP. The fact that these mutations (p.Ser84Ile and p.Val182Ala) did not segregate with the phenotype in corresponding families is consistent with a dominant mode of inheritance with incomplete penetrance.

(1) Pantel J et al., J Clin Invest 2006; 116:760

(2) Pantel J et al., J Clin Endocrinol Metab 2009; 94(11):4334

Nothing to Disclose: PNP-P, JPF, YZ, BBM, IJA, ASK, AAJ

OR35-4

Genetic Markers Are Associated with 1 Month Change in IGF-I and Growth Response at 1 Year in Growth Hormone Deficiency (GHD) but Not in Turner Syndrome (TS) during Treatment with GH: The PREDICT Study and Follow-Up.

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Background: PREDICT follow-up investigates relationships between short-term biomarker changes, long-term auxological changes and genomic markers during GH therapy in children with GHD or TS.

Objective: To evaluate prospectively the association of SNPs in 98 growth- and metabolism-related genes with 1-month change in IGF-I SDS and/or change in growth over 1 year in GH-treatment-naïve prepubertal children with GHD or TS.

Methods: Blood was taken at baseline for DNA extraction. 98 candidate genes, selected by an international advisory board, were analysed using 1536 SNPs. IGF-I levels (baseline and 1 month) were analysed centrally and converted to SDS. The association of genotypes at a given locus with changes in IGF-I SDS at 1 month and growth at 1 year was assessed by non-parametric tests adjusted for multiple testing ($p \leq 0.05$). GH dose (mcg/kg/day) was 35 for GHD and 50 for TS.

Results: In GHD, GRB10 and SOS2 correlate significantly with changes in IGF-I SDS, height SDS and HVSDS. In contrast, in TS, no genes are common across these 3 parameters. 6 genes are common to growth measures in GHD and 1 in TS. Genes associated with early change in IGF-I SDS, and also with HVSDS, are INPPL1 and WT1 in GHD and POU1F1 in TS. Comparing GHD with TS, CDK4 and CDK6 are both associated with IGF-I change. However, there is no overlap in the genes associated with growth measures between GHD and TS.

Table 1. Associated genes

| GHD | | TS | | | |
|-----------------------------|------------------------------|---------------|-----------------------------|-----------------------------|---------------|
| Change in IGF-I SDS (n=160) | Change in height SDS (n=110) | HVSDS (n=110) | Change in IGF-I SDS (n=139) | Change in height SDS (n=60) | HVSDS (n=60) |
| GRB10 | GRB10 | GRB10 | | | |
| SOS2 | SOS2 | SOS2 | | | |
| | IGFBP3 | IGFBP3 | | | |
| | GHRHR | GHRHR | GHRHR | | |
| | PIK3R1 | PIK3R1 | | | |
| | PIK3CA | PIK3CA | | TGF α | TGF α |
| INPPL1 | | INPPL1 | | | |
| WT1 | | WT1 | POU1F1 | | POU1F1 |
| CDK4 | | | CDK4 | | |
| CDK6 | | | CDK6 | | |
| TGF β 2 | GRB2 | SOCS2 | SOCS2 | LHX4 | TGF β 1 |
| SOS1 | RARB | SIRP α | INSR | | SIRP α |
| PTPN1 | | GAB1 | IGFALS | | ESR1 |
| CYR61 | | INS | FOS | | CCND3 |
| LEPR | | KRAS | PPP1CC | | LEPR |
| STAT cluster | | TP53 | BCL2 | | |
| GATA1 | | PDGFR β | PIK3CG | | |
| | | SHOX | PIK3CB | | |
| | | | PIK3R2 | | |
| | | | IGF1R | | |
| | | | ARRB1 | | |
| | | | SH2B2 | | |
| | | | AKT2 | | |

Conclusions: CDK4 and CDK6, early regulators of the cell cycle, are related to IGF-I generation in both GHD and TS. GRB10, an adaptor protein downstream of the IGF-I and insulin receptors, and SOS2, a signalling molecule within the MAPK pathway downstream of growth factor receptors, are implicated in IGF-I generation and growth in GHD only. Notably, the genes related to growth measures do not overlap between the two conditions. These data broaden the spectrum of genes and provide targets for pharmacoprediction.

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

Disclosures: PC: Investigator, Merck & Co.; Speaker, Merck & Co. LT: Investigator, Merck & Co.; Speaker, Merck & Co. SS-S: Employee, Merck & Co. JR: Analyst, Merck & Co. NM: Analyst, Merck & Co. CO: Employee, Merck & Co. PC:

Investigator, Merck & Co.; Consultant, Merck & Co.; Speaker, Merck & Co.
Nothing to Disclose: SQ, MC, JJ

OR35-5

First-Year Height Velocity and Safety Results from a Phase II, Randomized, Open-Label, Active-Treatment Controlled Trial of rhGH/rhIGF-1 Co-Administration in Short, Prepubertal Children with Low IGF-1 and Normal Stimulated GH Levels.

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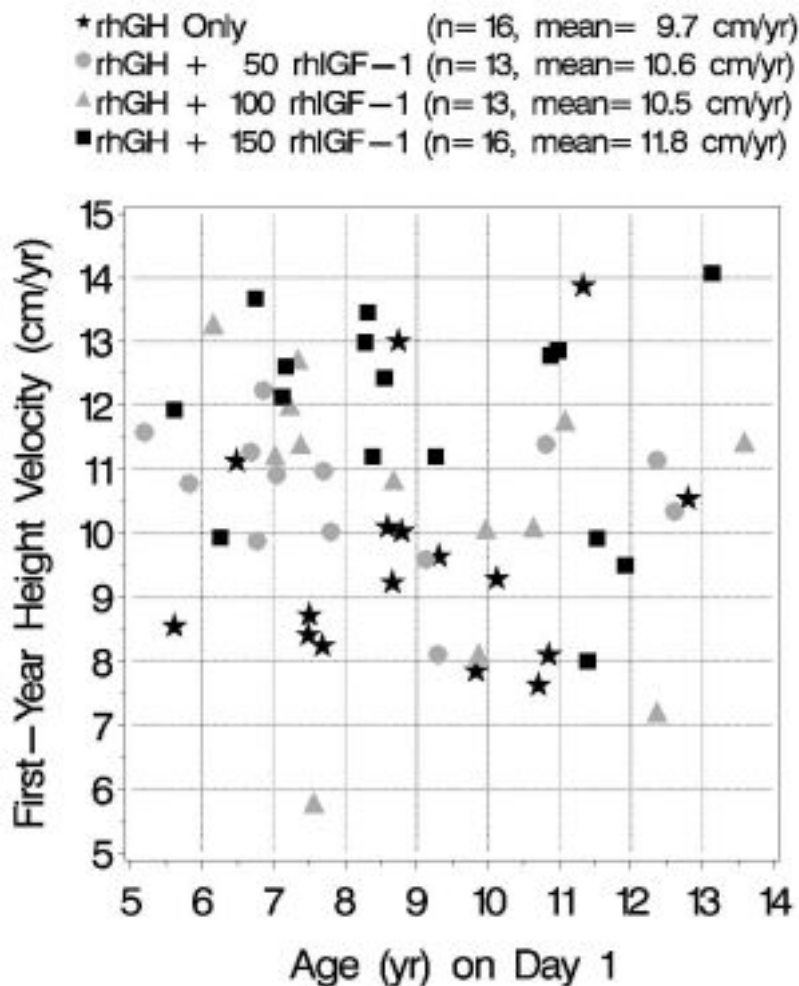
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Introduction: Both GH and IGF-1 are necessary for normal growth. Short, GH sufficient children with low IGF-1 levels may require very high doses of rhGH (>100 µg/kg/d) to raise IGF-1 levels to the upper half of the normal range (*JCEM* 2007;92:2480-6) and twice-daily rhIGF-1 dosing can suppress nocturnal GH secretion (*JCEM* 1993;77:273-80). This led to the hypothesis that rhGH/rhIGF-1 co-administration (co-admin) may outperform both monotherapies.

Methods: This ongoing, randomized, open-label study evaluates the efficacy and safety of co-admin versus rhGH alone in 106 treatment-naïve, prepubertal children with baseline height SDS ≤-2, IGF-1 SDS ≤-1, and maximum stimulated GH ≥10 ng/mL. Subjects receive morning injections of rhGH alone (45 µg/kg once-daily) or co-admin (45 µg/kg rhGH and 50, 100 or 150 µg/kg rhIGF-1 as 2 injections).

Results: As of 30 November 2009, safety data were available for 106 subjects (baseline mean age, 8.9 yr; mean height SDS, -2.5); first-year height velocity (HV) data were available for 58 subjects. The first-year HVs of co-admin subjects generally exceeded those of rhGH subjects (Figure 1). The mean changes in first-year height SDS were 0.76 for rhGH vs 0.91, 0.90 and 1.07 for the co-admin groups.

Figure 1. HV vs age.



The most common adverse events (AEs) were headache (42% rhGH vs 50% co-admin subjects) and vomiting (27% vs 15%). Hypoglycemia symptoms were equally reported (4% vs 5%); there were only 3 of 438 co-admin on-treatment blood glucose values <60 mg/dL. Two co-admin subjects had drug-related generalized urticaria and 2 had papilledema (probable intracranial hypertension; one considered a drug-related serious AE). Both co-admin subjects with papilledema stopped and restarted therapy without recurrence of the AE. Transient increases in mean serum AST and ALT levels (generally <2.5 x ULN) were observed during weeks 0-13 in some rhGH and co-admin subjects.

Conclusion: The data for this study so far show that co-admin is generally well tolerated and associated with first-year HVs that exceed those for rhGH alone in short children with low IGF-1 levels. The complete first-year data will be presented at the meeting.

Sources of Research Support: Ipsen, US and Genentech, Inc.

Disclosures: LKM: Study Investigator, Ipsen; Speaker, Ipsen. BR: Speaker, Ipsen; Study Investigator, Ipsen; Medical Advisory Board Member, Ipsen. JWF: Consultant, Ipsen, Genentech, Inc. GMB: Employee, Ipsen.

OR35-6

Growth Hormone Improves Growth in Duchenne Muscular Dystrophy Boys with Steroid-Induced Growth Failure.

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Background: Duchenne Muscular Dystrophy (DMD) is a progressive degenerative muscle disorder affecting 1 in 3500 boys. In the absence of a cure, daily high-dose glucocorticoids (GCs) are the mainstay of treatment, slowing disease progression and prolonging ambulation and survival. GCs cause growth failure, weight gain, absent puberty and osteoporosis, which negatively impact quality of life in DMD. Growth hormone (GH) offers potential benefit in DMD: it may help counter GC-induced growth failure, and could have positive effects on body fat, muscle strength and function. However, data regarding efficacy and safety of GH in DMD is lacking.

Objective: To evaluate efficacy and safety of GH in DMD boys with GC-induced growth failure during the first year of treatment.

Methods: We report a case-series of 29 DMD boys on daily GCs, treated in the Cincinnati Neuromuscular Comprehensive Care Center. The boys were treated with growth hormone for severe growth failure. Outcomes included growth velocity, height SD, weight, BMI, neuromuscular and cardiopulmonary function, and side effects.

Results: 29 prepubertal boys (mean age \pm SD 12.2 \pm 2.9y) were treated with GH for 4-32m (mean 12m). They had received daily GCs for 5.5 \pm 2.2y. Peak stimulated GH levels were 6.9 \pm 3.6 ng/ml. Height z-score (mean \pm SEM) was -3.1 \pm 0.2 and height velocity was 1.1 \pm 0.3 cm/y before GH. During the first year on GH, height velocity improved to 5.6 \pm 0.7 cm/y ($p < 0.0001$). Baseline decline in height z-score before GH ($p < 0.001$) was followed by stabilization at -3.0 \pm 0.2 ($p = 0.2$) on GH. There was a trend toward reduction in rate of weight gain at 1 year (2.8 \pm 0.7 to 0.6 \pm 1.1 kg/y, $p = 0.3$), with decreased weight z-scores from -0.6 \pm 0.3 to -1.2 \pm 0.4 ($p < 0.0004$). BMI z-score improved from 1.3 \pm 0.2 to 0.8 \pm 0.2 ($p < 0.0001$). There were no detrimental effects on neuromuscular or cardiopulmonary function attributable to GH. GH was well tolerated, with 3/29 experiencing side effects by 1y. One boy developed worsening insulin resistance / impaired glucose tolerance. Two boys had progression of scoliosis.

Conclusions: GH treatment of DMD boys with GC-induced growth failure improved growth during the first year. Rate of weight gain slowed for some, with improvement in BMI. GH was relatively safe, with no detrimental effects on neuromuscular and cardiopulmonary function. Further study is needed before conclusions can be drawn regarding longer-term safety and efficacy.

Nothing to Disclose: MMR, JC, JGW, SRR, HS, LHC, KJK, KH, BLW

OR36-1

Neurosurgical Hypothalamic Lesions and Postoperative Outcome in Childhood Craniopharyngioma - Results of the Multinational Prospective Trial KRANIOPHARYNGEOM 2000.

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Multivariable analyses of risk factors (age at diagnosis, degree of resection, irradiation, growth hormone treatment, gender) and descriptive analyses of overall (OS) and event-free survival (EFS) rates were performed in 117 patients from Germany, Austria and Switzerland, recruited prospectively during 2001 and 2006 and evaluated after 3 yrs of follow-up (**KRANIOPHARYNGEOM 2000**). Body mass index (BMI) and QoS (PEDQOL) at diagnosis, 12 and 36 mo after diagnosis were analyzed in relation to neuroradiological reference assessment of tumor localization and a score of post surgical hypothalamic damage (anterior, posterior or no hypothalamic lesions).

We observed a 3-yrs-OS of 0.97 ± 0.02 and a 3-yrs-EFS of 0.50 ± 0.05 , indicating high recurrence rates after complete resection (CR) (n=47; 3-yrs-EFS: 0.63 ± 0.09) and high progression rates after incomplete resection (IR) (n=66; 3-yrs-EFS: 0.31 ± 0.07). The risk of an event decreased by 80% after CR compared to IR (HR=0.20; p<0.001). Irradiation (XRT) had protective effects on EFS. XRT-patients had an 88% lower risk of progression compared to patients without/before XRT (HR=0.12, p<0.001). Substitution therapy with recombinant growth hormone had no impact on 3-yrs-EFS. BMI SDS at diagnosis was similar in patients without and with hypothalamic involvement of anterior or posterior hypothalamic areas. Surgical lesions of posterior hypothalamic areas (as detected in postsurgical imaging) were associated with increases in BMI-SDS during the first 12 mo (median increase +2.2 BMI SD; p<0.01) and 36 mo (+3.2 BMI SD; p<0.01). Postsurgical QoS deteriorated in patients with posterior hypothalamic lesions. Postoperative increases of BMI (>2SD) were associated with lowest QoS.

We conclude that tumor recurrences/progressions are frequent and occur early after initial treatment of childhood craniopharyngioma. Growth hormone therapy had no impact on high recurrence/progression rates observed during short-term follow-up. A radical surgical strategy leading to damage of posterior hypothalamic areas is not recommended due to associated severe obesity and impaired QoS. XRT was efficient in preventing recurrences/progressions. Accordingly, in **KRANIOPHARYNGEOM 2007** (www.kraniopharyngeom.net) QoS and survival rates after IR in patients with childhood craniopharyngioma (>5 yrs of age at dx) are analyzed after randomization of the time point of XRT (immediate XRT versus XRT at the time of progression of the residual tumor).

Sources of Research Support: Deutsche Kinderkrebsstiftung, Bonn, Germany (www.kinderkrebsstiftung.de).

Nothing to Disclose: HLM, UG, SS, FP, RDK, IZ, AF, MW-M, TP, GC, RK, CW, NS

OR36-2

Characterization of Prolactinomas Resistant to Dopaminergic Agonists.

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AIMS

The prevalence of pituitary adenomas has previously been underestimated. Recent data suggest a true prevalence of 1:1000 of the population; 60% are prolactinomas which have a female-to-male ratio 10:1. Dopamine agonists (DA) are often effective treatments for prolactinomas. However, cases of pharmacological resistance may occur. To date, the clinical, epidemiological and genetic characteristics of resistant prolactinomas have not been studied together in detail.

METHODS

Data on resistant prolactinomas were collected in 9 centres worldwide. Resistance was defined as a failure to normalize prolactin (PRL) levels or to decrease tumor size of macroprolactinomas by $\geq 50\%$ despite optimal DA therapy.

RESULTS

Pharmacological resistance occurred in 1% of cases. Among 80 patients with resistant prolactinomas, 55% were females. There were 68 macroadenomas (85%); 12 were giant adenomas. Macroadenomas occurred in 97.2% of males and 75% of females. Median PRL levels at diagnosis were higher in males (55000 $\mu\text{U/ml}$) than in females (10120 $\mu\text{U/ml}$). Late or secondary resistance (after initial response to therapy) was present in 8 cases (10%). At last follow-up only 16 cases demonstrated hormonal and radiological control. As in acromegaly, tumor debulking was effective in aiding better hormonal control with pharmacotherapy.

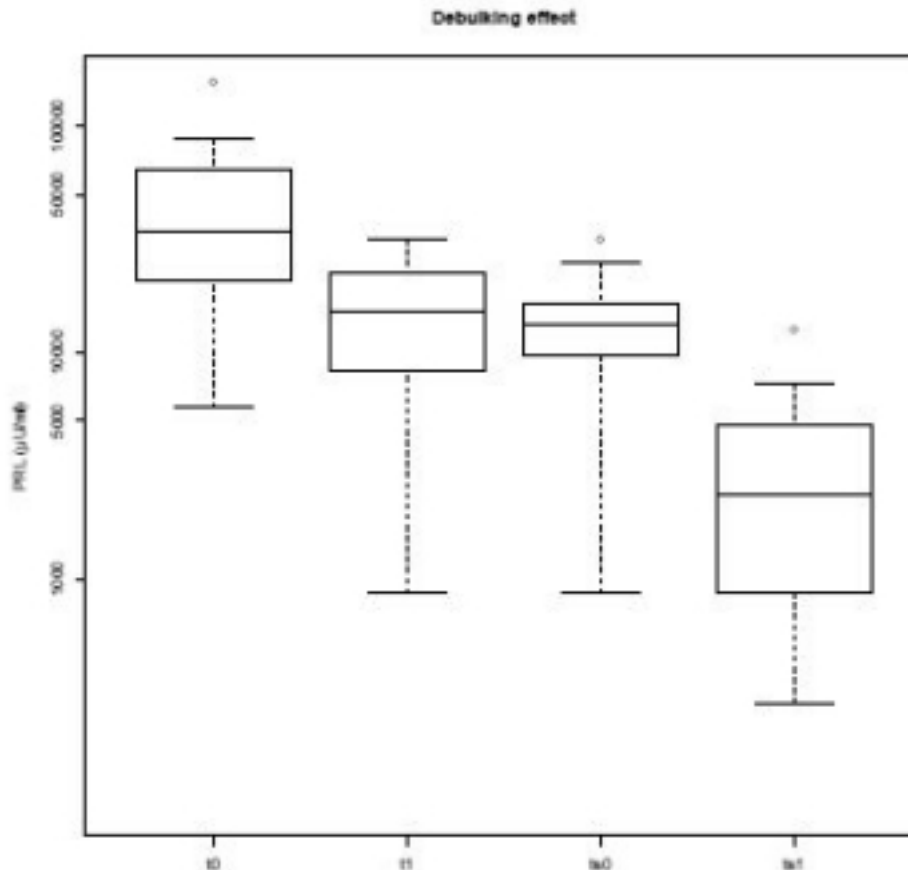


Figure 1: PRL levels at diagnosis (t0), pre-surgically (t1), post-surgically (ts0) and at last follow-up (ts1).

Four resistant cases occurred in the setting of FIPA (two had *AIP* mutations), three cases of *MEN1* and three malignant prolactinomas was seen. The mortality rate was 3.8%.

CONCLUSION

In this comprehensive study on the characteristics of 80 DA-resistant prolactinomas, there was a greater proportion of males than in prolactinomas generally and most presented as macroadenomas. PRL levels at diagnosis are higher in males than females. Prevalence of resistance of 1% is lower than previously described in the literature. Late resistance

to pharmacologic treatment can develop after an initial response. Tumor debulking is effective in improving the response to DA. Germline genetic mutations were present in 11% of resistant prolactinomas.

Disclosures: PC: Advisory Group Member, Pfizer, Inc.

Nothing to Disclose: LV, GT, LN, LV, JB, PP, AFD, TB, AB, BD, AT, MLJ-R, PC, PB-P, FB-C, AB

OR36-3

Should We Discontinue Dopamine Agonist Therapy after 3 or 5 Years in Patients with Macroprolactinoma and Microprolactinoma, as the Majority Have Recurrence of Hyperprolactinaemia within 12 Months?.

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Objective: To examine recurrence of hyperprolactinaemia following discontinuation of dopamine agonist (DA) therapy in patients with macroprolactinoma and microprolactinoma with prior treatment for 3-15 years, due to concerns of possible high recurrence-rates.

Methods: We identified adult patients (n=65) attending OCDEM (Churchill Hospital, Oxford) since 1985, with a confirmed diagnosis of macroprolactinoma (n=15) and microprolactinoma (n=50), who had been treated with DA therapy for ≥ 3 years (prolactin suppressed to normal range in all macroprolactinomas and 90% [n=45] microprolactinomas) and had had a trial off DA therapy. None had other treatments and none were pregnant. Data included: age at diagnosis; sex; tumour size/shrinkage with therapy (macroprolactinomas); length of DA therapy; prolactin levels at baseline, during DA therapy and at recurrence (defined by prolactin >375 mU/l [men] and >620 mU/l [women]); and time to recurrence.

Results: Hyperprolactinaemia recurred in 93% of macroprolactinomas (n=14) and 68% of microprolactinomas (n=34) within 12 months of DA discontinuation. Mean recurrence time was 8.8 months (SD 8.7) for macroprolactinomas and 5.1 months (SD 3.2) for microprolactinomas. Mean time on DA therapy was 7.5 years (SD 3.4) for macroprolactinomas and 3.9 years (SD 2.1) for microprolactinomas. For macroprolactinomas, mean initial tumour diameter was 2.0cm (SD 0.6), and most (n=14) had tumour shrinkage with DA therapy. In patients who recurred, mean prolactin level at baseline was 28,246mU/l (SD 24,569) for macroprolactinomas and 1873mU/l (SD 874) for microprolactinomas. Prolactin levels during DA therapy and at recurrence were: 144mU/l (SD 105) and 2236mU/l (range 411-12,847) respectively (P=0.05) for macroprolactinomas; 432mU/l (SD 379) and 1428mU/l (range 465-7413) respectively (P<0.005) for microprolactinomas. For microprolactinomas, prolactin levels on DA therapy differed between those who subsequently recurred off DA therapy (mean 432mU/l, SD 379) and those who did not recur (mean 233mU/l, SD 137), P=0.048.

Conclusions: In everyday Endocrine practice, hyperprolactinaemia recurs in most macroprolactinomas (93%) and microprolactinomas (68%) following discontinuation (3-15 years) of DA therapy, most within 6 months and all within 12 months. Our data argue against a trial off DA therapy for macroprolactinomas, and to consider a trial off DA therapy only in those microprolactinomas with a well-suppressed prolactin (<300 mU/l) on DA therapy.

Nothing to Disclose: TMB, JK, CG, RVS, JAHW

OR36-4

Hypocortisolaemia Is Common in Acute Traumatic Brain Injury and Predicts Mortality.

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Published data has demonstrated low random plasma cortisol (PC) and low cortisol responses to glucagon in the acute phase following traumatic brain injury (TBI). There is no sequential data on PC following TBI which is potentially important given the well-documented transient nature of pituitary deficiencies following TBI. We hypothesised that low PC may be a marker for more severe TBI and a predictor of mortality. 82 patients (69 men, median age 32.5, range 18-75) were recruited on admission with TBI (mean GCS +/- SD = 8.7 +/- 4.37). Patients with GCS >13, previous cerebral insult, age < 18 yrs or > 75 yrs, and those given dexamethasone or medications which interfere with cortisol metabolism, were excluded. Ethical committee approval was granted and informed consent obtained. Each patient had 0900h PC measured on day 1, 3, 5, 7, and 10 following TBI. Results were compared with 12 patients admitted to ITU following vascular surgery. An 0900h PC < 300nmol/L in a patient in ITU was regarded clinically as inappropriately low. None of the controls had a PC < 300 nmol/L in 10 days following surgery; all but one patient had a PC of > 500nmol/L on day 1 following surgery. In contrast, 63/82 TBI patients had at least one 0900h PC < 300 nmol/L (median = 161 nmol/L, range = 25-299 nmol/L) in 10 days of ITU admission. Most of the low PC concentrations occurred early after TBI, with recovery in many over the 10 days of the study, as shown in the table below.

| Day Post TBI | 1 | 3 | 5 | 7 | 10 |
|---------------------------------------|-------|-------|-------|-------|-------|
| Proportion with 0900h PC < 300 nmol/L | 28/82 | 38/81 | 27/81 | 17/78 | 19/75 |

Day 1 PC was independently and inversely predictive of mortality (Mann Whitney U test, p = 0.047). 17 patients died during follow up. 0900h PC was < 300 nmol/L in the measurement preceding death in 10/17, compared with 13/65 survivors who had 0900h PC readings < 300 nmol/L on day 10 (p = 0.019).

This is the first study to report sequential PC readings during the acute phase of TBI. Hypocortisolaemia is common in ITU patients with TBI compared with ITU patients following vascular surgery, though in the majority of patients it was transient. Low 0900h PC on day 1 following admission is predictive of mortality and patients who died are more likely to have had preceding hypocortisolaemia than survivors. Acute hypocortisolaemia is more common following TBI than is recognised and may be an important determinant of acute mortality.

Sources of Research Support: Unrestricted grant from Novo Nordisk.

Nothing to Disclose: MJH, LAB, RKC, EPO, DR, RO, AA, CJT

OR36-5

Increased Prevalence of Psychopathology and Maladaptive Personality Traits after Long-Term Cure of Cushing's Disease.

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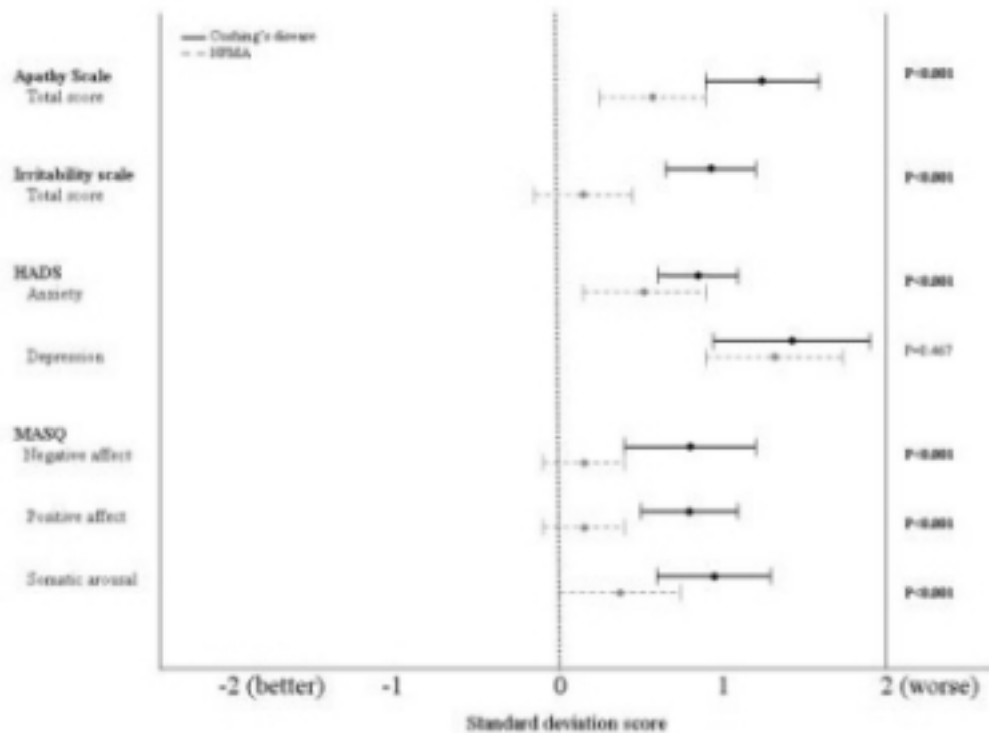
¹LUMC Leiden, Netherlands.

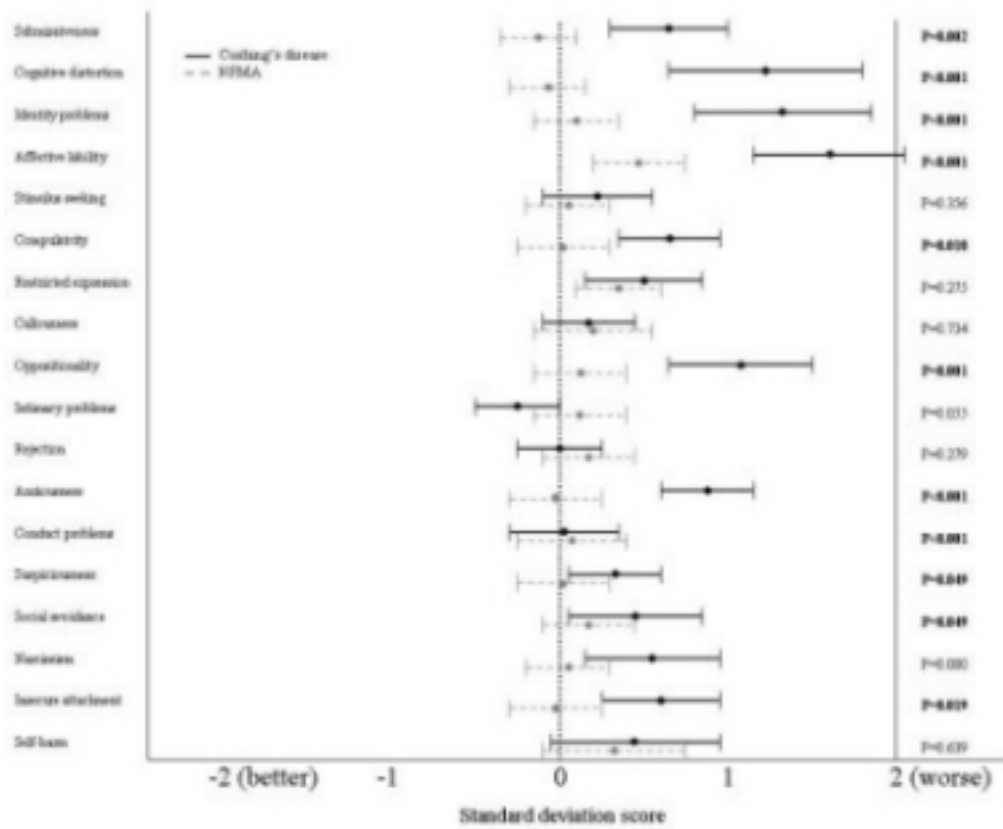
Background: Psychopathology and maladaptive personality traits are often observed during the active phase of Cushing's disease (CD). We hypothesized that patients with long-term cure of CD show persistent psychopathology and maladaptive personality traits.

Methods: We included 51 patients cured of CD (16% men, 53±13 yr) and 51 matched controls. In addition, we included 55 patients treated for nonfunctioning pituitary macroadenomas (NFMA) (55% men, 62±10 yr), and 55 matched controls. All patients and controls completed questionnaires on frequently occurring psychopathology in somatic illnesses, including the Apathy Scale, Irritability Scale, Hospital Anxiety and Depression Scale (HADS), and Mood and Anxiety Symptoms Questionnaire short-form (MASQ-30). Personality was assessed using the Dimensional Assessment of Personality Pathology short-form (DAPP).

Results: Mean duration of remission was 11yr (range 1-32yr). Compared to matched controls, patients cured from CD scored significantly worse on virtually all questionnaires. Compared to NFMA patients, patients treated for CD scored worse on apathy (p<0.001), irritability (p<0.001), anxiety (p<0.001), negative affect, and lack of positive affect (p<0.001 on both scales), somatic arousal (p<0.001), and eleven out of eighteen subscales of the DAPP (p<0.05). These results are shown in Figure 1 and 2.

Conclusions: Patients with long-term cured CD show an increased prevalence of psychopathology and maladaptive personality traits. These observations suggest irreversible effects of previous glucocorticoid excess on the central nervous system rather than an effect of pituitary tumors and/or their treatment in general. This may also be of relevance for patients treated with high doses of exogenous glucocorticoids.





Nothing to Disclose: JT, NRB, HAMM, RCvdM, JAR, AMP

OR36-6

Temozolomide Treatment in Aggressive Pituitary Tumors and Pituitary Carcinomas: A French Multicenter Experience.

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Context: To date 10 publications reporting only 16 patients with aggressive pituitary tumors or carcinomas treated with temozolomide are available. Expression of O⁶-methylguanine-DNA-methyltransferase (MGMT), a DNA repair protein implicated in the resistance to temozolomide, was studied in only 10 out of these 16 patients. It has been suggested that low expression of MGMT could predict temozolomide efficacy.

Objective: The aim of this study was to describe the effects of temozolomide treatment in a larger number of patients with aggressive pituitary tumors or carcinomas and to evaluate the possible prognostic significance of MGMT promoter methylation and protein expression.

Patient: From a French multicenter study, we report 8 patients; 5 carcinomas (3 PRL and 2 ACTH) and 3 aggressive pituitary tumors (1 PRL and 2 ACTH); treated with temozolomide administered orally for 4 to 24 cycles. Three out of the 8 patients were considered as responder to temozolomide because of significant tumoral shrinkage and decreased of PRL /ACTH secretion.

Design: MGMT expression, assessed by immunohistochemistry and MGMT promoter methylation analysed by pyrosequencing, were studied in 7 patients.

Results: Temozolomide treatment was effective in 3 out of the 8 patients (2 ACTH and 1 PRL tumors). Three cycles of temozolomide were sufficient to identify patients responding to temozolomide treatment; in non-responders, additional cycles did not improve treatment efficacy even in association with carboplatine and VP16. Tumoral response to temozolomide was not predicted by MGMT expression since MGMT expression was positive (30%) in 1 responder and negative in 2 out the 5 non-responders patients. Similarly, MGMT promoter methylation (3/7 tumors) and did not predict MGMT expression.

Conclusion: Temozolomide treatment could be an effective option for some aggressive pituitary tumors or carcinomas but MGMT status is a poor predictor of treatment outcome that cannot be used to select patients who may benefit from this treatment.

Nothing to Disclose: GR, NS, FD, MM, SS, PC, OC, PC, CC-R, RA, HD, SG, PF, EJ, JGP, MB, DF-B, CAM, JT, FB-C, TB

OR37-1

The Role of Glutamic or Aspartic Acid in Position Four of the Epitope Binding Motif and TSH-R-ECD Epitope Selection in Graves' Disease.

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Development of Graves' disease (GD) is related statistically to inheritance of HLA-DRB1*0301 (DR3), and more specifically of arginine at position 74 of the DRB1 molecule. ARG 74 is important in the shape and charge of pocket 4 in the binding cleft of DR3 protein, and is thus important in allowing or rejecting peptide binding and thus presentation on APCs. The extracellular domain (ECD) of human TSH receptor (hTSH) contains the target antigen in GD, segments of which end up on the cell surface of APCs bound to DR protein.

We analyzed the relation between hTSH-R-ECD peptides and DR molecules. We asked whether the presence of amino acids carrying a negative charge such as aspartic acid (D) or glutamic acid (E) at position four in the binding motif (cognate to pocket 4) influenced selection of functional epitopes.

Peptide epitopes from TSH-R-ECD with D or E in position four (D/E+) had higher affinity for binding to DR3 than peptides without D/E (D/E-) (IC_{50} = 29.3uM vs. 61.4uM, $P=0.0024$). HLA-DR7, negatively correlated with GD, and DRB1*0302 (HLA-DR18), not associated with GD, had different profiles of epitope binding.

PBMC from toxic GD patients who are DR3+ had higher responses to D/E+ peptides during in vitro proliferation assays than to D/E- peptides (SI; 1.42 vs. 1.22, $p=0.028$). All DR3+ GD patients (toxic + euthyroid) had higher responses, with borderline significance (SI; 1.32 vs. 1.18, $p=0.051$). DR3- patients did not have this association.

Splenocytes of DR3 transgenic mice immunized to TSH-R-ECD responded during in vitro proliferation assay to D/E+ peptides more than D/E- peptides (SI; 1.95 vs. 1.69, $p=0.036$).

Seven of 9 hTSH-R-ECD peptide epitopes previously reported to be reactive with GD patient's PBMC contain DR3 epitope binding motifs with D/E at position four.

Conclusions-TSH-R-ECD epitopes with D/E in position four of the DR3 peptide binding motif bind more strongly to DRB1*0301 than epitopes which are D/E-, are more stimulatory to GD patient's PBMC, and to splenocytes from mice immunized to hTSH-R. These epitopes appear important in immunogenicity to TSH-R, due to their favored binding to HLA-DR3, thus increasing presentation to T cells. This study presents a new paradigm in recognizing epitopes involved in autoimmune diseases, through recognition of specific interactions between aminoacids forming the disease related DR protein epitope binding cleft, and cognate aminoacids in the binding motif of the epitope.

Disclosures: WM: Researcher, EPIVAX,INC. MA: Researcher, EPIVAX,INC. ASDG: Speaker, EPIVAX,INC.

Nothing to Disclose: LJDG, HI

OR37-2

SNPs in the Intronic Regulatory Region of the Thyroid Hormone Receptor Gene Are Associated with a Tissue-Specific over Expression of the R338W Mutation in a Pituitary Selective Form of Resistance to Thyroid Hormone.

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Change of function mutations in thyroid hormone receptor- β (TR β) result in the syndrome of resistance to thyroid hormone that is manifested in autosomal dominant fashion (RTH). The clinical presentation varies from asymptomatic to diffuse hypothyroidism, to selective pituitary resistance, and is not completely attributable to specific genotypes. Recent observations indicate that an intron region in the mouse TR β gene plays a crucial role in the pituitary and retinal tissue-specific expression of the TR β -2 isoform (1). We speculated that polymorphisms in this regulatory region in the human gene could result in a modulation of the RTH phenotype.

The index case of selective pituitary resistance (2) is characterized by a R338W missense mutation in exon 9 which results in a reduced affinity for ligand and poor homodimer formation(3). We analyzed this case for mutations in the intronic regulatory region.

The human TR β 2 promoter and the intronic regulatory region were cloned into a pRep4 episomal luciferase reporter vector, which was transfected in GH3 pituitary-derived cells. Luciferase activity assays indicated that the human intronic regulatory region positively enhances expression of the reporter gene. No activity was present in kidney-derived HEK-293 cells. Direct sequencing of the patient's intronic region demonstrated the presence of three common SNPs (rs2596622, rs2596623, and rs17194828). By performing direct sequencing of the intronic region of the unaffected daughter we were able to determine the patient's haplotype as R338W- rs2596622C-rs2596623C. Luciferase reporter analyses in GH3 cells demonstrated that the patient's haplotype induces a 20% increase in transcription of the gene over the wild type.

The data suggest that the patient's haplotype induces pituitary over-expression of a previously described mutant TR β isoform, generating a tissue-specific dominant negative condition consistent with the patient's phenotype.

(1) Jones I et al., Mol Endocrinol 2007;21:1108

(2) Gershengorn MC et al., J Clin Invest 1975;56:633

(3) Sasaki et al., Mol Cell Endocrinol 1995;113:109

Sources of Research Support: Intramural Research Program of the NIDDK program Z01-DK047057-01.

Nothing to Disclose: ATA, VC, HL, PWB, MCS, OD, JL, DF, FSC

OR37-3

Distinct Roles of the Deiodinases on the Phenotype of Mct8 Defect.

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Previous studies on Mct8 knockout (KO) mice demonstrated increased 5'-deiodination, impaired thyroid hormone (TH) secretion and abnormal TH excretion. These and other unknown mechanisms result in the low serum T₄, high T₃, low rT₃ characteristic of the Mct8 defect. We investigated to what extent each of the 5'-deiodinases (D1, D2) contributes to the serum TH abnormalities of the *Mct8KO*, by generating mice with all combinations of Mct8 and D1 and/or D2 deficiencies, and comparing the resulting 8 genotypes. As in *D1KO* mice, the serum T₄ concentration in *Mct8D1KO* became higher (5.1±0.3 µg/dl) than in *Mct8KO* (1.2±0.1) and Wt mice (3.7±0.2), whereas the high serum T₃ of *Mct8KO* (131±6 ng/dl) normalized (109±4) to the Wt level (103±2). The low rT₃ of *Mct8KO* mice (3.2±0.4 ng/dl) also normalized (17±2) to Wt level (22±1). The thyroid function abnormalities of *Mct8KO* mice were maintained in *Mct8D2KO*, with more severe impairment of the hypothalamo-pituitary-thyroid axis, a serum TSH 59- and 27-fold higher than the Wt and *Mct8KO*, respectively. Serum T₃ was significantly higher (151±5 ng/dl) than that of the *Mct8KO* (131±6 ng/dl) mice, while serum T₄ and rT₃ remained comparable to *Mct8KO* (T₄ 1.6±0.1 vs. 1.2±0.1 µg/dl, rT₃ 3.1±0.4 vs. 3.2±0.4 ng/dl). The triple KO mice *Mct8D1D2KO* have high serum TSH levels, 100- and 46-fold that of the Wt and *Mct8KO*, respectively. Serum T₃ remains similar to that of *Mct8KO*, whereas T₄ was elevated (7.9±0.3 µg/dl), due to the failure of T₄ to T₃ conversion in the absence of D1 and D2, and in response to the elevated TSH. The significant decrease in serum rT₃ in *Mct8D1D2KO* (42±4 ng/dl) compared to *D1D2KO* mice (149±13) was similar to that observed in *Mct8D1KO* versus *D1KO* mice. This suggests that the low rT₃ is a Mct8-dependent feature, possibly due to decreased 5-deiodination by impaired TH access to D3-containing cells. For all genotypes, liver T₄ and T₃ content followed the pattern observed in serum, suggesting that TH uptake by the liver is less dependent on Mct8. *Mct8D1KO* mice showed improved brain markers compared to *Mct8KO*, whereas the brain of *Mct8D2KO* mice manifested a pattern of more severe TH deprivation. Our studies on these 8 genotypes provide a unique insight into the complex interplay of the deiodinases in the Mct8 defect, and suggest that D1 contributes to the increased serum T₃ in Mct8 deficiency, while D2 mainly functions locally, converting T₄ to T₃ to compensate distinct cellular TH depletion in *Mct8KO* mice.

Sources of Research Support: NIH DK15070 and DK020595, Sherman family, Esformes Endowment.

Nothing to Disclose: AMD, XHL, CDC, AH, DLSG, REW, VAG, SR

OR37-4

Increased Miscarriage Rate in Thyroid Antibody Negative Women with TSH Levels between 2.5-5.0 in the First Trimester of Pregnancy.

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Introduction:

Studies over the last two decades have demonstrated an increased miscarriage rate in euthyroid women who are thyroid antibody positive. Similarly, women with overt hypothyroidism have an increased rate of spontaneous pregnancy loss. The impact on pregnancy loss of TSH levels between 2.5-5.0 in thyroid antibody negative women is unknown.

Methods:

The present abstract is a component of a larger study in which 4562 women were screened for TSH and thyroid peroxidase in the first trimester of pregnancy in southern Italy. Women were randomly assigned to a universal screening (US) group or a case finding (CF) group and stratified as high risk or low risk for thyroid disease. All women in the US group and high-risk women in the CF group had TSH and thyroid peroxidase antibody performed immediately. Women in the CF low risk group had their sera assayed postpartum. Antibody positive women with a TSH above 2.5 were treated with LT4. The results on pregnancy outcome are in press (1). The present study evaluates the miscarriage rate in thyroid antibody negative pregnant women with TSH levels between 2.5-5.0 as compared to thyroid antibody negative women with TSH levels below 2.5. None of these women were treated with levothyroxine.

Results:

4123 in the first trimester of pregnancy women were TPO negative with a TSH of 5.0 or lower (mean time of screening was 8.8 weeks). The rate of spontaneous pregnancy loss was 6.1% (39/642) in women with a TSH between 2.5-5.0 and 3.6% (127/3481) in women with a TSH below 2.5 (p=0.006)

Discussion:

The present study demonstrates a significant increase in the rate of spontaneous pregnancy loss in antibody negative women who have first trimester TSH levels between 2.5-5.0 as compared to antibody negative women with first trimester TSH levels below 2.5. These data provide further evidence that the normal range for women in the first trimester of pregnancy is 2.5 and below. Future studies need to evaluate the impact on the miscarriage rate of LT4 treatment in antibody negative women with TSH between 2.5-5.0 in the first trimester of pregnancy.

1. Negro R, Schwartz A, Gismondi R. et al. Universal screening versus case finding for detection and treatment of thyroid hormonal dysfunction during pregnancy. J Clin Endocrinol Metab, in press.

Nothing to Disclose: RN, AS, RG, AT, TM, AS-G

OR37-5

Diagnostic Validation of Myocardial Performance Parameters by Echocardiography in Overt and Subclinical Hypothyroidism.

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Context: Thyroid hormone strongly affects cardiac function. Parameters of myocardial function have long been used as investigational tools to measure peripheral thyroid hormone action. In practice, serum TSH levels detect even mild/subclinical primary hypothyroidism, but are not diagnostic in central hypothyroidism. We have previously shown that some functional echocardiographic parameters are usually abnormal in central hypothyroidism with low FT4 and in ~40% of patients with hypothalamic-pituitary disease and "normal" FT4. Those abnormalities were corrected by proper thyroxine replacement (1,2). The aim of this study was to assess diagnostic performance of echocardiography in overt and subclinical primary hypothyroidism to further validate its use in the diagnosis of primary and central subclinical hypothyroidism.

Subjects & Methods: Adult patients with overt hypothyroidism (OH, high TSH/low FT4, n=20), subclinical hypothyroidism (SH, high TSH/normal FT4, n=10), before and after 4-6 weeks of L-thyroxine replacement, and 28 age- and sex- matched normal controls were submitted to echocardiographic and thyroid evaluation (serum TSH, FT4, T3 measured by routine assays).

Results: Hormonal and main echocardiographic results - myocardial performance index (MPI) and isovolumetric contraction time (ICT) - were (mean±SD) :

| | Controls | OH (before T4) | OH (after T4) | SH (before T4) | SH (after T4) |
|-------------|-----------|----------------|---------------|----------------|---------------|
| FT4 (ng/dl) | 1.02±0.15 | 0.29±0.12† | 1.14±0.18‡ | 0.94±0.24† | 1.14±0.18‡ |
| TSH (mU/l) | 1.0±0.2 | 97.2±53.8† | 2.4±1.3‡ | 14.3±5.6† | 2.3±1.5‡ |
| ICT (ms) | 39±10 | 74±22† | 43±22‡ | 53±11† | 40±13‡ |
| MPI | 0.40±0.05 | 0.65±0.15† | 0.44±0.11‡ | 0.48±0.09† | 0.40±0.07‡ |

†P<0.05 vs Controls; ‡P<0.05 vs before T4

Diagnostic sensitivities and specificities of MPI>0.45 and ICT >53 ms for OH were: 95% and 93%, and 85% and 96%, respectively. MPI correlated (P<0.01) with TSH (r=0.54), FT4 (r=-0.60) and T3 (r=-0.63). ICT correlated (P<0.01) with TSH (r=0.49), FT4 (r=-0.61) and T3 (r=-0.68). MPI and ICT measurements were able to confirm the diagnoses of OH and SH in 100% and 70% of cases, respectively.

Conclusion: MPI and ICT measurements by echocardiography were sensitive and specific for diagnosing tissue hypothyroidism in both overt and subclinical disease. These data give additional support for using echocardiography in the diagnosis of mild/subclinical forms of primary and central hypothyroidism.

- (1)Borges MR et al.,12th International Congress of Endocrinology, 2004, Lisboa. International Proceedings. Bologna, Italy: Medimond; 1191
 (2)Doin et al.,J Am Soc Echocardiogr 2004;17:622

Nothing to Disclose: FCD, MRM, EB, OC, AADP, ACC, VAM, JA

OR37-6

Subclinical Thyroid Disorders, Physical and Cognitive Function, and Mortality in Older Individuals.

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¹VU Univ Med Ctr Amsterdam, Netherlands and ²Leiden Univ Med Ctr Leiden, Netherlands.

Context: The clinical relevance of subclinical thyroid disorders in older individuals is still under debate.

Objectives: To determine associations between subclinical thyroid disorders and physical and cognitive function, depression and mortality in older individuals.

Design and subjects: The analyses were conducted in a population-based, prospective cohort of the Longitudinal Aging Study Amsterdam. We included 1267 individuals (aged 65 yr and older) with thyroid-stimulating hormone (TSH) measurements. Participants were classified according to clinical categories of thyroid function: euthyroidism, subclinical hypo- or hyperthyroidism, or overt hypo- or hyperthyroidism. The main outcome measures were physical function (physical performance tests, hand grip strength, functional limitations in daily life), cognitive function (minimal-mental state examination, Raven's colored progressive matrices, coding task and 15 words test), depressive symptoms (Center for Epidemiologic Studies-Depression Scale) and mortality.

Results: 69 participants (5.4%) were classified as subclinical hypothyroidism, 47 (3.7%) as subclinical hyperthyroidism, 9 as overt hypothyroidism and 10 as overt hyperthyroidism. As compared to euthyroidism, subclinical hypothyroidism was not associated with impairment of physical or cognitive function, or depression. Subclinical hyperthyroidism was associated with decreased muscle strength, but not with impairment of other measures of physical function, cognitive function or depression. Overt hypo- and hyperthyroidism had expected negative effects on physical and cognitive function, and depression. After a median follow-up of 10.7 years 632 participants were deceased. Subclinical hypo- and hyperthyroidism were associated with lower, although not significantly, risk of mortality (HR 0.75, 95% CI 0.49 to 1.15 and 0.75, 95% CI 0.47 to 1.21, respectively). Overt hyperthyroidism was related to increased mortality risk (HR 2.22, 95% CI 1.09 to 4.52).

Conclusion: Subclinical thyroid disorders were not related to disadvantageous effects on physical or cognitive function, depression or mortality risk. Consequently, usefulness of treatment of subclinical thyroid disorders may be limited.

Nothing to Disclose: RTdJ, PL, NMvS, KJR, DJD, MHK, JPV, OMD

OR38-1

Prolactin Cells Form a Highly Plastic Autonomous Network.

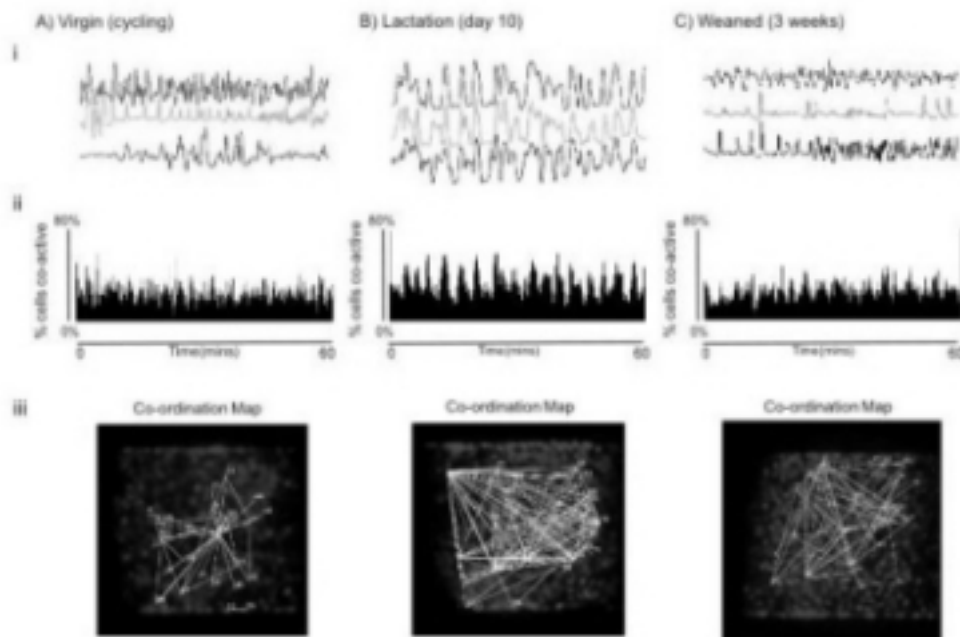
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We have previously shown that the pituitary gland is composed of multiple organised endocrine cell networks(1)(2). However, it remains unclear how cells belonging to the prolactin (PRL)-network, which is primarily under inhibitory control, are able to interact to secrete high basal levels of PRL as well as to up-regulate hormone output during periods of high physiological demand. Since intracellular Ca²⁺ fluctuations are intimately associated with exocytosis in pituitary cells, measurement of Ca²⁺ concentrations can be used to investigate how individual cells collectively communicate to release hormone. Here, using 2-photon Ca²⁺-imaging and mice with dsRED-tagged lactotrophs, we have been able to probe the structure-function relationships of the PRL-network.

In virgins, the connection pattern of the PRL-network obeys a power-law distribution, a hallmark of scale-free networks where a small number of cells host the majority of connections. These activity hubs may drive high basal PRL release by allowing the network to display some organised activity, as demonstrated by moderate levels of cell co-activity due to the firing of spontaneous Ca²⁺ spikes (Fig.1A). In contrast, during lactation, an increase in PRL-network density is associated with the appearance of multiple well-connected hubs which are able to pace the rhythm of co-ordinated cell activity between nearby and distant PRL-cells in an entirely autonomous manner (Fig.1B). The possession of such a coherently wired network is dependent on gap junction (GJ) signaling since application of a specific GJ blocker results in the re-emergence of a virgin connection pattern. Remarkably, 3 weeks after weaning, despite reversion of the PRL-network structure back to that observed in the virgin, the wiring pattern of the PRL-network still resembles that of the lactating animal (Fig.1C).

These results not only provide the first demonstration of an autonomous peripheral cell network in an adult animal which displays marked plasticity in response to physiological demand but also suggest the existence of a memory which may increase efficiency of PRL-network function following the first lactation.



(1) Bonnefont, X., Lacampagne, A., Sanchez-Hormigo, A., Fino, E., Creff, A., Mathieu, M.N., Smallwood, S., Carmignac, D., Fontanaud, P., Travo, P., Alonso, G., Courtois-Coutry, N., Pincus, S.M., Robinson, I.C., Mollard, P. (2005). Revealing the large-scale network organization of growth hormone-secreting cells. *Proc Natl Acad Sci U S A* 102:16880-16885
 (2) Hodson, D.J., Schaeffer, M., Birkenstock, J., Lafont, C., Fontanaud, P., Molino, F., Carmignac, D., Fernandez-Fuentes, M., He, Z., le Tissier, P., Mollard, P. (2009). Real-time 2-photon imaging of PRL-cell network function. 91st Meeting of the Endocrine Society, Washington D.C.

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

OR38-2

Somatotropes as Metabolic Sensors: Selective Deletion of Leptin Receptors Causes Obesity.

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Leptin, the product of the Lep gene, reports levels of adiposity to the hypothalamus and other cells that regulate adiposity, including pituitary somatotropes, which secrete growth hormone (GH). Global leptin or leptin receptor (LEPR) deficiency is associated with a decline in somatotrope numbers and function, suggesting that leptin may be important in the maintenance of this population. This hypothesis was tested in a new animal model in which exon 17 of the leptin receptor (Lepr) was selectively deleted in somatotropes by Cre-loxP technology, resulting in the production of a truncated LEPR protein lacking the JAK binding site. Organ genotyping proved that Cre-recombinase was expressed only in the pituitary. Pituitary cell number and size was normal in deletion mutants. However, deletion mutant mice showed a 72% reduction in percentages of pituitary cells bearing the leptin receptor b isoform (LEPRb) from 29% to 8% of pituitary cells. This correlated with a 43% reduction in LEPR proteins in the pituitary and no change in the hypothalamus. There was also a reduction in immunolabeled somatotropes from 30% of pituitary cells in wild type mice to 10% in deletion mutants. This reduction correlated with 50-72% reductions in serum GH in deletion mutant female or male mice. Dual immunolabeling for LEPRb and GH showed a 96% reduction in cells bearing both LEPRb and GH from 16% of total pituitary cells in controls to 1% in deletion mutant mice. Growth, insulin like growth factor-1, insulin, serum glucose, and the timing of puberty were normal. Although, growth curves do not differ between 21 days to 3 months of age, deletion mutant mice become ~30% heavier than controls with age (males 3-4 months, females 6 months). DEXA analysis showed that the weight gain correlated with an increase in percent body fat but no increase in lean mass. There was also no difference in food intake. Serum leptin levels were either normal or reflected the level of obesity. These studies have demonstrated that deleting the JAK binding site (exon 17) in LEPR of somatotropes has not interfered with roles played by GH in longitudinal growth, early weight gain or the timing of puberty. However, it has resulted in adult onset GH deficiency with a consequential reduction in lipolytic activity normally maintained by GH and increased adiposity. This highlights the importance of somatotropes as metabolic sensors in the maintenance of optimal body composition in the adult.

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Nothing to Disclose: GVC, NA, AH, ZB, MS, DG, LJS, NSA, HB, CC, RK, RL, SC

OR38-3

Enhancement of the Canonical Wnt Pathway in Rathke's Pouch Results in Pituitary Tumours Reminiscent of Human Adamantinomatous Craniopharyngioma.

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The canonical Wnt signalling pathway is required during embryonic development for normal cell proliferation and differentiation and for organ homeostasis in adulthood. Over-activation of this pathway has been implicated in human cancers such as colon or skin cancers among others. Here, we demonstrate that enhancement of the Wnt pathway in the embryonic Rathke's pouch causes over-proliferation of progenitor cells and severe differentiation defects in the *Pit1* -lineage, which results in extreme growth retardation and hypopituitarism. Mutant mice mostly die perinatally but those that survive weaning develop lethal pituitary tumors. Histopathological analysis revealed that these murine tumors most closely resemble human adamantinomatous craniopharyngioma rather than any other pituitary tumor, including pituitary adenomas, Rathke's cleft cysts, xanthogranulomas, posterior pituitary tumors (e.g. pituitocytomas) or even the adult (papillary) form of craniopharyngioma. This tumorigenic effect only occurs when Wnt pathway over-activation occurs in the early Rathke's pouch progenitors, but not when committed or differentiated cells are targeted. Finally, we demonstrate that genetic over-expression of the paired-like homeobox repressor *Hesx1* is able to delay tumor formation, by partially antagonising the activation of Wnt signalling downstream of beta-catenin. Together, our findings provide new insights into the roles of the Wnt pathway and HESX1 in the control of pituitary cell proliferation and demonstrate, for the first time, a causative role the Wnt pathway in an undifferentiated multipotent pituitary progenitor in the genesis of murine pituitary tumors that are reminiscent of human craniopharyngioma.

Nothing to Disclose: CG-M, CLA, MS, SJ, NC, PLT, MD, JPM-B

OR38-4

The Potential Role of miRNAs and the Underexpression of BTG2 in the Pathogenesis of Somatotropinomas.

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Progress in the pathogenesis of somatotropinomas has been observed in the later decades; however, some aspects still remain unsolved. Serial analysis of gene expression (SAGE) allows a comprehensive profiling of gene expression and also an assessment of transcript abundance. MicroRNAs (miRNAs), which contribute to cancer development and progression, also have been involved in pituitary tumorigenesis. Previous SAGE libraries from normal pituitary tissues and GH-secreting pituitary tumors identified genes differentially expressed. In the present study, we evaluated the expression of BTG2, RB1, and TP53 genes as well as a panel of miRNAs by real-time PCR in fifteen somatotropinomas and fifteen normal pituitaries. Relative quantification of genes and miRNA expression was calculated using the 2^{-DDCt} method. Fold change of the expression of each gene or miRNA was determined by the median of 2^{-DDCt} values of somatotropinoma related to median of 2^{-DDCt} values of normal pituitaries. We observed no different expression of RB1 and TP53 genes and an underexpression of BTG2 (-5.5-fold; $p < 0.0006$) in somatotropinomas compared to normal pituitaries. We observed no different expression of the miRNAs mir-15, mir-23a, mir-23b, mir-24-2, mir-145 and mir-150 and an underexpression of mir-141 (-100.0-fold; $p = 0.0001$), mir-16 (-16.7-fold; $p = 0.0006$), mir-143 (-2.9-fold; $p < 0.0007$), mir-21 (-2.0-fold; $p = 0.0276$), and let-7a (-2.0-fold; $p < 0.0001$) in somatotropinomas compared to normal pituitaries. We also observed a correlation of BTG2 gene and mir-15a ($r = 0.52$; $p = 0.04$) and mir-21 ($r = 0.61$; $p = 0.02$) in normal pituitary and a correlation of RB1 gene and mir-16 in tumoral tissue ($r = 0.55$; $p = 0.03$). In conclusion, let-7a, mir-16, mir-141 and mir-143 are tumor suppressor miRNAs and their underexpression has been described in somatotropinomas. They, potentially, target genes encode proteins with potential oncogenic functions. Indeed, somatotropinomas lost the correlation between miR-15 and BTG2 observed in normal tissues. These findings are interesting since the protein encoded by BTG2 appears to have antiproliferative properties. Therefore, besides the underexpression of miRNAs, the underexpression of BTG2 could have a role in the pathogenesis of somatotropinomas.

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Nothing to Disclose: TPFC, DSL, BMCP, ACM, MC

OR38-5

P53 Activation and Anti-Proliferative Functions of MEG3 Involve Different RNA Folding Structures.

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MEG3 is an imprinted gene encoding a non-coding RNA. It is highly expressed in normal pituitary, and as the first identified non-coding RNA linked to pituitary tumor pathogenesis, loss of MEG3 expression is specifically associated with clinically non-functioning adenomas of gonadotroph origin. MEG3 suppresses tumor growth, increases p53 levels, stimulates p53-mediated transactivation, and selectively activates p53 targets. To understand the novel molecular mechanisms by which a large non-coding RNA suppresses cell proliferation and activates p53 functions, we investigated the potential structure-function relationship of MEG3 RNA. The secondary structure of MEG3 RNA molecule contains several distinct folding motifs, named M1, M2, and M3, as predicted by the program mfold, which calculates RNA structures using thermodynamic methods. We generated deletion mutations of MEG3 RNA, in which each of these folding motifs was deleted, and tested them for p53 activation by reporter assays and anti-proliferation by BrdU incorporation assays. We find that removal of the folding structure M1 does not affect p53 activation, but abolishes anti-proliferative function. In contrast, deletion of the folding structure M2 results in the loss of p53 activation, but has no effect on anti-proliferative function. Therefore, different folding structures of the MEG3 RNA molecule are associated with different functions. These data suggest that MEG3 can suppress cell proliferation in a p53-independent manner, thus confirming that MEG3 is able to suppress proliferation of both p53-positive and p53-negative cells. Furthermore, we generated hybrid mutant MEG3 RNA molecules in which the original primary sequence corresponding to the M1 or M2 region was replaced by artificially synthesized and unrelated sequence. These hybrid mutants still fold into a secondary structure similar to wild-type. In functional assays, these hybrid mutants are fully capable of activating p53 and suppressing cell proliferation like wild-type. In summary, our data show that the functions of p53 activation and anti-proliferation of MEG3 involve different RNA folding structures, and the RNA folding structure is more important to its biological functions than the primary sequence. This study provides a first look into the novel molecular mechanisms of the biological functions of a large non-coding RNA we hypothesize to be involved in the pathogenesis of clinically non-functioning adenomas.

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Nothing to Disclose: XZ, KAR, YZ, YN

OR38-6

Her2/Neu Receptor Signaling in Rat and Human Prolactinoma Cells: Novel Strategy for Targeted Prolactinoma Therapy.

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Objective: We recently reported pathways underlying *in vitro* and *in vivo* regulation of pituitary tumor gene expression and cell proliferation by EGF, heregulin and ErbB receptor ligand signaling. As Her2/Neu, an ErbB receptor family member, is overexpressed in prolactinomas, we now test the role of Her2/Neu in prolactinoma hormone regulation and cell proliferation to support the rationale for targeting this receptor for drug therapy of those tumors.

Methods: We generated constitutively active Her2/Neu stable GH3 cell transfectants (Her2CA-GH3), and tested PRL gene expression and cell proliferation. We inoculated hormone-secreting Her2CA-GH3 cells to WF rats, and treated them with oral lapatinib, a dual tyrosine kinase inhibitor of Her1/EGFR and Her2/Neu, or gefitinib, a tyrosine kinase inhibitor of Her1/EGFR. We also treated primary cultured pituitary cells derived from human prolactinomas with lapatinib.

Results: After selection and propagation, MAPK phosphorylation, and PRL mRNA levels were markedly enhanced (~250-fold) in Her2CA-GH3 compared to empty vector stable transfectants (EV-GH3). PRL secretion was induced 100-fold in Her2CA-GH3 cells ($p < 0.01$), and stable transfectants exhibited increased cell proliferation (1.8 fold, $p < 0.01$). Her2CA-GH3 cells also showed higher colony formation in soft agar (31 ± 1.6 vs 12 ± 1.3 control colonies per field, $p < 0.01$). Lapatinib blocked Her1/EGFR and Her2/Neu signaling molecules, and suppressed PRL expression > gefitinib (~50% suppression with gefitinib, $p < 0.05$; ~70% suppression with lapatinib, $p < 0.01$). Lapatinib suppressed colony formation in soft agar (~80%, $p < 0.01$) more than gefitinib (~50%, $p < 0.01$) and induced cleaved-caspase 3, a marker of apoptosis. Tumors in rats implanted sc with Her2CA-GH3 were larger than those implanted with EV-GH3 (766 ± 53 vs 568 ± 38 mm³, $p < 0.05$). Her2CA-GH3 tumor transfectants implanted in rats decreased in size (~40%, $p < 0.05$) after 1 week of lapatinib treatment. In human primary prolactinoma tumor cell cultures derived from resected prolactinoma tissue, lapatinib dose-dependently suppressed both human PRL mRNA expression and PRL secretion (~80%, $p < 0.01$, and ~70%, $p < 0.01$).

Conclusions: Her2/Neu potently induces PRL secretion and regulates experimental prolactinoma cell proliferation. As Her2/Neu pituitary signaling is abrogated by tyrosine kinase inhibitors, this receptor could be an effective target for medical therapy of prolactinomas.

Nothing to Disclose: HF, GV, OC, JM, SR, SM

OR39-1

Twice-Weekly Administration of Kisspeptin-54 for Eight Weeks Stimulates Reproductive Hormone Release in Women with Hypothalamic Amenorrhea.

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BACKGROUND: Hypothalamic amenorrhea (HA) accounts for over 30% of cases of amenorrhea in women of reproductive age(1). Current treatments have limited success rates and side effects. We have recently shown that a single injection of the novel hormone kisspeptin(KP) potently stimulates reproductive hormone release in women with HA(2). However, twice-daily KP administration to women with HA results in tachyphylaxis(2). Less frequent administration of KP may lead to sustained reproductive hormone release in women with HA, which would have therapeutic implications.

AIMS:(i)Determine the time-course of desensitization to twice-daily KP injections; (ii)Investigate if twice-weekly KP injections chronically stimulate reproductive hormone release in women with HA.

METHODS:(i)Patients with HA received twice-daily subcutaneous(sc) KP-54 injection (6.4nmol/kg; n=5) for 14 days. On the 1st, 2nd, 3rd, 4th and 14th injection days, blood was sampled at regular intervals over 4 hours post-injection for plasma luteinizing hormone(LH) and follicle stimulating hormone(FSH). (ii)Patients with HA were randomized to receive twice-weekly sc KP-54 (6.4nmol/kg) or saline injection (n=5/group) for 56 days. On days 1, 14, 28, 42 and 56, blood was sampled for 4 hours post-injection for LH and FSH.

RESULTS:(i)Twice-daily KP injections: LH responsiveness diminished gradually during the 14 day injection period. LH release following KP on the 2nd, 3rd, 4th and 14th injection days was 40%, 75%, 88% and 91% lower than on the 1st injection day, respectively. Desensitization of the FSH response following KP occurred rapidly; FSH release on the 2nd injection day was 84% lower than on the 1st injection day.

(ii)Twice-weekly KP injections: patients with HA were more responsive to KP on day 1 than day 14 (mean maximal LH increase in iU/L: day 1, 21.5±10.7; day 14, 10.0±4.3; P<0.001). No further significant drop in responsiveness to KP was observed beyond day 14 (mean maximal LH increase in iU/L: day 28, 9.0±4.1; day 42, 8.9±3.5; day 56, 7.9±4.5; P>0.05 vs. response on day 14). On the last (56th) day, KP injection raised LH 16 fold higher than saline control.

CONCLUSION: We have determined the time-course of desensitization to twice-daily KP-54 injections. We have also performed the first long-term study of KP administration to women with HA. Twice-weekly KP administration stimulates reproductive hormone release over a 2 month period. Thus KP may be a future novel therapy for reproductive disorders.

(1)Reindollar RH et al. Am J Obstet Gynecol 1986; 155:531-543.

(2)Jayasena CN et al. J Clin Endocrinol Metab 2009; 94:4315-23.

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Nothing to Disclose: CNJ, GMKN, AA, KGM, AL, DAP, AM, CT, MD, GT, MAG, SRB, WSD

OR39-2

Specific Alterations in the Epigenome of Polycystic Ovary Syndrome (PCOS)-Like Female Rhesus Monkeys.

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PCOS is a heterogenic common genetic disorder with a poorly understood pathogenesis. A PCOS-like phenotype, however, can be produced by prenatal androgenization (PA) of female rhesus monkeys. We hypothesize that perturbation of the epigenome, through altered DNA methylation, is the mechanism whereby PA reprograms monkeys to develop PCOS. Archived infant visceral fat tissues from 7 PA and 5 control monkeys were initially studied because of (a) the association between visceral fat and metabolic disease, and (b) the likelihood that epigenetic alteration in 2-month old infants would reflect changes induced by intrauterine events, rather than aging after birth. Bisulfite treated samples were subjected to genome-wide CpG methylation analysis, which simultaneously measured methylation levels at 27,578 CpG sites in 14,475 genes, using the Infinium HumanMethylation27 BeadChip (Illumina, San Diego, CA), providing a beta value of methylation percentage at each site. Analysis was carried out using Bayesian Classification with Singular Value Decomposition (BCSVD) (1), a method developed for association studies with a large number of independent predictors, but a small sample size. BCSVD tests all probes simultaneously in a single test, avoiding the multiple testing problem. Stringent criteria were then applied to filter out invalid probes due to sequence dissimilarities between human probes and monkey DNA. This yielded 163 differentially methylated loci between PA and control infant visceral fat (BCSVD $P < 0.05$), most of which manifested greater methylation in PA samples than in controls. We examined pathways related to these 163 genes using the PANTHER (Protein ANalysis THrough Evolutionary Relationships) Classification System. Pathways that were identified included oxidative stress response, DNA replication, B cell activation, Wnt signaling, glutamine/glutamate conversion, and Notch signaling. Our results suggest that PA may modify DNA methylation of fetal visceral adipose, likely perturbing gene expression. This is the first evidence that excess androgen exposure in nonhuman primates may predispose to PCOS via epigenetic mechanisms in adipose, and warrants further investigations of different infant and adult tissues of PA monkeys to determine the evolution of epigenetic alterations that manifest the adult phenotype. Epigenetic alteration in PA monkeys may provide a novel avenue to understand PCOS in humans.

(1) Kwon S et al., BMC Proc 2009;3(Suppl 7):S9

Nothing to Disclose: NX, DHG, SK, XG, DHA, DAD, RA, MOG

OR39-3

Abnormal Expression of Genes in Inflammation, Lipid Metabolism, and Wnt Signaling in the Lean Women with Polycystic Ovary Syndrome (PCOS).

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Objective: 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 this study we determined the expression of genes related to lipid, inflammation, and adipogenesis pathways in the adipose tissue of lean women with PCOS and matched controls.

Methods: Subcutaneous lower abdominal fat tissues were obtained from 11 PCOS subjects and 12 body mass index (BMI)-matched controls (BMI: 21-26 kg/M²) and gene expression profiling was performed using Affymetrix Human Genome U133 arrays. Differentially expressed gene function was classified by gene ontology. Microarray results for selected genes were confirmed by quantitative real-time PCR (q-RTPCR).

Results: Microarray analysis identified 96 genes whose expression was altered ≥ 2.0 -fold in lean PCOS adipose tissues vs. controls. Inflammatory response genes (cytokines IL6 and IL8; chemokines CCL2 [MCP-1], CCL3, CCL4, and CXCL2; and SOCS3) were significantly down-regulated in lean PCOS adipose tissue. Decreases in the expression of IL-6 (12.3-fold), CXCL2 (15.9-fold), and SOCS3 (22.6-fold) were confirmed by q-RTPCR. Lipid metabolism genes associated with insulin resistance (DHRS9, UCLH1, FADS1 and LIPE) were significantly upregulated in PCOS adipose tissue; q-RTPCR confirmed the increases in DHRS9 (2.6-fold), UCLH1 (2.6-fold), and FADS1 (2.8-fold) expression. Genes related to Wnt signaling known to affect adipogenesis and/or insulin resistance (DKK2, JUN, and FOSB) were also differentially expressed; q-RTPCR confirmed changes in DKK2 (1.9-fold increase), JUN (4.1-fold decrease) and FOSB (60-fold decrease) expression.

Conclusions: Select genes involved in inflammation, lipid metabolism, and Wnt signaling, are differentially expressed in lean PCOS adipose tissue. These data suggest that adipose tissue dysfunction and abnormal gene expression, regardless of the degree of obesity, may contribute to the metabolic abnormalities observed in PCOS women.

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Disclosures: CW: Consultant, Clarus.

Nothing to Disclose: GC, MP, JX, Y-HC, J-ML, RA

OR39-4

Effect of Simvastatin on Human Theca-Interstitial Cells Proliferation and Apoptosis.

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Objectives: Polycystic ovary syndrome (PCOS) is associated with ovarian enlargement and prominent theca-interstitial hyperplasia. Our recent clinical studies have demonstrated that simvastatin, an inhibitor of the rate-limiting step of the mevalonate pathway, improves several aspects of PCOS including reduction of serum testosterone and decrease of ovarian volume. This study was designed to determine whether simvastatin directly affects growth of ovarian theca-interstitial cells and whether the effects of simvastatin are related to a reduced supply of products of the mevalonate pathway: cholesterol, farnesyl pyrophosphate (FPP) and/or geranylgeranyl pyrophosphate (GGPP).
Methods: Human theca-interstitial cells isolated from ovaries of PCOS (N=4) and non-PCOS patients (N=4) were cultured with 10% FBS until 60-70% confluency and subsequently incubated for 48h in serum-free medium without additives (control), with simvastatin (10-30 μ M), FPP (30 μ M), GGPP (30 μ M), and/or the cell- and mitochondrion-permeable cholesterol (22-hydroxycholesterol; 10 μ g/ml). Proliferation was evaluated by DNA synthesis assay (radiolabelled thymidine incorporation) and quantification of viable cells using MTS assay. Apoptosis was evaluated by determination of caspases 3/7 activity expressed per number of viable cells. Each treatment was performed in at least 6 replicates.
Results: Simvastatin induced an inhibition of DNA synthesis in all experiments on cells from control and PCOS subjects by 73 \pm 13.1% (mean \pm SD) for simvastatin at 10 μ M (P<0.001) and by 78 \pm 16.4% (mean \pm SD) for simvastatin at 30 μ M (P<0.001). This effect was associated with corresponding reduction of the number of viable cells. Addition of 22-hydroxycholesterol did not reverse this effect. Simvastatin induced activity of executioner caspases 3/7 by up to a 3-fold increase (P<0.0001). These apoptotic effects were reversed in the presence of intermediate products of the mevalonate pathway (and substrates of isoprenylation): FPP and GGPP.
Conclusions: Simvastatin inhibits growth of theca-interstitial cells irrespective of the availability of cholesterol. This effect is, at least in part, due to induction of apoptosis. Since FPP and GGPP abrogate effects of simvastatin, we propose that apoptotic actions of simvastatin are related to reduction of isoprenylation. The present findings provide a novel explanation for the beneficial effects of simvastatin on PCOS.

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Nothing to Disclose: AS, AC, PP, IR, DHW, JV, AJD

OR39-5

Metformin and Insulin Signalling in the Human Ovary.

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Hyperinsulinaemia in PCOS is now widely treated with metformin which increases insulin sensitivity, reduces hyperandrogenism and may increase ovulation rate. Polycystic ovarian cells are insulin resistant, leading to insufficient glucose uptake and metabolism. Our aim was to investigate metformin's effects on key components of the ovarian insulin-signalling pathway, from the insulin receptor (IR) to the insulin-sensitive glucose transporter GLUT4. Human granulosa-luteal cells (GLC) were cultured with metformin (10^{-7} M), insulin (10ng/ml) or metformin+insulin. To investigate IR involvement, cells were cultured with the above treatments and an anti-IR antibody (MAB1137). mRNA for IR substrates (IRS-1, -2) was detected via PCR analysis, and protein expression via antibodies against total IRS-1, IRS-2, phospho-Akt and pan-Akt. To investigate GLUT4 expression KGN granulosa cells were cultured with metformin for 24h, serum-starved for 3h and exposed to insulin for 30min. The protein lysates were fractionated into cytoplasmic and membrane-enriched fractions for GLUT4 immunoblotting. IRS-1 mRNA was expressed with all treatments. In contrast IRS-2 mRNA transcription was only switched on in the presence of metformin with/without insulin. IRS-1 protein was increased significantly over other treatments by combined metformin and insulin, but surprisingly reduced to basal levels with all treatments by the IR (blocking) antibody. Similarly MAB1137 practically abolished the treatment-induced increase in IRS-2 protein, suggesting that IRS-2 had a greater IR dependence than IRS-1. As expected the addition of insulin increased the ratio of phosphorylated to pan Akt expression, though this was unaltered by the addition of metformin. Immunoblotting for GLUT4 showed a heavily glycosylated form which increased in the cytosol in the presence of metformin compared to control. Exposure to insulin increased the expression of glycosylated GLUT4 even further in both cytosol and membrane fractions, and interestingly when metformin was added to insulin, glycosylated GLUT4 in the membrane fraction increased even further. This implies that metformin may increase glucose uptake and metabolism in these cells by several mechanisms: by increasing IRS-1 and switching on IRS-2: both processes apparently IR-dependent, and by enhancing insulin-mediated GLUT4 trafficking to the membrane of ovarian cells. These effects may facilitate follicular growth.

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Nothing to Disclose: SR, LP, SJB, SAW, HDM

OR39-6

A Mouse Model of Polycystic Ovary Syndrome Due to Enhanced Pituitary Insulin Signaling.

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Polycystic ovary syndrome (PCOS) is the most common reproductive disorder in women. To study the consequences of obesity in the reproductive axis, we developed a mouse model of diet induced obesity (DIO) in a mixed background strain of female mice that displays a significantly impaired reproductive phenotype compared to lean littermates. DIO mice fed a high fat diet for 12 weeks starting at 8 weeks of age display fasting hyperglycemia, hyperinsulinemia, and hyperleptinemia. DIO females show an impaired reproductive phenotype compared to lean littermates, as DIO females have fewer pregnancies, fewer ovulations, 5.4-fold fewer corpora lutea, and spend significantly less time in the estrus stage of the estrous cycle. Similar to women with PCOS, female DIO mice display elevated basal serum luteinizing hormone (LH) and testosterone (T) levels. LH levels in DIO female mice were found to be 1.5-fold higher and T levels were 2-fold higher in DIO mice over lean females. We have previously shown that WT DIO female mice have an elevated response to acute gonadotropin-releasing hormone (GnRH) stimulation due to chronically elevated insulin signaling in the pituitary gonadotroph. This 2-fold increased response to GnRH in DIO mice indicates that on an obese, hyperinsulinemic background, the pituitary is more sensitive to GnRH. To explore an acute role for insulin signaling on pituitary LH secretion, mice were treated with 1.5Units/Kg insulin for 40 minutes. Serum LH was increased in both lean (1.5-fold) and DIO mice (1.3-fold). In contrast, mice with a pituitary-specific knockout of the insulin receptor had no significant increase in LH after insulin stimulation. These results indicate a direct role for insulin signaling at the level of the pituitary. Although a direct affect of insulin at the level of the ovarian theca cell cannot be excluded, the increased serum LH levels in the DIO mice were associated with elevated T levels, suggesting a role for elevated neuroendocrine activity in the hyperandrogenism of our model of obesity and infertility.

Nothing to Disclose: KJB, SWW, RSM, SR, FEW, AW

OR40-1

G_sα Deficiency in the Paraventricular Nucleus of the Hypothalamus Contributes to the Parent-of-Origin Effect of G_sα Mutations on Energy Metabolism.

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The ubiquitously expressed G protein α -subunit G_sα couples hormone and other receptors to the generation of intracellular cAMP. Heterozygous G_sα mutations lead to obesity in Albright hereditary osteodystrophy patients and mice, but only when the mutation is on the maternal allele due to genomic imprinting with preferential expression of G_sα from the maternal allele in a small number of tissues (1). We recently showed that this parent-of-origin effect on energy balance (and glucose metabolism) is due to imprinting within the central nervous system (CNS) and provided evidence for G_sα imprinting in the paraventricular nucleus of the hypothalamus (PVN) (2). In addition we showed that CNS-specific G_sα deficiency leads to impaired stimulation of energy expenditure by a central melanocortin (MC4R) receptor agonist (MTII) with no effect on food intake (2). We have now derived mice with G_sα gene deletion in the ventral medial hypothalamus (VMH) or PVN by crossing G_sα-floxed mice with Sf1-cre or Sim1-cre transgenic mice, respectively. VMH-specific G_sα deletion on either the maternal or paternal allele had no effect on body weight when mice were maintained on either regular or high fat diet (HFD). In contrast, male mice with PVN-specific G_sα deletion on the maternal allele (mPVN-GsKO) gained significantly greater body weight and fat mass on both regular and HFD, which was associated with reduced energy expenditure and no change in food intake or locomotor activity. Consistent with our prior observations in mice with G_sα deficiency throughout the CNS, the ability of MTII to acutely inhibit food intake was unaffected in mPVN-GsKO mice. In addition, mPVN-GsKO mice became glucose intolerant and insulin resistant. PVN-specific G_sα deletion in the paternal allele (pPVN-GsKO) had no effect on energy balance or glucose metabolism. Female mPVN-GsKO mice only showed a mild weight increase on HFD with no effect on glucose metabolism. G_sα expression level in PVN was markedly reduced to 31% of controls in mPVN-GsKO mice while only reduced to 76% of controls in pPVN-GsKO mice, confirming that G_sα is imprinted in PVN. These results show that G_sα imprinting in PVN plays a role in the regulation of energy expenditure and glucose metabolism (particularly in males). Central melanocortin MC4R receptor regulation of energy homeostasis may involve both G_sα-dependent pathways which stimulate energy expenditure and G_sα-independent pathways which inhibit food intake.

(1) Weinstein LS et al., *Int J Obesity (Lond)* 2010; 34:6.

(2) Chen M et al., *Cell Metab* 2009; 9:548.

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Nothing to Disclose: NMN, MC, EM, JW, AK, LSW

OR40-2

A DNA-Hypermethylation Polymorphism in the POMC Gene Is Associated with Childhood Obesity and Affects a P300 Binding Site.

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Mutations in the POMC and MC4R gene, that are embedded in the leptin-melanocortin signalling cascade of central body weight regulation, lead to severe early onset obesity. We tested the hypothesis, if in addition to classical genetic defects also epigenetic alterations of the POMC gene locus might be associated with human obesity. We investigated the DNA methylation pattern of the POMC CpG islands by bisulfite-sequencing. Functional studies of P300 binding were performed with ChIP analysis and qPCR. First we described the methylation pattern of the POMC gene locus which revealed a distinct methylation pattern at the 5' POMC CpG island in human peripheral blood cells (PBC), which was conserved in mice PBC. In the 3' CpG island, we obtained in human PBC a sharp boundary of DNA methylation with a hypermethylated intron 2 and a completely hypomethylated exon 3. Both DNA methylation patterns were reproducible in microdissected β -MSH positive human postmortem brain samples and PBC-DNA extracted from newborn screening cards, indicating a stable pattern, which is present directly after birth. We further analyzed the DNA methylation in PBC of 100 obese childhood patients and 54 normal weight individuals with two independent bisulfite-based methods. We found a significant hypermethylation of 10 CpG positions at the intron2-exon3 intersection in obese patients ($p < 0,001$). Moreover at the first CpG position within the exon3, which is significantly hypermethylated in the obese cohort, we confirmed a binding site of the histone acetyltransferase P300 by ChIP analysis and reveal a reduced P300 binding capacity in hypermethylated, obese patients in qPCR analysis. Therefore we describe for the first time a DNA-hypermethylation polymorphism that is significantly associated with childhood obesity. The hypermethylation polymorphism is located within a P300 binding region of the 3' POMC CpG island suggesting an effect on chromatin formation and altered POMC gene expression.

Nothing to Disclose: PK, MM, BH, AG, HK

OR40-3

Melanocortin 4 Receptor Signaling Is Required for Weight Loss and Thermogenesis after Roux-En-Y Gastric Bypass in the Mouse.

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Recent studies suggest that Roux-en-Y gastric bypass (RYGB) induces weight loss by altering physiologic mechanisms regulating energy balance. We and others have shown that RYGB increases total and resting energy expenditure (EE). This increase occurs only in the fed state, suggesting that RYGB augments diet (feeding)-related thermogenesis. Brown adipose tissue (BAT) activation after feeding is a critical thermoregulatory mechanism in rodents, and signaling through melanocortin receptor 4 (MC4R) is a key component of this thermogenic pathway. We sought to determine (1) whether RYGB in rodents leads to BAT activation and (2) whether signaling through MC4R is essential for the weight loss and thermogenic effects of RYGB. *Methods.* We compared the effects of RYGB in C57BL/6 mice with diet-induced obesity (RYGB-DIO; initial body weight 50 ± 1 g) with those of a sham operation (SO-DIO). We then compared the effects of RYGB and SO in wild-type mice and in mice genetically deficient in MC4R (MC4RKO). *Results.* One year after RYGB, RYGB-DIO mice weighed 35% less than SO-DIO animals (52 ± 3 vs. 34 ± 4 g; $p < 0.001$) and had a >75% reduction in total fat mass. Total oxygen consumption in RYGB-DIO was 21% greater than in SO-DIO (1196 ± 62 vs. 983 ± 37 ml/h/kg(0.75); $p < 0.05$). RYGB-DIO showed distinctive changes in BAT histomorphology, including a significant reduction in lipid droplets and enhanced vascularity, as well as a 1.8-fold increase in BAT UCP1 gene expression and a substantial increase in intracellular phospho-p38 MAPK, PGC1 α and UCP1. These animals also demonstrated increased ¹⁸F-FDG uptake into BAT by PET. In contrast, MC4RKO mice exhibited substantially less RYGB-induced weight loss. One year after RYGB, MC4RKO mice weighed 16% less than SO MC4RKO mice (55 ± 6 vs. 46 ± 11 g; $p < 0.001$). RYGB in MC4RKO mice was associated with only a minimal decrease in total fat mass. *Conclusions.* RYGB increases EE in DIO mice and leads to metabolic activation of BAT, which appears to be an important mechanism by which EE is increased after surgery in this model. Signaling through MC4R is essential for RYGB-induced weight loss and thermogenesis. The activation of BAT and exploitation of MC4R signaling suggest that RYGB acts by influencing the central mechanisms of energy balance. These observations underscore the utility of GI manipulation as a means of studying central metabolic regulation, as well as identifying and validating targets for pharmacological therapy of obesity.

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Nothing to Disclose: NS, XBZ, A-LB

OR40-4

Central BDNF Differentially Regulates Energy Expenditure in Male and Female Rats.

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Experimental evidence implicates brain-derived neurotrophic factor (BDNF) in the regulation of energy balance. Mice with decreased expression of BDNF, or of its high-affinity receptor TrkB, are hyperphagic and obese; interestingly, this phenotype is more pronounced in females. BDNF and estrogen receptor α are highly expressed and partially co-localized in the ventromedial hypothalamus, raising the possibility that BDNF gene expression is regulated in an estrogen-sensitive manner. We first asked whether estradiol (E2) modulates BDNF expression at mediobasal hypothalamus by comparing BDNF mRNA levels in females across different phases of the estrous cycle as well as in ovariectomized (OVX) females vs. OVX females with E2 replacement. BDNF mRNA levels were similar across the estrous cycle and did not correlate with circulating E2 levels. However, OVX females in which E2 was replaced to supra-physiological levels had significantly more BDNF mRNA than OVX females.

BDNF has been proposed as a downstream effector of the melanocortinergic system, and the influence of BDNF to cause weight loss depends on the activity level of the melanocortin agonist-producing proopiomelanocortin (POMC) neurons which receive energy homeostatic information from circulating peripheral signals such as leptin. Leptin affects energy balance in a sexually dimorphic way in rodents with females being more sensitive to leptin than males. We previously reported that mice lacking leptin receptors selectively in POMC neurons increased adiposity through sex distinct physiological mechanisms. Specifically, lack of leptin receptors in POMC neurons led to conservation of energy expenditure in females but not in males. We hypothesized that, since BDNF is a direct downstream effector of the melanocortinergic leptin-POMC signaling, BDNF would differentially regulate energy expenditure between males and females. In the current study, we administered 0.1 $\mu\text{g}/\mu\text{l}$ BDNF or its vehicle saline into the third ventricle of age-matched male and female rats and measured energy expenditure using indirect calorimetry during the first 24 hrs following the injection. BDNF-injected female rats, but not male rats, significantly increased oxygen consumption during both light and dark phases comparing to vehicle-injected same sex controls, indicating increased energy expenditure. These findings are novel in that they reveal sex-specific underlying physiology used to achieve weight loss following central BDNF in rats.

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Nothing to Disclose: HS, JGB, SCW, RJS

OR40-5

Ghrelin Mimics Fasting in Biasing Food Appeal towards High-Calorie Foods.

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Introduction: Ghrelin, a circulating orexigenic stomach-derived hormone, declines after meals and may influence eating behaviour in part through stimulation of dopaminergic brain reward systems. Fasting biases activation in reward systems and food appeal towards high-calorie foods in humans (1). We therefore investigated whether ghrelin mimics this effect.

Methods: After an initial practice visit, 18 healthy, non-obese adults (mean \pm SEM age 23 ± 1 y, BMI 24.0 ± 0.7 kg/m², 13 male) fasted overnight before 3 randomized, double-blind study visits on separate days at least a week apart. Women were scanned in the follicular phase of the menstrual cycle. Subjects remained fasted or ate a fixed breakfast (730kCal), and 55mins later received a subcutaneous injection of saline or octanoylated ghrelin (3.6nmol/kg). 40mins later subjects viewed high-calorie or low-calorie food pictures (n=60 each) within a functional MRI protocol over 20mins, and rated their appeal (score 1-5).

Results: The appeal of low-calorie foods did not differ significantly between visits (fed-saline: 3.76 ± 0.15 ; fasted-saline: 3.91 ± 0.11 , fed-ghrelin: 3.85 ± 0.13). By contrast, high-calorie foods were more appealing on the fasted-saline (4.28 ± 0.11 , $P=0.006$) and fed-ghrelin (4.15 ± 0.12 , $P=0.02$) visits compared to the fed-saline visit (3.95 ± 0.12). High-calorie foods were of similar appeal to low-calorie foods on the fed-saline visit ($P=0.22$). By contrast, high-calorie foods, especially chocolate and non-chocolate sweet foods, were of greater appeal than low-calorie foods on both fasted-saline ($P=0.006$) and fed-ghrelin ($P=0.025$) visits.

Conclusion: Ghrelin mimicked fasting in biasing food appeal towards high-calorie rewarding foods. Changes in food hedonics when missing or eating meals may be explained by associated changes in circulating ghrelin as part of a homeostatic process. Functional MRI data analysis will enable identification of the brain reward systems through which ghrelin achieves this effect.

(1) Goldstone AP et al., Eur J Neuroscience 2009; 30:1625-35

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Nothing to Disclose: APG, CGPdH, SS, GD, SSD, TW, DA, GF, SRB, JDB

OR40-6

The Effects of a Kisspeptin-Cell-Specific Deletion of the Leptin Receptor on the Hypothalamic-Pituitary-Gonadal (HPG) Axis of Female Mice.

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Nutritional status is an extremely important determinant of reproductive development and fertility. Although the mechanisms mediating metabolic influences on fertility remain unclear, a strong association between metabolic and reproductive disorders has been demonstrated. In mammals, kisspeptins and their receptor, GPR54, are essential for the initiation and maintenance of fertility. Recent studies suggest that the hypothalamic kisspeptin system may also participate in conveying metabolic signals to gonadotropin-releasing hormone (GnRH) neurons. For example, conditions of negative energy balance reduce hypothalamic expression of the kisspeptin gene, and central administration of kisspeptin effectively rescues suppression of gonadotropin release by these metabolic challenges. A leptin-kisspeptin pathway has been suggested as an important mediator of suppressive effects of negative energy balance on reproduction. Leptin receptors are expressed in kisspeptin neurons of the arcuate nucleus of the hypothalamus (ARC) and leptin modulates kisspeptin expression in this key center for control of metabolism and reproduction. Here we selectively eliminated the leptin receptor (Lepr) in kisspeptin cells (Kisspeptin-CRE+/Leprflx/flx) in mice and analyzed the reproductive axis of female animals. There were no differences in body weight, pubertal onset, or estrous cyclicity between females of both genotypes. However, a modest but significant decrease in basal serum luteinizing hormone (LH) levels was observed in Kisspeptin-CRE+/Leprflx/flx females compared to WT (Kisspeptin-CRE-/Leprflx/flx) littermates (0.36 ± 0.08 ng/ml Cre+ vs. 0.69 ± 0.10 ng/ml Cre-). To investigate the effects of negative energy balance on the HPG axis, ovariectomized WT and Kisspeptin-CRE+/Leprflx/flx mice were fasted for 48hr. While fasting significantly suppressed LH levels, no difference was observed in the magnitude of this effect between WT and Kisspeptin-CRE+/Leprflx/flx females. Consistent with this, leptin receptor signaling was not activated in kisspeptin neurons after leptin administration in fasting females. Our results suggest that while Lepr signaling in kisspeptin cells may contribute to the maintenance of normal basal gonadotropin secretions, it does not play an essential role in attaining or maintaining fertility, or in the modulation of gonadotropin secretion under conditions of acutely altered energy balance.

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Nothing to Disclose: CM, MA-M, JEL, SCS, UB

OR41-1

The Set Point of the Thyroid Axis Is Determined by a Balance between CoActivators and CoRepressors *In Vivo*.

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It is well known that mice lacking steroid receptor coactivator 1 (SRC-1), a nuclear receptor coactivator, manifest resistance to thyroid hormone (RTH) with elevated peripheral T4 and T3 levels and normal TSH levels. Consistent with this, SRC-1 knockout (KO) mice cannot suppress TSH subunit gene expression in the face of increasing thyroid hormone (TH) levels implying, surprisingly, that SRC-1 is required for negative regulation by the thyroid hormone receptor (TR). We hypothesized that the inability of SRC-1 KO mice to repress gene transcription through the TR originates, paradoxically, from unbalanced nuclear corepressor action. To test this, we crossed SRC-1 KO mice with mice that globally express a modified nuclear receptor corepressor (NCoR), which cannot interact with the TR (NCoRΔID mice). Interestingly, NCoRΔID mice have low peripheral thyroid hormone levels with normal TSH levels suggesting that the inability of NCoR to bind to the TR allows for enhanced sensitivity to TH. To prevent maternal TH levels from influencing the developing hypothalamic-pituitary-thyroid (HPT) axis, all SRC-1 KO/NCoRΔID mice were generated by crossing mice heterozygote for both SRC-1 and NCoRΔID that have total T4 (TT4) levels analogous to WT mice. SRC-1 KO/NCoRΔID mice were born at the expected Mendelian frequency and developed normally. To evaluate the HPT axis, TT4 levels were measured at 9 weeks of age in male mice. As expected SRC-1 KO mice had elevated TT4 levels (5.81 +/- 0.34 μg/dL) compared to WT mice (2.49 +/- 0.11 μg/dL), whereas NCoRΔID mice had low TT4 levels (1.55 +/- 0.19 μg/dL). Remarkably SRC-1 KO/NCoRΔID mice had normal TT4 levels (2.91 +/- 0.38 μg/dL) compared to WT mice and SRC-1 KO mice with one NCoRΔID allele showed a partial correction in their TT4 levels (4.28 +/- 0.41 μg/dL). Similar findings were also seen in female mice. Thus, decreasing available NCoR allows for enhanced sensitivity to TH in SRC-1 KO mice and allows circulating T4 levels to normalize in this model of RTH. Therefore, a balance between SRC-1 and NCoR determines the set point of the HPT axis *in vivo*. Further work is required to determine whether these coregulators are mediating their actions on the HPT axis at the level of the TRH neuron in the hypothalamus or the thyrotroph in the pituitary.

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Nothing to Disclose: KRV, IA, JCH, KAH, ANH

OR41-2

Novel Expression of Type 2 Deiodinase along the Blood-Brain Barrier during Bacterial Endotoxin Challenge.

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Type 2 deiodinase (D2) is the major enzyme in the brain that converts thyroxine (T4) to the more biologically active form, tri-iodothyronine (T3). D2 is expressed by astroglial cells in several brain areas and by specialized ependymal cells, tanycytes, lining the infralateral walls and floor of the third ventricle. We previously reported that bacterial lipopolysaccharide (LPS) administration, a well-accepted experimental approach to simulate infection, robustly increases D2 expression in tanycytes, resulting in local T3 generation that may contribute to the nonthyroidal illness syndrome by inhibiting the hypothalamic-pituitary-thyroid (HPT) axis. We now report that in addition to upregulating D2 in tanycytes, LPS treatment induces a marked transcriptional increase in D2 activity in specific cell types of the brain that normally express little or no D2. Within 9h of LPS administration, D2 mRNA was highly expressed in the leptomeninges, (pia-arachnoid) of rats to levels almost as high as in tanycytes. D2 enzymatic activity was also dramatically increased in leptomeninges removed from the cortex and basal regions of the brain in LPS-treated rats, but not in controls. Fluorescent *in situ* hybridization revealed that D2 mRNA is transported to the cellular processes of pial and arachnoid fibroblasts, including pial processes that coat large blood vessels entering the brain. In addition to leptomeningeal cells, some of the LPS-treated animals had a similarly strong D2 expression in pericytes found along several brain capillaries. As in meningeal cells, D2 mRNA was also transported into the pericyte processes surrounding the microvessel. We conclude that LPS treatment causes widespread D2 expression at strategic loci along the blood brain barrier. This previously unrecognized phenomenon raises the possibility that meningeal cells and/or pericytes increase T3 levels in brain tissue during endotoxemia, which may aid in adaptation to the adverse effects of infection. Furthermore, T3 generated by meningeal cells and/or pericytes may also contribute to the nonthyroidal illness syndrome by inhibiting the HPT axis, which was previously thought to be an exclusive function of tanycyte-derived T3.

Sources of Research Support: Grant NIHDK-37021.

Nothing to Disclose: GW, PSS, SSN, PRL, JH, RML

OR41-3

Impairment of Type 3 Deiodinase (D3) Contributes to the Hypersensitivity to Thyroid Hormone (TH) of TR α Knock out (TR α KO) Mice.

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TR α KO mice are hypersensitive to TH. We determined whether the basis for TH hypersensitivity is due to altered TH metabolism. D3 metabolically inactivates T4 and T3. Accumulating evidence indicates that increased expression of D3 mRNA during hyperthyroidism contributes to TH homeostasis protecting the tissues from an excess of TH. However, the mechanism of this regulation remains unknown. In this study we investigated whether TR α is involved in D3 regulation and its contribution to the phenotype observed in TR α KO mice.

D3 mRNA expression in mouse embryonic fibroblasts (MEFs) devoid of TR α or TR β was compared to wild type mice (WT), after treatment with T3. In addition, D3 mRNA expression in GH3 cells after overexpression of TR α was determined. Furthermore we examined D3 expression in vivo in brain and pituitary in TR α KO mice. T3 treatment increased D3 transcript level in MEFs obtained from WT or TR β KO mice, but not in TR α KO mice (2nM of T3 induced 2.29 ± 0.10 , 2.29 ± 0.06 and 1.01 ± 0.03 fold change respectively). T3 stimulated D3 promoter activity in GH3 cells only after cotransfection with human TR α , but not human TR β (2.14 ± 0.024 and 0.67 ± 0.003 fold increase, respectively). Moreover, treatment of GH3 cells with increasing doses of T3 significantly increased D3 mRNA when these cells were transfected with human TR α , but not with an empty vector. Together, these results indicate that the transcriptional regulation of D3 by THs is mediated through TR α . TR α KO mice display impairment in the transcriptional regulation of D3 by THs in both brain (0.86 ± 0.039 vs 2.18 ± 0.21 of WT), and pituitary (0.77 ± 0.42 vs 3.37 ± 1.13 of WT) and confirmed transcriptional regulation of D3 by THs is TR α dependent. The rate of THs clearance from the serum in TR α KO mice was compared to WT mice. TR α KO mice had significantly higher levels of T3 at 4, 8 and 16 hours after the last injection of L-T3. After administration of T4, TR α KO mice have significantly lower ratio rT3/T4 at 16 and 24 hours. These results demonstrate TR α KO mice have decreased clearance of THs as a consequence of D3 deregulation. In summary, we demonstrated that the up regulation of D3 transcript levels by T3 is TR α isoform-dependent. Therefore, the absence of TR α results in decreased clearance of THs and TH hypersensitivity.

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Nothing to Disclose: OB-M, X-HL, MA-S, CDC, SR, REW

OR41-4

Fundamentally Different Roles for Thyroid Hormone Receptor Isoforms in Thyroid Hormone Pituitary Feedback.

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Thyroid hormone (TH) stimulates and inhibits gene transcription during development and during changes in the environment and nutritional state. TH binds to three distinct isoforms derived from two different genes: TR- α , TR- β 1 and TR- β 2. The hypothalamic-pituitary-thyroid (HPT) axis is one of the most important targets for TH negative feedback regulation, and a crucial role for TR- β negative regulation has been well established using several TR- β knockout/knockin mouse models. In contrast, the role of TR- α in the HPT axis remains unclear since TR- α knockout mice have either no change or a mild reduction in serum TH levels. Unfortunately, most of the studies of TSH subunit gene regulation have used heterologous cell lines, and the physiological relevance of these studies remains uncertain. In this study, we utilized a novel subclone of the T α T1 mouse thyrotroph cell line (T α T1.1), which expresses TR- α , TR- β 1 and TR- β 2, both TSH subunits and TRH receptor 1 (TRH-R1) and displays physiologically relevant regulation by thyroid hormone and TRH. TH reduced TSH- β and TRH-R1 mRNA levels in this cell line but had no effect on TSH- α mRNA levels. Using chromatin immunoprecipitation (ChIP) assays, we identified areas of interaction between TR- α and TR- β and the proximal 5' flanking region of the TSH- β subunit gene; interestingly, the same region of the TSH- α subunit gene did not display TR binding. We next eliminated TR protein expression in T α T1.1 with specific adenoviral shRNAs. We found that TH reduced TSH- β and TRH-R1 mRNA levels after treatment with shRNA against TR- β but TSH- β and TRH-R1 mRNA levels were unchanged after treatment with shRNA against TR- α . ChIP assays confirm comparable binding of both TR- α and TR- β 1/2 to the proximal promoter of the TSH- β but not TSH- α gene, and loss of specific isoform binding in T α T1.1 cells after treatment with a specific shRNA. In summary, our data show that both TR- α and TR- β 1/2 bind to the proximal promoter of TSH- β but not TSH- α gene in T α T1.1 cells and that TH negative regulation of TSH- β and TRH-R1 genes is dependent on TR- β but not by TR- α . In conclusion, our data support a DNA-binding dependent mechanism for negative regulation of the TSH- β gene by TR- β . Given that both TR- α and TR- β isoforms bind to the TSH- β promoter our data suggest, for the first time, a fundamental difference in mechanism of action of these isoforms at least with respect to negative TH regulation.

Sources of Research Support: DK 49126/53036.

Nothing to Disclose: MIC, SM, TMO-C, FEW

OR41-5

Pitx2 Deletion in Thyrotrophs Using a New Specific Cre Recombinase Causes Growth Deficiency.

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Pitx2 is a homeodomain transcription factor required in a dose dependent manner for the development of multiple organs. Homozygotes for a *Pitx2* null allele (*Pitx2*^{-/-}) have severe pituitary hypoplasia, while mice with reduced function alleles (*Pitx2*^{neo/neo}) exhibit modest hypoplasia, reduction in the *Pou1f1* lineage, and absence of the gonadotroph lineage (1,2). PITX2 is expressed in Rathke's pouch and becomes enriched in adult gonadotrophs and thyrotrophs (3). Gonadotroph specific deletion of *Pitx2* has no effect on puberty or fertility, possibly due to an overlapping role with the related *Pitx1* gene (4).

We hypothesize that *Pitx2* has a role in thyrotroph function because: 1) most thyrotroph cells express *Pitx2*; 2) *Pitx2*^{-/-} mice have decreased expression of *Tshb* and transcription factors required for thyrotroph cell differentiation and/or function: *Prop1*, *Pou1f1*, *Gata2*; 3) *Pitx2* transactivates *Tshb* and *Pou1f1* promoters in cell culture (5). To test this hypothesis we developed thyrotroph-specific cre transgenic mice with a recombineered *Tshb* BAC that ablates floxed genes in differentiated pituitary thyrotrophs, *Tg(Tshb-cre)*. We used a cre reporter strain to select the transgenic line with the most robust thyrotroph specificity, most precise developmental regulation, and least ectopic expression. We crossed the best *Tg(Tshb-Cre)* strain to *Pitx2*^{+/-} mice. *Pitx2*^{+/-}; *Tg(Tshb-Cre)* progeny were mated to *Pitx2*^{flox/flox} mice to generate thyrotroph-specific *Pitx2* deficient offspring, *Pitx2*^{flox/-}; *Tg(Tshb-Cre)*. Double immunohistochemistry confirmed that *Pitx2* is deleted in the majority of thyrotroph cells. *Pitx2*^{flox/-}; *Tg(Tshb-Cre)* mice have growth insufficiency evident by 3-4 wks, and at 8 wks males and females have 11 and 20% mean weight reduction, respectively. This is consistent with expectations for a moderate TSH deficiency. The pituitary glands have fewer thyrotroph cells, suggesting that *Pitx2* may be required for thyrotrope maintenance. The thyroid glands are disproportionately smaller, although preliminary measurements indicate that circulating T3 and T4 levels are in the normal range at 8 wks. We are comparing the levels of *Tshb* transcripts, and circulating T3-T4 at 4 wks, at the time of growth failure onset. In conclusion, *Pitx2* appears to have an important role in regulation of the pituitary- thyroid axis and growth, in addition to its requirement for expansion of Rathke's pouch and developing the gonadotrope lineage.

(1) Gage PG et al., Development 1999; 126:4643

(2) Suh H et al., Development 2002;129:329

(3) Charles MA*, Suh H* et al., Mol Endocrinol 2005; 19:1893

(4) Charles MA*, Mortensen AH*, Potok MA* et al., Genesis 2008; 46:507

(5) Tremblay JJ et al. 2000; 71:277

*co-first

Sources of Research Support: NIH HD034283.

Nothing to Disclose: FC, MLB, DFG, JMK, ECR, SAC

OR41-6

GATA2 Mediates the Thyrotropin-Releasing Hormone-Induced Transcriptional Activation of the Thyrotropin β Gene.

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Thyrotropin-releasing hormone (TRH) generated from the hypothalamus activates not only the secretion of thyrotropin (TSH) but also the transcription of the its β subunit (TSH β). However, the mechanism of TRH signaling in the TSH β gene has been elusive. It was reported that Pit1 and GATA2 are the major determinants of the thyrotroph development in pituitary and that the main activator of TSH β gene is GATA2 while Pit1 relieves the inhibition of GATA2 function by the suppressor region (SR), which locates downstream to GATA-responsive element (GATA-RE). However, previous studies have been designed without consideration of GATA2. Here, we investigated role of GATA2 in the TRH signaling in TSH β expression using kidney-derived CV1 cells transfected with the expression plasmid for TRH receptor (TRH-R). We found that the TSH β promoter that lacks SR was activated by the GATA2 without Pit1 and this activity was potentiated by TRH treatment. GATA2-dependent promoter activities of the glycoprotein hormone α subunit and endothelin-1 promoters were also enhanced by TRH/TRH-R. Phorbol ester, TPA, but not protein kinase A activator, forskolin, stimulated the GATA2/Pit1-dependent promoter activity, suggesting the involvement of protein kinase C in TRH signaling. The deletion analysis of GATA2 indicated the critical role of its zinc finger domain in the TRH-induced activation. Gel-shift study revealed that TRH as well as TPA enhance the GATA2 binding with GATA-RE. We found that the transcriptional inhibition by thyroid hormone (T3) was dominant over the activation by TRH/TRH-R. However, unliganded T3 receptor or the reported AP-1-like sequence overlapping with putative negative regulatory element (NRE) was not required for the TRH-induced transactivation of the TSH β promoter. Studies using somato-mammotroph-derived GH3 transfected with GATA2 revealed that, unlike the transactivation of the prolactin gene, the effect of TRH on the TSH β promoter is independent of the mitogen-activated protein kinase signaling pathway. Finally, we found that GATA2 is the limiting factor for TRH-dependent transcription of the TSH β gene in thyrotroph cell line, T α T1. These results indicate that GATA2 plays a pivotal role in the TRH signaling for the transactivation of the TSH β gene.

Sources of Research Support: In part by a Health Sciences Research Grant to H.N. and a Grant-in Aid for Scientific Research to S.S. and H.N. from the Ministry of Education, Culture, Sports, Science and Technology in Japan.

Nothing to Disclose: KO, SS, AM, HI, HM, SS, HM, HN

OR42-1

Testosterone with Dutasteride Improves Insulin Sensitivity in Young Obese Men.

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Introduction: Testosterone (T) administration to men has been postulated to improve insulin sensitivity (IS) but clinical trials have not shown a consistent pattern. We examined the effects of T administration to young, obese men with low to low normal free T levels and determined whether inhibiting metabolism of T to estradiol (E₂) or dihydrotestosterone (DHT) would change outcomes.

Methods: This was a 98 day prospective, randomized, double-blind, parallel group, placebo-controlled clinical trial.

Subjects: Men 24-51y of age, BMI $\geq 30\text{kg/m}^2$ and waist circumference $\geq 100\text{cm}$. 57 men were randomized to 1 of 4 treatment groups (Table 1). IS by a two step euglycemic hyperinsulinemic clamp and fat mass (FM) and fat-free mass (FFM) by DXA scan were performed at baseline and day 98. Glucose disposal rate (GDR) was used as a measure of IS (1).

Treatment groups with number of subjects

| Groups | Placebo | Testosterone | Anastrozole/T | Dutasteride/T |
|------------------|---------|--------------|---------------|---------------|
| n | 13 | 14 | 14 | 16 |
| GnRH antagonist | - | acyline | acyline | acyline |
| Testosterone | - | T gel | T gel | T gel |
| Enzyme inhibitor | - | - | anastrozole | dutasteride |

Results: IS: GDR for Step 1 of the clamp was significantly increased from baseline to day 98 in Dutasteride/T group (6.2 ± 0.73 to 7.5 ± 1.9 mg/kg FFM*min, $p < 0.05$). The percent change in Step 1 GDR from baseline to day 98 for the Dutasteride/T group ($33.1 \pm 14.7\%$) was significantly higher compared to the Placebo ($-4.2 \pm 3.6\%$, $P < 0.05$) and T group ($-7.6 \pm 5.2\%$, $P < 0.05$). The percent change in Step 2 GDR from baseline to day 98 for the Dutasteride/T group was significantly higher compared to the Anastrozole/T group (19.4 ± 9.3 vs $-6.8 \pm 7.6\%$, $P < 0.05$). **Body composition:** The Dutasteride/T group had a significant increase in FFM (63.5 ± 1.2 to $65.7 \pm 1.4\%$, $P < 0.05$) and decrease in FM (33.9 ± 1.3 to $31.7 \pm 1.5\%$, $P < 0.05$) similar to the T group (FFM: 63.2 ± 1.7 to $64.8 \pm 1.9\%$, $P < 0.05$ and FM: 34.1 ± 1.8 to $32.6 \pm 2.0\%$, $P < 0.05$). There was no significant change in FFM or FM in the Placebo or Anastrozole/T groups. **Correlative Data:** Day 98 E₂ but not DHT levels, were highly correlated with Step 2 GDR (ρ 0.43, $P = 0.03$), change in FFM (ρ 0.53, $P = 0.003$) and FM (ρ -0.47, $P = 0.01$).

Conclusions: Testosterone's effects of increasing GDR and FFM and decreasing FM in young, obese men are enhanced by dutasteride but not by anastrozole. These results suggest that the conversion of T to E₂ is crucial in the beneficial effects of T on metabolism and body composition. Testosterone plus dutasteride may become a novel treatment of insulin resistance and obesity.

(1) DeFronzo RA et al., Am J Physiol Endocrinol Metab 1979; 237:E214

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Nothing to Disclose: PSL, KLH

OR42-2

Continuous Improvement in Insulin Secretion Following Laparoscopic Ileal Interposition in Type 2 Diabetic Patients: A 3-Year Evaluation.

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Results of different bariatric surgeries in type 2 diabetic morbidly obese patients had shown to decrease fasting insulin concentration, insulin secretion rate and total insulin output in response to intravenous or oral glucose or mixed meals. On the other hand HOMA β , acute insulin response (AIR) and the insulinogenic index increased to a variable extent. Laparoscopic ileal interposition associated to sleeve gastrectomy (II-SG) had demonstrated to be a safe and effective operation for type 2 diabetic patients with BMI bellow 35 kg/m². The aim of this study was to evaluate the impact of this operation in insulin secretion and its sustainability of effect.

A total of 446 OGTT (3-hour oral glucose tolerance test) were evaluated. Patients were divided in 4 groups. Group I - preoperative; II - 1-12 months after the procedure; III - 13-24months; IV - 25-38 months. We used C-peptide and insulin area under the curve (AUC), and the relation of both with glucose-AUC, as the secretory indexes that reflected long-term adaptation. We used the insulinogenic index to assess the dynamic behavior of the β -cell. OGIS was used as a measurement of insulin sensitivity. HOMA IR and HOMA β were also calculated.

Results summary

| | Group 1 | Group 2 | Group 3 | Group 4 | Units |
|-----------------------------|--------------|-------------|--------------|--------------|---------------------------------------|
| N | 265 | 91 | 63 | 27 | |
| Time | Preoperative | 1-12 months | 13-24 months | 25-38 months | months |
| BMI | 30.2 | 22.8 | 23 | 23.8 | Kg/m ² |
| C-peptide AUC | 697 | 1175 | 1118 | 1299 | pmol.l ⁻¹ .180min |
| C-peptide AUC / glucose AUC | 0.016 | 0.037 | 0.035 | 0.040 | pmol.mmol ⁻¹ |
| Insulin AUC | 4293 | 4402 | 4051 | 5853 | pmol.l ⁻¹ .180min |
| Insulin AUC / glucose AUC | 0.104 | 0.139 | 0.129 | 0.189 | pmol.mmol ⁻¹ |
| Insulinogenic index | -0.8 | 0.23 | 0.26 | 0.29 | mg.mUi ⁻¹ |
| OGIS | 250 | 421 | 443 | 457 | ml.min ⁻¹ .m ⁻² |
| HOMA IR | 6.1 | 1.5 | 1.0 | 0.9 | % |
| HOMA β | 72 | 40 | 28 | 27 | % |

BMI - Body mass index; AUC - Area under the curve

Ileal interposition associated with sleeve gastrectomy showed a global and continuous improvement in β -cell function as demonstrated by the above mentioned indexes.

De Paula et al - 2009 - Prospective randomized controlled trial comparing two versions of laparoscopic ileal interposition associated with sleeve gastrectomy for patients with BMI 21-34 kg/m². Soard - nov 2009 - Article in press Doi: 10.1016/j.soard.2009.10.005

Nothing to Disclose: SV, AS, AH, ADP

OR42-3

Gender Dimorphic Relationships of Adiposity, Fitness, and Physical Activity with Indices of Skeletal Muscle and Hepatic Insulin Sensitivity in Healthy Older Subjects.

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Background: The age-related increases in total and visceral adiposity, and declines in physical fitness and activity are associated with insulin resistance. However, the effects of these factors on the two major sites of insulin action: liver and skeletal muscle are unclear.

Objective: We examined the relationships among adiposity, fitness, physical activity, and skeletal muscle and liver insulin sensitivity in 114 healthy older (65-82 yr) women (n = 46) and men (n = 68).

Methods: Adiposity was measured by body mass index (BMI), waist to hip ratio (WHR), skin-fold thickness, DEXA (total body fat), and abdominal MRI (total, subcutaneous and visceral fat); fitness, by treadmill (VO2 max) and fast-pace step-time (FSST); total muscle strength (STR) by isotonic testing; and physical activity by questionnaire (PASE). Surrogate indices of muscle (skeletal muscle insulin sensitivity, SMIS) and liver insulin resistance (Hepatic Insulin Resistance Index, HIR) were estimated from plasma insulin and glucose levels during OGTT.

Results: BMI, WHR, abdominal visceral fat (AVF), VO2 max, STR, and physical activity scores were lower, whereas total body fat (TBF, estimated by skin fold thickness or DEXA) and total and subcutaneous abdominal fat were higher in women compared with men. Fasting blood glucose (FBG), fasting insulin (FI), and 2-hr glucose values were higher (P <0.05) in men (FBG, Mean ± SD: 99 ± 9 vs. 95 ± 11; FI: 12.5 ± 8.1 vs. 9.8 ± 3.1; 2-hr PG: 153 ± 41 vs. 135 ± 43). SMIS and HIR values were not significantly different in men versus women. Bivariate analyses revealed that adiposity [BMI, WHR, TBF, and total abdominal fat percent (TAF)], VO2 max, and FSST were correlated with SMIS only in women (BMI: r = -0.27; WHR: r = -0.32; TBF: r = -0.37; TAF: r = -0.35; VO2 max: r = 0.39; and FSST: r = -0.30). In contrast, adiposity (BMI, WHR, TBF, TAF, and AVF) but not VO2 max or activity were related to HIR only in men (BMI: r = 0.32; WHR: r = 0.24; TBF: r = 0.25; TAF: r = 0.34; and VFT: r = 0.35). Stepwise forward regression analysis revealed that the strongest predictors of SMIS in women were TBF, STR, and FSST, accounting for 36.6 % of its variation. Similarly, the strongest predictors of HIR in men were TBF, AVF, and STR, accounting for 16.8 % of its variation.

Conclusions: These results suggest that there are gender dimorphic effects of adiposity, fitness and physical activity on skeletal muscle and hepatic insulin sensitivity in healthy older individuals.

Sources of Research Support: In part by grants from National Institute on Aging.

Nothing to Disclose: HWL, RM, KP, SMH, JDS, MRB

OR42-4

Partial Sleep Restriction Decreases Insulin Sensitivity in Type 1 Diabetes.

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Objective:

Sleep restriction results in decreased insulin sensitivity and glucose tolerance in healthy subjects. We hypothesized that sleep duration is also a determinant of insulin sensitivity in patients with type 1 diabetes.

Research design and methods:

We studied 7 patients (3 men, 4 women) with type 1 diabetes: mean age 44±7 yr, body mass index (BMI) 23.5±0.9 kg/m² and HbA1c 7.6±0.3 %. They were studied once after a night of normal sleep duration, and once after a night of only 4 hours of sleep. Sleep characteristics were assessed by polysomnography. Insulin sensitivity was measured by hyperinsulinemic euglycemic clamp studies with infusion of [6,6-²H₂] glucose.

Results:

Sleep duration was shorter in the night with sleep restriction than in the unrestricted night (469±8.5 vs. 222±7.1 min, P= 0.001). Sleep restriction did not affect basal levels of glucose, non-esterified fatty acids (NEFA) or endogenous glucose production. Sleep restriction did not alter endogenous glucose production during the hyperinsulinemic clamp compared to the unrestricted night (6.2±0.8 vs 6.9±0.6 μmol.kgLBM⁻¹.min⁻¹, NS). In contrast, sleep restriction decreased the glucose disposal rate during the clamp (25.5±2.6 vs. 22.0±2.1 μmol.kgLBM⁻¹.min⁻¹, P=0.04), reflecting decreased peripheral insulin sensitivity. Accordingly, sleep restriction decreased the rate of glucose infusion by ~21% (P=0.04). Sleep restriction did not alter plasma NEFA levels during the clamp (143±29 vs. 133±29 μmol/L, NS).

Conclusions:

Partial sleep deprivation during a single night induces peripheral insulin resistance in patients with type 1 diabetes. Therefore, sleep duration is a determinant of insulin sensitivity in patients with type 1 diabetes.

Nothing to Disclose: ED, MvD, JGvD, NRB, GJL, KvK, RPLMH, EPMC, JAR

OR42-5

Vaspin Is Associated with Female Sex, Puberty, Obesity and Insulin Sensitivity in Children.

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Visceral adipose tissue-derived serine protease inhibitor (vaspin) has been suggested as a novel adipocytokine that is related to parameters of obesity and that might exert insulin sensitizing effects in adults. We aimed (i) to assess physiological changes of vaspin levels during development in normal healthy children, (ii) to assess the association with obesity, insulin resistance and cardiovascular parameters in children, and (iii) evaluate vaspin levels during an oral glucose tolerance test to assess the specific clinical interaction of vaspin with insulin metabolism.

Vaspin serum levels were determined by ELISA (ng/mL) in lean (n=65, BMI SDS=-0.26±0.09, age 12.8±0.4y) and obese (n=67, BMI SDS=2.28±0.06, age 12.1±0.3y) children.

Vaspin levels were significantly higher in girls compared to boys (1.27±0.21 vs. 0.40±0.04, $P<0.0001$). In girls, vaspin correlated with age ($r=0.38$, $P=0.014$) and increased with pubertal stage ($P=0.031$), whereas there was no dynamic with age and puberty in boys.

Obese children had lower vaspin serum levels than their lean peers (0.55±0.06 vs. 0.92±0.14, $P=0.013$). This difference was restricted to girls. Lower vaspin serum levels were associated with better insulin sensitivity ($r=-0.23$, $P=0.010$), but with higher systolic blood pressure and impaired endothelial function ($r=-0.21$, $P=0.018$). Multiple regression analyses confirmed the associations of Matsuda ISI and systolic blood pressure with vaspin independent from sex, age and BMI. To obtain deeper insight into the relation of vaspin to insulin and glucose metabolism, we analyzed vaspin levels during an oGTT in 20 obese adolescents. Vaspin levels significantly declined to 75% of baseline in hyperinsulinemic adolescents ($P=0.0010$), while there was no significant decline in normoinsulinemic patients. We did, however, not identify significant direct correlations between vaspin and insulin or glucose levels during the oGTT. In conclusion, the sexual dimorphism of vaspin develops during puberty. Vaspin is decreased in obese children, particularly in girls, and is independently associated with insulin sensitivity. In response to an oral glucose load and/or the resulting increase in insulin, vaspin declines in insulin resistant obese adolescents.

Sources of Research Support: German Research Council KO3512/1-1 & Else Kröner-Fresenius Foundation Memorial Grant awarded to AK; German Research Council & German Competence Net "Obesity" awarded to WK, AK, MB, MS.

Nothing to Disclose: AK, MB, SE, PK, DF, KD, SB, TMK, MS, WK

OR42-6

Sleep Quality Predicts Glycemic Control in Type 2 Diabetes.

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There is good evidence for a link between poor sleep quality and reduced glucose tolerance. In healthy adults, we showed that suppression of slow-wave activity (SWA), a marker of sleep depth or intensity, results in decreased insulin sensitivity without beta cell compensation (1). Epidemiologic studies have shown that individuals reporting sleep disturbances have an increased risk of type 2 diabetes (T2DM). Patients with T2DM have a high prevalence of sleep disturbances, particularly obstructive sleep apnea (OSA). There is a robust graded relationship between increasing OSA severity and poorer glucose control in T2DM, independent of adiposity and other confounders (2). Here we examined whether levels of SWA, a highly stable and heritable trait (3,4), may also predict glycemic control in T2DM, independent of OSA.

57 patients with T2DM underwent in-laboratory polysomnography to assess for the presence and severity of OSA. Levels of SWA derived from EEG spectral analysis were calculated during the first two sleep cycles (when SWA is most abundant). Glucose control was assessed by hemoglobin A1c (HbA1c). We performed multivariate regression analyses, with HbA1c as the dependent variable and age, sex, race, BMI, years of diabetes, exercise, insulin use as well as OSA severity (quantified by apnea-hypopnea index) and levels of SWA as predictors of glycemic control (Table 1). Only 34% of the variance in HbA1c was predicted by the traditional risk factors (Model 1). When OSA severity and SWA were included in the regression model (Model 2), both markers of sleep quality were significant independent predictors of HbA1c levels and together they accounted for an additional 18% of the variance in HbA1c.

These findings confirm an important role of sleep quality in glucose control in T2DM and support the presence of a previously unrecognized sleep-related, genetic predisposition to poor glycemic control independent of the presence and severity of OSA and of known risk factors.

Table 1

| Multivariate regression model | Overall Model | OSA severity | SWA(μv^2) |
|--|---------------|--------------|------------------------|
| Model 1: Age, sex, race, BMI, years of diabetes, exercise, insulin use | | | |
| R ² | 0.34 | | |
| p level | 0.0032 | | |
| Model 2: Age, sex, race, BMI, years of diabetes, exercise, insulin use, OSA severity and SWA | | | |
| R ² | 0.52 | | |
| p level | <0.0001 | 0.0092 | 0.0013 |
| β coefficient* | | 0.060 | - 0.133 |

* β coefficients are for log transformed values for OSA severity and SWA

- (1) Tasali E et al., Proc Natl Acad Sci 2008; 105(3): 1044-9
- (2) Aronsohn RS et al., Am J Respir Crit Care Med 2009
- (3) Dauvilliers Y et al., Sleep Med Rev 2005; 9(2): 91-100
- (4) Tan X et al., Clinical Neurophysiology 2001; 112:1540-1552

Sources of Research Support: NIH Grants P01 AG11412, P60 DK20595, R01 HL086459, UL1RR024999; American Academy of Sleep Medicine/Pfizer Scholars Grant in Sleep Medicine; ResMed Foundation.

Nothing to Disclose: RSA, HW, FC, EVC, ET

OR43-1

Metformin Treatment Delays Menarche and Augments Adult Height in Low-Birthweight Girls with Precocious Pubarche: A Pilot Study over 6 Years.

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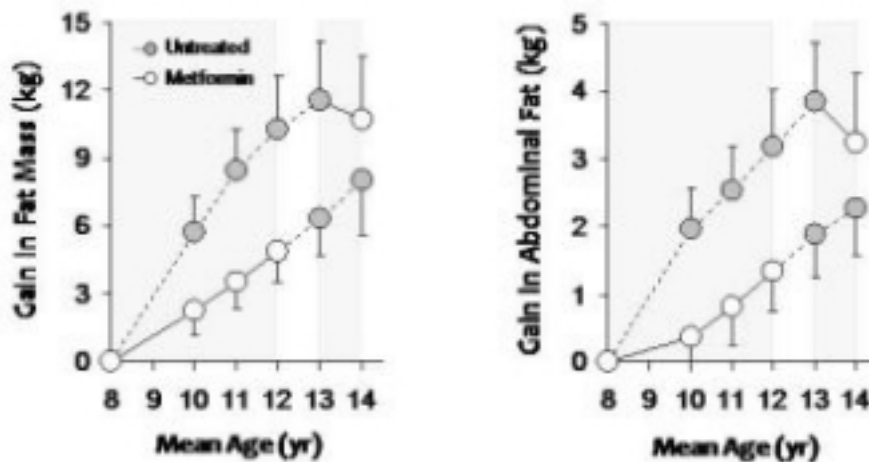
¹Hosp Sant Joan de Deu, Univ of Barcelona Esplugues, Spain ; ²Hosp Dr Josep Trueta Girona, Spain ; ³Hosp de Terrassa Terrassa, Spain and ⁴Univ of Leuven Leuven, Belgium.

Context & Aim: Low-birthweight (LBW) girls with precocious pubarche (PP; pubic hair before 8 yr) are at risk for developing an early and rapidly progressive puberty leading to an early menarche and to an adult height below target level. Hyperinsulinemic insulin resistance is thought to drive such rapid maturation. We studied the effects of an early versus a late insulin-sensitizing intervention with metformin.

Study Population & Design: 38 nonobese LBW-PP girls were followed for 6 yr (age 8-14 yr) in an open-labelled study. Girls were randomly assigned to remain untreated or to receive metformin for 4 yr. In year 5, all the girls were followed without intervention. In year 6, the treatment cross-over was completed so that the initial metformin subgroup remained untreated, and the initially untreated girls received metformin (850 mg/d) for 1 yr. We focus here on the results at the end of year 6.

Main outcomes: Age at menarche; height; weight; endocrine-metabolic state (fasting blood); body composition; abdominal fat partitioning; hepatic adiposity.

Figure 1



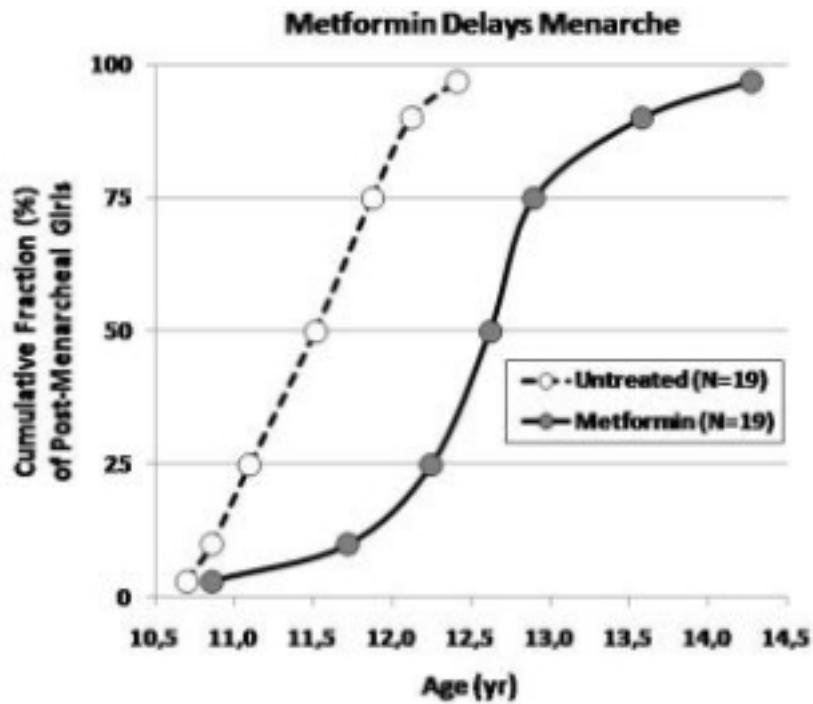


Figure 2

Results & Conclusion: The two subgroups diverged between 0-5 yr for many features (including insulin, testosterone, and visceral fat) and converged again in year 6. However, at least two features will remain different: age at menarche was 11.4 ± 0.1 yr in late-treated girls and 12.5 ± 0.2 yr in early-treated girls ($P < 0.0001$); data on height velocity and on near-adult height indicate that early metformin therapy prolongs statural growth and will augment adult height (by an estimated 4 cm) up to target level.

Nothing to Disclose: LI, AL-B, MD, MVM, FEDZ

OR43-2

Earlier Age at Pubertal Onset in Contemporary US Children.

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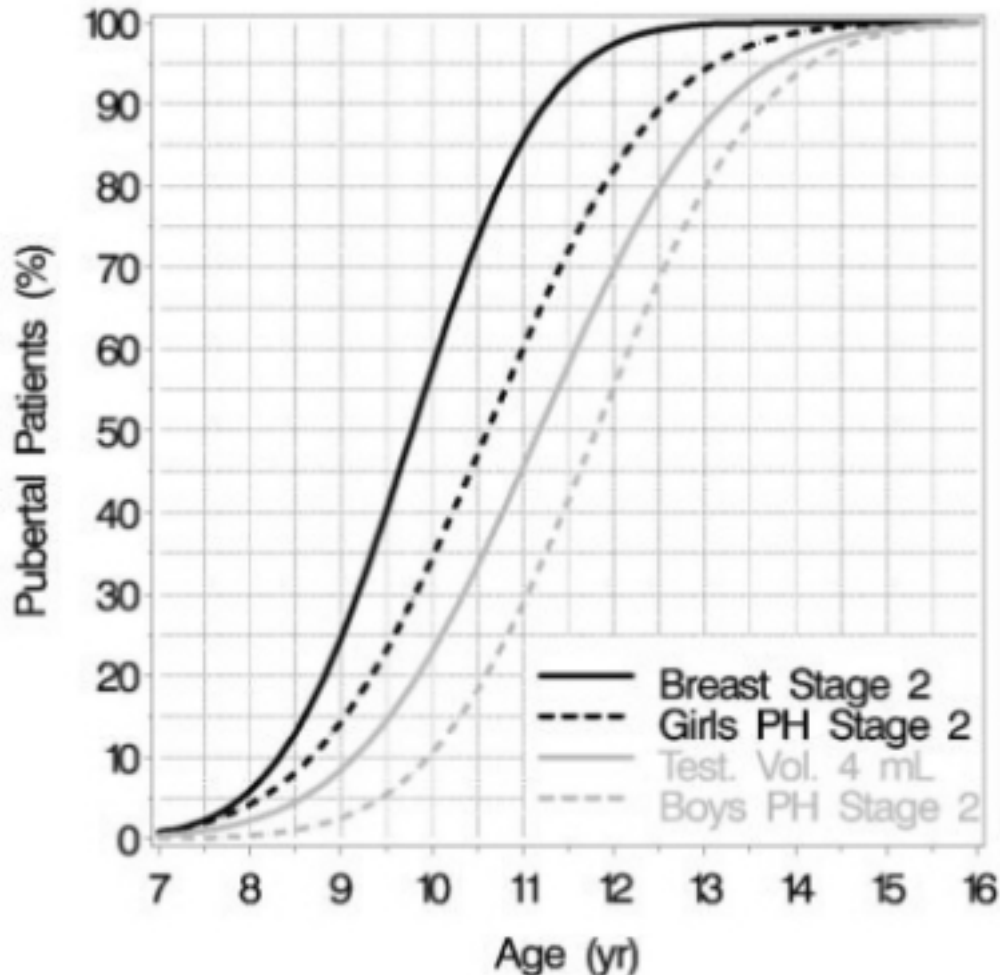
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Introduction: Previous studies suggest a secular trend towards an earlier age at pubertal onset (1-3). This study provides new information about the normative age ranges for pubertal onset in contemporary US children.

Methods: In 2007, 1229 healthy subjects (age range, 3-18 years) were examined by pediatricians at centers in Arizona, Florida and Ohio for pubertal characteristics including testicular volume and Tanner staging for pubic hair and breast development. The ages at pubertal onset were estimated using probit analysis as in a previous study (1) in order to accommodate for the fact that the actual age of pubertal onset was not observed.

Results: Breast development in girls was seen as early as age 7.6 (estimated 3rd percentile) and in 97% of girls by age 11.9 years. Testicular volume ≥ 4 mL in boys was seen as early as age 8.2 and in 97% of boys by age 14.2 years. Tanner stage 2 pubic hair in boys was seen as early as age 9.1 and in 97% of boys by age 14.5 years. 69% of subjects were non-Hispanic Caucasian, 16% were non-Hispanic black, 7% were Hispanic and 8% were members of other ethnic/racial groups. The distributions of the age at pubertal onset for non-Hispanic Caucasians are shown in Figure 1.

Figure 1. Age at Pubertal Onset for non-Hispanic Caucasians



The mean age at pubertal onset for Tanner stage 2 breast development and testicular volume = 4 mL were similar across ethnic/racial groups, but Tanner stage 2 pubic hair occurred one year earlier for non-Hispanic black boys and girls compared with non-Hispanic Caucasians and Hispanics.

Discussion: These data suggest that girls have earlier breast development and boys have testicular volume = 4 mL earlier than previously reported (50th percentiles were 0.3 and 0.2 years earlier, respectively) (2). Pubic hair development in boys and girls was also earlier than reported for one study (2) but similar to another study (1). Overall these data suggest an earlier age of testes and breast development in contemporary US children than previously reported.

1) Sun, et al. 2002 Pediatrics; 110:911-9. 2) Tanner, et al.1985 J Pediatr; 107:317-29. 3) Euling, et al. 2008 Pediatrics; 121:S172-91

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OR43-3

Baseline Inhibin B Levels Discriminate in Boys Constitutional Delay of Puberty from Hypogonadotropic Hypogonadism, Better Than Testosterone, Anti-Mullerian Hormone and Gonadotropin Levels.

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The differential diagnosis between constitutional delay of puberty (DP) and hypogonadotropic hypogonadism (HH) may be difficult in boys, especially when HH is partial, allowing initiation of puberty but not completion. Baseline testosterone and gonadotropin levels overlap between constitutional DP and HH and dynamic testing is usually needed for discriminating the two conditions. We assessed the usefulness of baseline inhibin B (InhB) and anti-Mullerian hormone (AMH) measurements to discriminate HH from constitutional DP using Receiver Operating Characteristic analysis. Both hormones are produced by Sertoli cells and are detectable in serum before puberty. InhB is highly FSH-dependent. AMH levels are high before puberty, as a reflection of Sertoli cells integrity, and decrease thereafter upon testosterone downregulating effect.

We studied 82 boys aged 14 to 18 years with DP: 16 had isolated HH, 15 congenital HH within combined pituitary hormone deficiency (PHD), and 51 constitutional DP, as confirmed by long term outcomes. Subjects were genital stage 1 (testis volume <3 mL; 9 isolated HH, 7 combined PHD, and 23 constitutional DP) or early stage 2 (testis volume 3-6 mL, 7 isolated HH, 8 combined PHD, and 28 constitutional DP).

Age and testis volume were similar between the 3 groups. Compared to constitutional DP, isolated HH and combined PHD subjects had lower InhB, testosterone, FSH, and LH levels ($p < 0.05$), whereas AMH concentration was lower only in isolated HH and combined PHD subjects with genital stage 1, likely reflecting a lower pool of Sertoli cells in deep HH. In isolated HH and combined PHD boys with genital stage 1, sensitivity and specificity were 100% for InhB level <35 pg/mL. In isolated HH and combined PHD boys with genital stage 2, sensitivities were 90% and 80% whereas specificities were 92% and 88%, respectively, for InhB level <65 pg/mL. The performance of testosterone, AMH, FSH, and LH measurements was lower.

Our study shows that baseline InhB level was a reliable marker of FSH production and provides excellent discrimination between HH and constitutional DP in subjects with genital stage 1, while baseline FSH level does not differentiate the two conditions with the needed sensitivity and specificity. In subject with genital stage 2 InhB is also a better index of pituitary defect than the other hormones. Therefore baseline InhB measurement is a simple and reliable alternative to dynamic testing in boys with pubertal delay.

Nothing to Disclose: RC, EB, CB, NB-N, FG, SD, SR, NL

OR43-4

The Identification of 180 Genetic Loci Involved in Adult Height Variation Highlights Biological Pathways and Provides Insights into the Contribution of Common Genetic Variation to Human Growth.

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Idiopathic short stature is one of the most common reasons for visits to pediatric endocrinologists, and is strongly influenced by genetic factors. Indeed, up to 90% of variation in height is attributable to inherited variation. Height is a classic polygenic trait: it has been proposed for nearly 100 years that variants in multiple genes influence height. As part of the Genetic Investigation of ANthropometric Traits (GIANT) Consortium, we performed a meta-analysis of genome-wide association studies of adult height, encompassing 2.8 million single nucleotide polymorphisms (SNPs) and 133,800 individuals of European ancestry from 50 individual studies. We detected 207 distinct regions of the genome with a SNP associated with height at $P < 5 \times 10^{-6}$ (versus 5 expected by chance). When we examined the 207 SNPs in a replication dataset of over 51,000 samples, 180 reached a combined $P < 5 \times 10^{-8}$, a commonly used threshold for genome-wide significance. Together, the associated variants explain up to 12% of height variation. The associated loci are strongly enriched for genes known to be mutated in monogenic disorders of skeletal growth ($P < 0.001$), and implicate genes in relevant biological pathways, including hedgehog signaling, TGF-beta signaling, extracellular matrix, and growth hormone signaling. The regions include associated variants near many biologically relevant genes, including those encoding the growth hormone secretagogue receptor (*GHSR*, $P = 3 \times 10^{-18}$), insulin-like growth factor receptor (*IGF1R*, $P = 3 \times 10^{-21}$), and two independent signals ($r^2 < 0.01$) in the estrogen receptor 1 gene (*ESR1*, $P = 1 \times 10^{-17}$ and $P = 6 \times 10^{-12}$). Many loci have multiple associated variants: conditional analysis on 207 lead SNPs identified 22 regions that have additional independent strong associations. In conclusion, we have identified nearly 200 genetic loci influencing adult height. These loci are enriched for genes in biological pathways relevant to human growth, and support a notion that allelic heterogeneity (multiple variants at the same genetic locus) may be a common feature of polygenic traits. Furthermore, many of the associated variants are not near genes known to be relevant to stature, suggesting that further investigation of these loci in patients and in model systems may uncover new biological aspects of human growth.

Nothing to Disclose: JNH

OR43-5

Three Novel *GLI2* Mutations (L788fsX794, L694fsX732 and E380X) Presenting as Variable Penetrance of Pituitary Hormone Deficiencies without Holoprosencephaly.

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Introduction: *GLI2* is a zinc finger transcription factor downstream in Sonic Hedgehog signaling, expressed in ventral diencephalon and primitive Rathke's pouch of mice. Roessler et al described *GLI2* mutations in patients with holoprosencephaly and pituitary abnormalities. Hormonal data and pituitary MRI of these patients have not been published.

Objectives: to characterize genotype and phenotype associated with 3 novel *GLI2* mutations.

Material and Methods: DNA was extracted from a cohort of patients with hypopituitarism. The complete coding sequence of *GLI2* was amplified by PCR using intronic primers followed by automatic sequencing.

Results: The index case of family 1 was a 7.1 yr girl with short stature (height SDS -5.4), polydactyly, hypoglycemia and seizures. Hormonal tests showed GH, TSH, PRL, ACTH and GnRH deficiencies. *GLI2* sequencing revealed a heterozygous 7 bp deletion (c.2362_2368del) resulting in a frameshift and stop codon (p.L788fsX794). This mutation was present in 5 relatives with polydactyly (including the mother), in 3 with polydactyly and hypopituitarism (2 uncles with IGHD; one cousin with GH, TSH and GnRH deficiencies), and absent in 3 normal relatives.

In family 2, a 4.5 yr boy had growth failure (height SDS -4.5), cryptorchidism and cleft lip and palate. Tests revealed isolated GHD. *GLI2* analysis revealed a heterozygous 4 bp deletion (c. 2081_2084del) leading to a frameshift and truncated protein (p. L694fsX732). His apparently healthy father (height SDS -1.2) carries the same mutation. *GLI2* mutations in families 1 and 2 predict loss of the C-terminal activator domain.

In family 3, an 8 months-old girl had height SDS of -2.9, seizures associated with hypoglycemia, excessive thirst and urine volume. Tests showed TSH, ACTH, GH and ADH deficiencies. *GLI2* study identified a heterozygous c.1138 G>T, resulting in a stop codon (p.E380X) predicting lack of zinc fingers and the C-terminal activator domain. Her normal mother (height SDS -0.5) also carries the mutation.

MRI in 5 patients with *GLI2* mutations and hypopituitarism showed a hypoplastic anterior pituitary and ectopic posterior lobe without holoprosencephaly.

Conclusion: We expand the spectrum of *GLI2* mutations describing 3 novel mutations predicting truncated proteins lacking the DNA-binding and activator domains. Data from these 3 families supports partial penetrance, variable pituitary hormone deficiencies, including diabetes insipidus, polydactyly, and midline facial abnormalities.

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OR43-6

Skeletal Muscle Mitochondrial Function in Children, Young and Middle Age Adults.

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Background: Elderly subjects have reduced mitochondrial function. However, it remains unclear whether the decline in mitochondrial function begins earlier in the lifespan.

Objective: To determine skeletal muscle mitochondrial oxidative phosphorylation *in vivo* by ³¹P-magnetic resonance spectroscopy (MRS), across a variety of age groups, from children to middle aged adults.

Design: Cross sectional study of 121 healthy normal weight and overweight individuals from age 8 to 55 years.

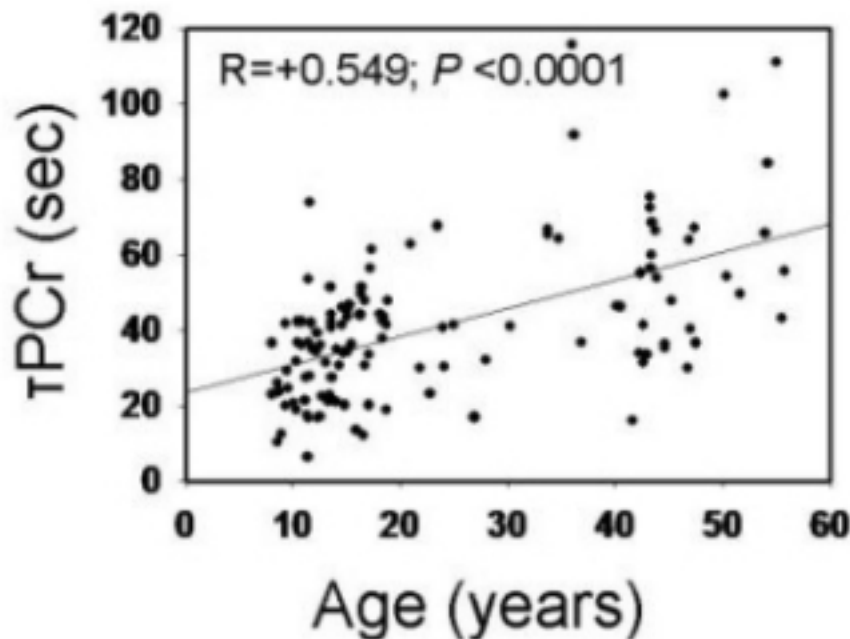
Setting: A single center university medical center in Boston, MA.

Participants: 68 children and 53 adults from the Boston community.

Measurements: Mitochondrial function was determined by measuring phosphocreatine recovery after sub-maximal exercise by ³¹P-MRS. Subjects were also evaluated with anthropometric measurements and metabolic profiles.

Results: Decreased mitochondrial function, described as increasing τPCr, was positively associated with increasing age.

Figure 1.



A significant relationship between age and mitochondrial function was seen in both normal weight and overweight subjects. In multivariate regression analysis, age remained highly significant and the strongest predictor of τPCr when controlling for gender, race, ethnicity, HOMA IR, BMI, and measures of work load during sub-maximal exercise. In addition, dietary intake, and physical activity did not significantly contribute to the model.

Conclusions: Skeletal muscle mitochondrial function declines with aging even in children and young adults, and this may have significant implications for the biology of aging.

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