Whilst individuals with either the PPARy (n=3) or PP1R3 (n=2) gene defects alone exhibit normal insulin sensitivity, compound heterozygosity for both genetic abnormalities (n=5) cosegregates completely with severe insulin resistance, representing the first description of a digenic form of this disorder.

M57

Naturally-Occurring Mutations in Steroidogenic Factor-1 (SF-1) Provide Insight Into Dose-Dependent Regulation of Gene Transcription by Monomeric Orphan Nuclear Receptors

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Steroidogenic factor-1 (SF-1, NR5A1) is an orphan nuclear receptor that regulates the transcription of multiple target genes involved in steroidogenesis, reproduction and male sexual differentiation. Unlike most nuclear receptors, SF-1 binds to the response elements of these genes as a monomer and recognizes variations on the extended half site, PyCA AGGTCA. Recently, we described a heterozygous G35E mutation in the P-box of SF-1 in a patient with severe primary adrenal failure, complete XY sex-reversal and persistent Mullerian structures (Nat Genet 1999;22: 125-6). The P-box amino-acid sequence is critical for recognizing the core half-site element of SF-I responsive promoters, particularly when “ imperfect” binding sites are present, and a heterozygous mutation is sufficient to cause a severe phenotype in this patient. We have now identified a homozygous R92Q mutation in the A-box of SF-1 in a baby with a similar phenotype and consanguineous parents. This A-box arginine residue is highly conserved among nuclear receptors that bind to DNA as monomers (e.g., SF-1, LRH, ERR, NGFIb) and is thought to interact primarily with the 5’ flanking sequence within the minor groove of DNA (PyCA) to stabilize the receptor-DNA complex. Functional studies reveal that the R92Q mutant has partial loss of binding to and transactivation of several SF-1 target genes when compared to the G35E P-box change. Further, heterozygous carriers of the R92Q mutation have a normal phenotype and adrenal function. Thus, a homozygous R92Q A-box mutation is necessary for full phenotypic penetrance and autosomal dominant inheritance is seen. Taken together, these two naturally-occurring SF-1 mutations reveal the relative importance of the P-box and A-box regions for monomeric binding by nuclear receptors, demonstrating that functional gene dosage effects are important when one factor controls the transcription of many different target genes in humans.

M58

Cell Cycle Dysregulation in Human Pituitary Tumours

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Pituitary adenomas in humans are slow-growing, relatively benign tumours which only very rarely metastasise and such tumours are likely to offer very useful model systems of the early stages of oncogenesis. Transgenic or ‘knock-out’ models of many of the proteins involved in the regulation of the cell cycle, such as retinoblastoma (Rb), p27, p16, CDK4 develop abnormalities of different endocrine tissues, most importantly, pituitary. We therefore speculated that abnormalities in these pathways may be important aetiologic features in sporadic pituitary tumours. p27 is a cyclin-dependent kinase inhibitor which inhibits the progression of the cell cycle in the G1 phase by inhibiting the action of cyclin E. Using semi-quantitative, immunocytochemistry, we found that p27 expression is progressively lowered as one passes from normal to benign pituitary adenomas, and is especially low in corticotroph tumours and pituitary carcinomas. Corticotroph tumours also express relatively more cyclin E. We were unable to identify parallel changes in p27 mRNA, or mutations in the p27 transcript, suggesting that the post-translational degradation of p27 is enhanced in corticotrophomas and malignant tumours. Both of these tumour subtypes over-express Ki-67, indicative of increased proliferative activity. The malignant tumours are also associated with diminished phospho-p27 but increased nuclear staining for jun-activation binding protein-1, JAB1, a proto-oncogene which accelerates phosphorylation and removal of p27 from the nucleus and hence its ubiquitination in the cytoplasmic proteasome. Conversely, the phospho-p27/p27 ratio is massively increased in corticotrophomas, but is not associated with changes in JAB1. JAB1 is bound to and inactivated by the macrophage inhibitory factor, MIF. We speculated that even where JAB1 was apparently present at normal levels, decreased MIF would increase its bioactivity in pituitary adenomas; unexpectedly, we noted that MIF immunostaining was increased in such tumours, suggesting that it may be part of a compensatory mechanism to moderate JAB1 activity. Proteasomal degradation requires preparation for ubiquitination by binding to the SKP complex, with specific addressing by the F-box protein Skp2 and the recently described Cks1. We are currently studying the expression of these two agents as well as the PIK3/Akt/forkhead-TF pathway, which is the major upstream regulator of p27 degradation, to see whether they are up-regulated in pituitary adenomas. We hope to identify abnormalities causing the fall in p27, and hence the principal pathogenic mutations causing pituitary adenomatosis.

M59

Autocrine Activation of Glucocorticoids in Osteoblasts Increases With Age and Glucocorticoid Exposure

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The risk of glucocorticoid-induced osteoporosis increases substantially with age but there is considerable individual variation. In recent studies we have shown that glucocorticoid effects on bone may be dependent upon expression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) and expression of 11β-HSD1 modulates osteoblastic proliferation and differentiation. Using primary cultures of human osteoblasts we have now characterised age-specific variation in osteoblastic 11β-HSD1 and defined enzyme kinetics and regulation using natural and therapeutic glucocorticoids. 11β-HSD1 reduces activity (cortisone to cortisol) was present in all osteoblast cultures and activity correlated with age (r=0.58 all cultures, p<0.01, r=0.97 calcaneal derived cultures, p<0.001, n=14). A similar increase in 11β-HSD1 mRNA expression with age was suggested by real-time PCR (r=0.35, n=10). Glucocorticoids caused time and dose dependent increases in 11β-HSD1 activity over control (e.g. dexamethasone (1µM) 2.6-fold±0.5 (mean±SE), p<0.001; cortisol (100nM) 1.7-fold±0.1, p<0.05). Similar increases in 11β-HSD1 mRNA expression were demonstrated (3.5-fold with DEX, p<0.01; 2.5-fold with cortisol, p<0.05). The capacity of 11β-HSD1 to metabolise prednisone and prednisolone was investigated in human osteoblasts and fetal kidney cells stably transfected with human 11β-HSD1 cDNA. These cells interconverted prednisone and prednisolone with reaction kinetics indistinguishable from cortisone/cortisol. To assess in vivo availability of substrates for
osteoblastic 11β-HSD1, plasma cortisone and prednisone levels were measured in 12 normal males before and after 5mg prednisolone orally. 0900 cortisone levels were 110±5nmol/L and prednisone levels peaked at 78±33nmol/L 120min after prednisolone. Thus, therapeutic use of steroids increases substrate availability for 11β-HSD1 in bone. These studies indicate that autocrine activation of glucocorticoids within bone is likely to play an important role in the age-related decrease in bone formation and increased risk of glucocorticoid-induced osteoporosis.

M60

A<sub>2B</sub> adenosine receptors mediate the adenosine-induced increase in cell growth and IL-6 expression in pituitary folliculostellate cells

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Coordinated interaction between the immune and neuroendocrine systems is of key importance in regulating the host’s response to inflammation and anoxic stress. Adenosine, released under such conditions, modulates a number of inflammatory processes and can regulate the activity of the hypothalamo-pituitary-adrenal (HPA) axis. Although adenosine receptors (ARs) have been described in the pituitary gland, the distribution of the receptor subtypes (A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> or A<sub>3</sub>) has not been defined. Accordingly, we have analysed the distribution of ARs in primary rat anterior pituitary (AP) cells, and pituitary endocrine and folliculostellate (FS) cell lines, and compared their effects on cell growth, IL-6 and VEGF expression. In AP and FS cells, adenosine and AR agonists (NECA, a universal AR agonist, and CGS 21680, a selective A<sub>2B</sub>R agonist) stimulated CAMP production with a potency order (NECA>adenosine>CGS 21680) indicating the presence of functional A<sub>2B</sub> receptors. This stimulation was not observed in endocrine cells where adenosine and the selective A<sub>1</sub>R agonist CCPA inhibited forskolin stimulated CAMP production. Gene and protein expression for the A<sub>2B</sub>R and A<sub>1</sub>R in FS cells, and the A<sub>1</sub>R in endocrine cells was confirmed by RT-PCR, immunocytochemistry and ligand binding. Adenosine stimulated the growth of FS cells via the A<sub>2B</sub>R but in higher concentration inhibited cell growth in endocrine cells via the A<sub>1</sub>R. Adenosine and NECA stimulated IL-6 (up to 10- and 30-fold) and VEGF (up to 1.5- and 3-fold) production in FS but not in endocrine cells, effects that were reversible by dexamethasone. These data highlight the differential actions of adenosine in FS and endocrine cells mediated respectively via the A<sub>2B</sub>R and A<sub>1</sub>R. The effects on FS cell growth and IL-6 release support important paracrine roles for adenosine in pituitary physiology and pathophysiology.

M61

DIABETES IN AN HIV POPULATION: A HIDDEN EPIDEMIC?

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Background Several groups have been identified to be at high risk of developing diabetes. There is emerging evidence in the treatment of HIV that some antiretroviral therapies (ART) adversely affect the glucose-insulin axis and lipid metabolism.

Methods A retrospective database analysis of HIV positive individuals attending an HIV tertiary referral centre was conducted. Demography and ART were ascertained. Diabetes prevalence was determined by ≥1 random blood glucose ≥11.1mmol/L.

Results There were 6259 patients on the database, mean age 45.8 (range 24-82) years, 288 (4.6% prevalence) had ≥1 glucose measurements (92% had ≥2 readings) in the diabetes range. Previous diabetes diagnosis was not established. From this hyperglycaemia group, 57% received ART (33% were on a combination that contained a protease inhibitor (PI), 17% were on nucleoside analogues (NA) alone and 7% had a NA/non-nucleoside reverse transcriptase inhibitor combination). 15% were on no ART therapy.

Conclusion The diabetes prevalence in this cohort is at least twice the frequency expected of a younger general population. The contribution of antiretroviral treatments, HIV infection, diabetogenic drugs eg steroids, concurrent infections promoting insulin resistance and high caloric diets may all have contributed to the observation. As half this diabetes group were on a PI or a NA, this highlights the importance of drug induced alterations of glucose metabolism with these therapies.

With the success of antiretroviral therapies in HIV disease, chronic metabolic disorders, in particular diabetes, may have important future implications in terms of management and clinical outcome.

M62

Reduced adipose tissue sympathetic nervous system (SNS) activity in human obesity despite increased whole body norepinephrine spillover.

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The SNS is thought to be a principal regulator of energy expenditure, substrate deposition and adipose tissue (AT) function (e.g. leptin secretion). However, its role in human obesity has been much disputed. Some authors invoke increased activity to explain obesity related hypertension whilst others point to low activity in many animal models of obesity and suggest that this would have the potential to contribute to the development or maintenance of the obese state itself via effects on lipolysis, thermogenesis and cell signalling.

We have applied isotope dilution methodology to human whole body, subcutaneous abdominal AT and skeletal muscle norepinephrine (NE) arterio-venous differences to determine spillover rates and thus estimate total and regional SNS activity in 11 lean and 11 age and sex-matched obese human subjects. Whilst whole body NE spillover was increased in the obese (5.03±0.84 vs. 2.67±0.33 nmol.min<sup>-1</sup>, p<0.02), forearm muscle NE spillover was similar in both groups. AT NE spillover was significantly reduced in the obese both under fasting (54.2±15.3 vs. 98.8±17.8 pg/100g AT.min<sup>-1</sup>, p=0.05) and post-prandial (68.8±27.3 vs. 174.1±37.6 pg/100g AT.min<sup>-1</sup>, p<0.05) conditions. Furthermore, whilst AT NE spillover increased in lean subjects following meal ingestion (p<0.02), no significant increase was evident in the obese. Fasting arterial epinephrine concentrations (an index of adrenal medullary activity) were significantly higher in the obese (p<0.02). We also report highly significant correlations between whole body NE spillover and both systolic and diastolic blood pressures (p<0.01).

We conclude that despite increased whole body SNS activity (which correlates with blood pressure), local AT SNS activity is reduced in human obesity. Moreover, it also fails to respond normally to meal stimulation in obese humans. Underactivity of the SNS within AT may contribute to the development or pathophysiology of obesity.