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MICROARRAY ANALYSIS OF GROWTH HORMONE RESPONSIVE GENES IN PERIPHERAL BLOOD LEUKOCYTES IN VIVO

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There is strong evidence that growth hormone (GH) activates the immune system both directly, and indirectly through IGF-1 and the cytokine network. Little is known, about what genes are regulated by GH in immune cells in vivo. The aim of this study is to investigate the effects of GH on gene expression in leukocytes.

Healthy male subjects, recruited as part of a intervention study aimed at developing a GH doping test, were administered 2mg/day GH for eight weeks followed by a 6 week washout period. Total RNA was extracted from white blood cells collected at baseline (week 0), weeks 4 and 8 (GH treatment) and week 14 (GH washout). Gene expression analysis was performed using Affymetrix HG-133 Plus 2.0 human genome arrays, which consist of 54925 probe sets, and the data analysed by GeneSpring software. Differential expression was analysed by one-way ANOVA.

In preliminary analysis of data from 4 subjects, GH induced significant change in 1049, 1463 and 690 probe sets at weeks 4, 8 and 14, respectively, compared to baseline ($p < 0.05$). Of these, 11, 16 and 1 corresponding genes were up or down-regulated by greater than 2-fold at weeks 4, 8 and 14, respectively. Consistent changes in six of these genes were present at both weeks 4 and 8 and these genes are involved in biological process-metabolism (n=3), cellular component-golgi stack (n=2) and molecular function-lipid binding (n=1) and -catalytic activity (n=2), using Gene Ontology.

This data indicates that GH induces the expression of genes in peripheral leukocytes during GH treatment. Since peripheral blood is easily accessible, identification of a gene expression fingerprint in leukocytes could lead to the development of a GH doping test.

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GENERATION AND VALIDATION OF AN FSH-β-PROMOTER CONSTRUCT TO TARGET CRE RECOMBINASE EXPRESSION TO ANTERIOR PITUITARY GONADOTROPES

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Activins and inhibins are members of the TGF-β superfamily which positively and negatively regulate pituitary follicle stimulating hormone (FSH) synthesis respectively. FSH is composed of an α and a β subunit, the latter of which is produced exclusively in pituitary gonadotropes. This expression pattern makes the FSH-β promoter ideal for targeting gene-specific expression in the gonadotropes. The currently available pituitary-specific Cre transgenic mouse is the alpha-GSU-Cre which expresses Cre recombinase in all the five cell types of the adult anterior pituitary. No gonadotrope-specific Cre transgenic mouse has been described. To enable the future creation of conditional gonadotrope-specific gene knockout mice, we are engineering a transgenic mouse line which expresses Cre recombinase mediated by the FSH-β promoter. To generate the FSH-β-Cre construct, a 5.5kb fragment of the ovine FSH-β promoter was fused to the Cre recombinase gene in the pGL3-basic vector. To confirm that the oFSH-β promoter is functional in gonadotrope cells, the same promoter driving luciferase gene expression (oFSHβ-lux) was transfected into the LβT2 mouse gonadotrope cell line. Activin (1 nM) increased the activity of this promoter 5-fold compared to untreated cells. Inhibin (0.5 nM) suppressed this activity by 52 ± 11%. The oFSH-β-Cre construct was tested *in vitro*, by co-transfecting LβT2 cells with a Cre reporter construct (pSV-paX1), containing a floxed stop codon that prevents transcription of β-galactosidase. Cre activity will excise the stop codon, allowing β-galactosidase to be expressed. Treatment of LβT2 cells with 1 nM activin for 24 h, increased the proportion of β-galactosidase-positive cells compared to untreated controls, confirming that activation of the FSH-β promoter is driving Cre expression in these cells. This construct will be used to create transgenic mice for future studies of inhibin and activin actions in the pituitary.

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PREGNANCY-INDUCED LEPTIN RESISTANCE INVOLVES A LOSS OF APPETITE SUPPRESSION BY ALPHA-MELANOCYTE STIMULATING HORMONE

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Despite elevated plasma leptin concentrations, food intake and fat deposition is increased during pregnancy. In fact, during pregnancy a state of leptin resistance develops. We have demonstrated that intracerebroventricular (i.c.v.) leptin administration is unable to suppress food intake in pregnant rats, as it does in non-pregnant animals. One of the major neuronal populations involved in mediating leptin action in the hypothalamus are the proopiomelanocortin neurons in the arcuate nucleus, which produce the anorectic peptide αMSH. Leptin-induced phosphorylation of STAT3 (pSTAT3) in αMSH neurons is normal during pregnancy, suggesting that factors downstream of αMSH may be altered to account for the leptin resistance seen in pregnant animals. To test this hypothesis, we examined the effect of i.c.v. αMSH on food intake in diestrous (n=16) and day 14 pregnant (n=16) Sprague Dawley rats. Infusion cannulae were surgically implanted into the rats, followed by daily food intake measurements during a recovery period of about one week. On diestrus and day 14 of pregnancy, after a 24-hour fast, αMSH (10 µg) or vehicle (aCSF) was injected into the left lateral ventricle. One hour later, at the time of lights off, food was returned and food intake was measured 3 and 24 hours later. During the 24 hours preceding treatment, food intake was significantly greater in pregnant rats compared to cycling rats. In diestrous rats, αMSH treatment (n=8) resulted in significantly reduced food intake compared to vehicle-treated diestrous rats (n=8) both 3 and 24 hours later. In the pregnant rats however, there was no difference in food intake between the αMSH-treated or vehicle-treated rats. These results indicate that not only is pregnancy characterised by leptin resistance, but it is also an αMSH-resistant state. This loss of response to αMSH would therefore contribute to the hyperphagia of pregnancy, an important biological adaptation to help the mother prepare for the metabolic demands of lactation.

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CUSHING'S SYNDROME IN A DIABETIC CLINIC POPULATION

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Introduction: The diagnosis of Cushing's syndrome (CS) is often not straight forward. However, it is important to recognise and diagnose this condition as there is significant morbidity associated with the disease. Recent publications have suggested that a significant proportion of patients with type 2 diabetes mellitus (T2DM) may have undiagnosed occult CS and hence it may be worth screening for CS in patients with DM.[1]

Methods: Overweight patients (BMI>25) (n= 179) with T2DM who had no history of alcohol abuse or psychiatric illness, were recruited from our diabetes clinics. 171 were evaluated with the low-dose (1mg) dexamethasone suppression test (DST). The DST was considered positive if the morning plasma cortisol was greater than 50nmol/L. These patients were further evaluated using 24-hour urinary free cortisol (UFC) collection.

Results: A positive DST was recorded in 31/171 patients. Of these, 3 had elevated UFC. Clinical follow-up identified that 2 of these patients had excessive alcohol consumption as a likely cause of their abnormal investigations. The third patient had 4 UFC collections, 3 of which were elevated. He does not have any stigmata of CS and subsequent radiological investigations have not revealed any pituitary or adrenal pathology.

There were 27 false-positive DST. We postulate that there are a number of mechanisms which may contribute to this including: use of medications metabolised by the cytochrome P450 3A4 (eg Statins, carbamazepine), non-alcoholic hepatic steatosis, obesity, or poor glycaemic control. Interestingly, we did not find any correlation between either BMI and DST cortisol level ($r^2 = 0.017$) or glycosylated haemoglobin and DST cortisol level ($r^2 < 0.001$) in our cohort.

Conclusion: We were unable to identify any individuals from our cohort who had occult CS in the context of documented T2DM. Our experience suggests that widespread screening of asymptomatic diabetic populations is not justified.

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PROGESTERONE WITHDRAWAL TRIGGERS INCREASED PROLACTIN SECRETION AND INDUCTION OF SOCS mRNA IN THE ARCUATE NUCLEUS DURING LATE PREGNANCY

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Under normal conditions prolactin stimulates hypothalamic dopaminergic neurons. In turn, dopamine inhibits prolactin secretion; thereby prolactin regulates its own secretion via negative feedback. During late pregnancy hypothalamic dopaminergic neurons become unresponsive to prolactin, resulting in the antepartum prolactin surge. Suppressors of cytokine signalling (SOCS) proteins have been implicated in mediating this change of dopaminergic neuron response. Around day 20 of pregnancy, progesterone concentrations decrease while estrogen remains high. We hypothesized that the initiation of the prolactin surge and consequential increased SOCS expression is triggered by progesterone withdrawal. Thus, by delaying the fall in progesterone during late pregnancy we should abolish changes in prolactin regulation and SOCS mRNA expression. Sprague Dawley rats were ovariectomized on day 18 of pregnancy and received progesterone and estrogen implants. Progesterone implants were removed on day 21, to mimic the fall in progesterone, or day 22 of pregnancy, to delay the fall in progesterone by 24 hours. Control animals remained intact. We observed a significant increase in prolactin during day 22 compared to day 18 of pregnancy in intact animals (123.5 ± 18.0 ng/ml vs. 6.7 ± 1.3 ng/ml; $P < 0.05$). Following progesterone withdrawal on day 21 we observed a similar antepartum prolactin surge, however it was not as marked as in intact animals (53.6 ± 7.6 ng/ml; $P < 0.05$). No surge was observed following delayed progesterone withdrawal. In addition, we observed a 3-fold increase ($P < 0.05$) in SOCS-1 and CIS mRNA levels in the arcuate nucleus during the prolactin surge in control animals and following progesterone withdrawal on day 21. No significant change in SOCS mRNA was seen following delayed progesterone withdrawal. These results support our hypothesis that the timing of the antepartum prolactin surge and the associated change in arcuate SOCS-1 and CIS mRNA levels depend on the withdrawal of progesterone.

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IMPACT OF NEONATAL INFECTION ON CRH AND GLUCOCORTICOID RECEPTOR mRNA ABUNDANCE IN THE MOUSE BRAIN.

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Background: Prenatal and neonatal infection has been shown to alter glucocorticoid receptor function and the adult stress response in a number of animal models. However no studies have examined whether neonatal infection also alters the stress response in the mouse. The current study aims to examine alterations to glucocorticoid receptors (GR), mineralocortoid receptors (MR) and corticotropin releasing hormone (CRH) mRNA after neonatal exposure to Chlamydia in the mouse.

Method: At birth neonates were exposed to Chlamydia Pneumoniae (intranasally, 400 ifu). Control animals received no treatment. At six and nine weeks old animals were euthanised and brains removed and snap frozen. RNA was extracted and GR, MR, and CRH abundance was measured with β actin as the reference gene using real-time polymerase chain reaction.

Results: Neonatal infection resulted in a significant ($p = 0.01$) decrease in MR and CRH abundance when compared to controls for both male and female mice. Male mice had a significant ($p = 0.01$) decrease in MR and CRH compared to female mice in response to neonatal infection. There was no change in GR abundance in response to neonatal infection in either male or female mice.

Discussion: The current study suggests that changes to the stress response in the mouse may be mediated by alterations to MR and CRH rather than GR at the level of mRNA. The current study suggests that the mouse is a suitable model to examine the role of neonatal infection in programming alterations to the adult stress response.

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ACTIVATION OF CORTICOTROPHIN-RELEASING HORMONE (CRH), ARGININE VASOPRESSIN (AVP) AND ENKEPHALIN (ENK) NEURONES LOCATED IN THE PARAVENTRICULAR NUCLEUS BY ISOLATION AND RESTRAINT STRESS IN SHEEP

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The hypothalamo-pituitary-adrenal (HPA) axis is activated during stress and there are sex differences in the activation of the HPA axis which are reflected in plasma concentrations of cortisol. The basis for these differences is not known. Corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) producing neurones of the paraventricular nucleus (PVN) are activated during stress and control the HPA axis. Enkephalin producing neurones of this region are also activated during stress. We tested the hypothesis that there is a sex difference in the activation of CRH, AVP and enkephalin neurones during isolation/restraint stress in sheep. Blood samples (3h) were taken to monitor plasma cortisol concentrations and stress was imposed for 1.5h after 1.5h of control blood sampling. The brains of gonadectomised male and female sheep (n=3/sex/group) were collected after isolation/restraint stress (stress group) and from contemporaneous controls. Double-labelling immunohistochemistry for Fos and either CRH, AVP or enkephalin was undertaken to quantify the number of each type of neurones that was activated during stress. No sex differences were observed in cortisol concentrations in control or stressed animals. Isolation and restraint caused an increase in the number of CRH and AVP cells with Fos-immunoreactive (-ir) nuclei without sex differences. The number of enkephalin cells with Fos-ir positive nuclei was increased in male but not female sheep. We conclude that isolation/restraint stress activated similar numbers of CRH and AVP neurones in both sexes but activates enkephalin neurones in males only.

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THE EFFECTS OF A HIGH PHYTOESTROGEN DIET ON THE TESTES OF THE RAT

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Environmental oestrogens have been suggested to have deleterious effects on male reproduction. This study investigated the effects on the testes of exposure to a high phytoestrogen (PO) diet at various stages in the development of the rat. Male rats were exposed to a high PO diet (465microgram/g) from either: conception to birth, birth to weaning, weaning to adulthood or from conception to adulthood. Control animals received a low PO diet (112 microgram/g) from conception to adulthood. Groups of male rats (n=10) were killed at 18 days, 6 and 16 weeks postpartum and the testes removed for histology and subsequent stereological analysis.

At 18 days spermatocytes were present in all testes, but spermatids were only identified in animals exposed to a low PO diet during conception. At 6 weeks significantly less residual bodies were present in tubules of animals that had received a high PO from conception ($P < 0.05$). Epididymal sperm numbers were also lower in these animals.

No differences in the number of Leydig cells were observed between any of the groups at 16 weeks. A significant reduction ($P<0.01$) in the number of Sertoli cells was observed in animals exposed to a high PO diet continually from conception. These animals also had reduced numbers of spermatogonia ($P<0.002$) and spermatocytes ($P<0.04$) compared to the control animals, however, no significant difference in the number of spermatids was observed. No changes in Sertoli or germ cell number were seen in any of the other treatment groups.

These data suggest that a high PO diet during pregnancy and/or lactation may affect the onset of puberty. Continuous exposure to a high PO diet from conception may affect the number of Sertoli cells, spermatogonia and spermatocytes in the adult animal. However, mechanisms appear to exist to prevent significant reduction in postmeiotic germ cell numbers.

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DO EXOSOMES FROM PLACENTAL EXPLANTS CARRY SYNCYTIN?

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Background: In general, retroviral infection has been related to immunosuppression and it has been shown that the cause of this immunosuppression is the presence of a highly conserved immunosuppressive peptide (ISU) in the transmembrane subunit (TM) of the retroviral envelope protein. Human placenta expresses high levels of endogenous retroviral envelope protein and the presence of these proteins have been hypothesised to promote cell-cell fusion during placental formation and immunosuppression during pregnancy. In particular, Syncytin, a human endogenous retrovirus envelope protein belonging to the HERV-W family is expressed at high levels in the placenta and has been

demonstrated to have fusogenic properties as well as a putative ISU within the TM of the protein. We raised antibodies to target the putative ISU of Syncytin. We hypotesised that the human placenta may produce exosomes that express the retroviral protein Syncytin and that expression of this protein may be regulated by cAMP.

Methods: Placental explants were collected and cultured for 24 hours and then treated with Forskolin (50 mM), CRH (100nM), and a combination of Forskolin (50 mM) and CRH (100nM) for 24, 48 and 72 hours at 37^{o}C (5% CO_2). At each end point, the supernatant was removed and exosomes were enriched by ultracentrifugation, proteins extracted and analysed using Western blotting to detect the presence of Syncytin's TM subunit.

Results: Western Blotting analysis of enriched placental exosomes showed cross-reactivity with a 24kDa protein, the expected size of Syncytin's TM subunit, which increased in a time dependent manner and appeared to be independent of Forskolin or CRH treatments. Also, preliminary observations show cross-reactivity of our antibody with a 24kDa protein present in human plasma from pregnant women.

Conclusions: Using antibodies we raised to target the putative ISU of Syncytin, we found cross-reactivity with a 24kDa protein corresponding to the expected molecular weight of the TM subunit of Syncytin in exosomes produced by human placental explants. No increase in Syncytin expression in an exosomal fraction was observed in response to agents known to increase cAMP. These results suggest a novel mechanism by which the placenta may influence the immunology of pregnancy.

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TRANSGENERATIONAL EFFECTS OF DEVELOPMENTAL PROGRAMMING ON THE FETAL AND PLACENTAL CHARACTERISTICS OF THE RAT.

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Maternal under-nutrition during early gestation can alter both fetal growth rate and endocrinology. Fetal growth restriction during pregnancy is associated with an increased risk of disease in adult life, particularly type II diabetes. The current study focuses upon the effects of developmental programming on the reproductive potential of the subsequent generation. Female Wistar rats were assigned to receive either a standard diet *ad libitum* (AD group) or 30% of the *ad libitum* standard diet (UN group) throughout gestation. Litter size was standardised and female offspring from these pregnancies (F1 generation) were then fed the standard diet *ad libitum* until day 140. At this time these females were mated with AD males and subjected to one of the two dietary regimes described during gestation. A cohort of pregnancies was terminated at day E20 with fetal and placental parameters recorded. When the remaining animals gave birth their litter size was standardised and the F2 pups (males and females) were nursed by their dams until weaning. This produced 3 groups of offspring (F2 generation) (AD-AD, AD-UN, UN-AD). Despite being fed *ad libitum* since weaning F1 UN-AD dams demonstrated significant ($P<0.05$) hyperphagia during both pregnancy and lactation relative to control AD-AD dams. Moreover, fetal reabsorptions were significantly increased (more than 3 fold higher) in UN-AD compared to AD-AD dams. Pup weights were not different between these treatments, however, placentae of UN-AD pups were significantly smaller ($P<0.05$) and consequently the fetus to placenta ratio of these pups was equivalent to AD-UN rather than AD-AD pups. In addition, the litters of UN-AD dams were significantly skewed ($P<0.05$) towards males (59%) compared to AD-AD litters (50%). These results clearly identify transgenerational effects of developmental programming on maternal appetite and reproductive characteristics. *Supported by Maurice and Phyllis Paykel Trust.*

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DIFFERENTIAL EXPRESSION OF THE BETAGLYCAN GENE IN THE MURINE OVARY AND TESTIS DURING GONADOGENESIS.

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Betaglycan (BG) binds inhibin and transforming growth factor-beta (TGF-β) with high affinity and is a key modulator of the actions of many TGF-β superfamily members. Abundant expression of BG is observed in both sexes in the adult gonads, suggesting important roles in regulation of reproduction in both the male and female. The significance of this cell surface receptor during gonadogenesis is yet to be elucidated. In this study, we characterised the gonadal expression pattern of BG during murine embryonic development ($n\geq2$ for each study). The expression of BG mRNA was detected using wholemount in situ hybridisation in the bipotential gonad at day 11.5 days post-coitum (dpc) and by semi-quantitative reverse transcription polymerase chain reaction in male gonads at 12.5-18.5 dpc. Low levels of BG mRNA were detected in the developing female gonad at 12.5-14.5 dpc, with reduced expression at later ages prior to birth. Both the male and female gonads expressed BG on the day of birth, neonatally and in adulthood.

Immunohistochemistry data indicated that BG protein was low in the embryonic ovary compared to the developing testis, for which protein expression was detected in interstitial somatic cells from 12.5-16.5 dpc. Expression was also localised to the cells surrounding the developing seminiferous cords. Low BG protein expression was detected in the male germ cells and pre-Sertoli cells. These data indicate that BG is differentially expressed between the sexes during gonadogenesis, and suggest a role for BG in male gonadal differentiation.

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DIFFERENCES IN TUMOUR NECROSIS FACTOR ALPHA PRODUCTION BY TERM AND PRETERM PLACENTAE

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Background: Placental cytokines may play a role in the initiation of spontaneous labour in term and preterm pregnancies. The production of tumour necrosis factor (TNF) alpha and other Th1 cytokines by the placenta are associated with chorioamnionitis and preterm delivery [1]. It has been observed that TNF alpha levels are higher in placentas from normal deliveries compared to preterm and premature rupture of membrane deliveries [2]. We were interested in characterising the response to an inflammatory challenge in placentae collected from term and preterm deliveries and whether there were any differences in the inhibitory effect of glucocorticoids on this pathway with gestational age.

Methods: Placentae were collected from normal term deliveries and preterm deliveries of pregnancies complicated by pre-eclampsia and IUGR. Placental explants were cultured for 24hrs and then exposed to lipopolysaccharide (LPS) (10μg/ml), in the presence and absence of 100nM dexamethasone, 1μM cortisol or 10μM cortisone. After 24hrs the supernatants were assayed for TNF alpha by sandwich ELISA.

Results: Placentae from term deliveries had a significantly higher TNF alpha response to LPS than preterm placentae ($p<0.05$). Glucocorticoid inhibition of placental TNF alpha production was significantly greater in term placentae than preterm placentae ($p<0.05$). Cortisone did not have any effect on term or preterm placental TNF alpha response.

Conclusion: These data suggest that placental TNF alpha production in response to inflammation increases with increasing gestational age. The inhibition of this response by glucocorticoids is significantly greater in term than preterm placentae suggesting an inflammatory challenge in preterm placentae may be significantly more detrimental.

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PLACENTAL GENE EXPRESSION IS ALTERED BY FETAL SEX, MATERNAL ASTHMA AND INHALED GLUCOCORTICOID INTAKE

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Background: Previous research examining the effect of maternal asthma during pregnancy on placental function and fetal outcome indicated there were sex specific differences in how the fetus responds to maternal asthma. These data suggested male and female fetuses initiate different mechanisms to the same stress and raised the question of whether there were global differences in placental gene expression in relation to fetal sex, maternal asthma and inhaled glucocorticoid treatment.

Methods: Using microarray we determined the gene expression profiles of placentae collected from male or female fetuses of normal, human pregnancies and pregnancies complicated by asthma in the presence and absence of inhaled glucocorticoid intake. Data was analysed using a Binary Tree Structured Vector Quantization algorithm which generates a gene expression map. Sites on the map where there were obvious differences in gene expression were selected for analysis.

Results: Placentae from female fetuses of asthmatic mothers that did not use inhaled steroids during pregnancy had 37 gene alterations relative to the control population. Placentae from male fetuses of asthmatic mothers that did not use inhaled steroids during pregnancy had 6 gene changes relative to the control population. Placentae from female fetuses of asthmatic mothers who did use inhaled steroids during pregnancy had 22 gene alterations relative to the control population and placentae of male fetuses had no gene changes. There were 10 placental genes altered in the presence of maternal asthma that were common to both male and female fetuses.

Conclusion: This data indicates that there are significant differences in how a placenta from a male fetus and female fetus respond to a maternal disease and raises the question of whether we are significantly compromising our interpretation of human placental data when we do not take the sex of the fetus into account.

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LOBE-SPECIFIC REDUCTION IN MATURE PROSTATE STRUCTURAL AND FUNCTIONAL DIFFERENTIATIATION IN PROSTATE EPITHELIAL SPECIFIC ANDROGEN RECEPTOR KNOCKOUT (PEARKO) MICE

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A functional androgen receptor (AR) is crucial for mesenchymal cell-dependent paracrine pathways that induce prostate development and cellular differentiation, but the distinct roles of AR in stromal and epithelial cells in the mature prostate remains to be defined. To study the physiological function of prostate epithelial AR in the mature prostate, we established a novel mouse model with targeted disruption of prostate epithelial AR. Floxed-AR allele carrying mice (exon3 flanked by loxP sites) were crossed with transgenic probasin promoter-driven Cre (Pbsn-cre) mice to generate epithelial-specific AR knockout (PEARKO) mice. Eight week old PEARKO males had significantly ($p<0.05$) decreased weight of all prostate lobes (36-80% of control), while serum testosterone levels and testis weight were unaltered. Functional cytodifferentiation of PEARKO prostate epithelial cells was reduced ($p<0.05$) in all lobes with most prominent effects in dorsolateral and anterior lobe epithelium. To evaluate the prostate structural differentiation, real-time RT-PCR was used to analyze mRNA abundance of cytokeratin 8 (CK8) for mature luminal epithelial cells and smooth-muscle α-actin (SMA) for smooth muscle. Real-time RT-PCR results were normalized against cyclophilin mRNA expression. Relative abundance of CK8 was unaltered while SMA was significantly ($p<0.05$) decreased in anterior and dorsolateral prostate, 46 and 53% of control, respectively. For functional cytodifferentiation, mRNA abundance was analyzed for prostate lobe-specific, androgen-dependent secretory protein genes: renin-1, probasin, and MP25 for anterior, dorsolateral and ventral prostate, respectively. The most significant changes were observed in anterior and dorsolateral prostates while ventral prostate was least affected. Relative expression of androgen responsive, lobe specific mRNA for AP, DLP and VP was significantly ($p<0.05$) decreased 72, 88 and 39% respectively. These results indicate that epithelial AR is required to maintain full prostate structural and functional differentiation, highlighting that prostate lobes are distinct in their morphology and hormonal responsiveness. (Funded by Cure Cancer Australia)

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UTERINE EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR-A mRNA IN HORMONE TREATED OVARIECTOMISED MICE

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Endometrial angiogenesis induced by oestrogen and progesterone is mediated by vascular endothelial growth factor (vegf-A) but the molecular mechanisms involved remain unclear. In this study we quantified relative changes in mRNA expression levels of total vegf-A, the individual vegf-A isoforms (120, 144, 164, 188 and 205) and the vegf-A receptors (flk-1 and associated receptors nrp-1 and nrp-2) in whole mouse uteri following oestrogen and progesterone treatment. *Animal Models: Progesterone Regime*: Mice (CBA x C57, n=9) were treated with a single injection of 100 ng of estradiol on day eight following ovariectomy, followed by a day with no treatment and three consecutive daily injections of 1 mg progesterone. Two groups were treated with either the vehicle (n=5) or progesterone only (n=10). All mice were dissected on day 13 after ovariectomy. *Short-term Oestrogen Regime:* Mice (n=9) were given a single injection of 100 ng of estradiol and dissected 24 hours later. mRNA expression was quantified by real time RT-PCR. All results were normalized against 18S rRNA. Relative levels of total vegf-A, flk-1, nrp-1 and nrp-2 mRNA were significantly lower ($p=0.01$) in those animals dissected 24 hours following oestrogen treatment compared with the mice treated with vehicle or progesterone only. Although there was no significant difference in the mRNA expression levels of the vegf-A isoforms 120, 164 and 188 between treatment groups, the highest levels of mRNA expression were detected for vegf 164 and the lowest levels for vegf 188. vegf-A 144 and 205 were not detected. We conclude that treatment of ovariectomized mice with oestrogen produces a concurrent reduction in total vegf-A, flk-1, nrp-1 and nrp-2 mRNA expression in whole uterine tissue.

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DEVELOPMENTAL EXPRESSION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS α AND γ IN THE BOVINE PLACENTA

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Peroxisome proliferator-activated receptors (PPARs) constitute a subfamily of nuclear hormone receptors that are involved in lipid metabolism, differentiation, proliferation and inflammation. PPAR α is expressed in highly oxidative tissues, and plays a key role in regulation of cellular uptake, activation and β-oxidation of fatty acids. PPAR γ is present in adipose tissue, and promotes adipocyte differentiation and lipid storage. Fatty acids (FAs), FA-derived compounds and eicosanoids such as prostaglandins are natural ligands for both PPARs. Gene expression of PPARs α and γ have been reported in human and rodent placenta and potentially play a role in placental development, but their role in bovine pregnancy is unclear.

This study aims to investigate the placental gene expression patterns of PPARs α and γ during early bovine pregnancy. Partial bovine PPARs α and γ genes were successfully cloned using RT-PCR and mRNA expression levels measured in developing bovine placenta (fetal cotyledons and maternal cotyledons) at Days 50, 100 and 150 gestation using RT-PCR and northern blot analyses. In maternal cotyledons, PPAR α expression peaked at Day 100 of gestation whereas very low levels were observed at Days 50 and 150. Very low levels of PPAR α expression was also detected in Day 150 fetal cotyledons. PPAR γ expression was observed only in maternal cotyledons at Day 50, and was present in both fetal and maternal cotyledons at Days 100 and 150 of gestation.

The presence of PPAR α and γ in the bovine placenta during early gestation suggest that both PPARs maybe required for metabolism of fatty acids in the bovine placenta. Our work provides a framework for the further investigation of PPARs and their specific role in regulation of placental development and function to ensure successful bovine fetal development.

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STEROIDOGENIC POTENTIAL OF THE BOVINE FETAL ADRENAL AT EARLY TO MID-GESTATION

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Steroid hormone synthesis from cholesterol involves the activity of several enzymes. During fetal development, the placenta and fetal adrenal complement each other in steroidogenic activity during pregnancy. This presentation focuses on determining the ontogeny of steroidogenic potential in the fetal bovine adrenal during early to mid-gestation. Fetal adrenal tissues were collected between Days 138 and 165 of gestation (full term ~270 days) from bovine fetuses generated by artificial insemination. The expression of the steroidogenic enzyme genes involved in glucocorticoid, mineralocorticoid and sex steroid synthesis were investigated in the fetal adrenal gland using Northern blotting with total RNA. The genes examined were: cholesterol desmolase (*Cyp11A*), 17α-hydroxylase (*Cyp17*), 3β-hydroxysteroid dehydrogenase (*3β-HSD*), 11β-hydroxylase (*Cyp11B*), steroid 21-hydroxylase (*Cyp21*) and cytochrome p450 aromatase (*Cyp19*). The cellular localization of these mRNAs was determined by *in situ* hybridization with digoxigenin-labelled probes. *Cyp11A*, *3β-HSD, Cyp21* and *Cyp11B* were all expressed in fetal adrenals from Days 138 to 165. There was no detectable *Cyp17* or *Cyp19* mRNA in the fetal adrenals at these stages. *In situ* hybridization showed that *Cyp11A*, *Cyp11B*, *Cyp21* and *3β-HSD* mRNA were all localized to the developing fetal adrenal cortex. Thus, *de novo* synthesis of progesterone, corticosterone and aldosterone from cholesterol can occur in the fetal adrenal from around mid-gestation. However, the lack of *Cyp17* and *Cyp19* expression suggests that the bovine fetal adrenal cannot synthesize cortisol and estrogens at this stage. The placenta likely complements the adrenal gland with the synthesis of the sex steroids, progesterone and estrogen, during bovine fetal development.

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THE IMPACT OF ANTENATAL CORTICOSTEROIDS ON UMBILICAL VENOUS AND ARTERIAL CORTISOL IN NEONATES 23-41 WEEKS GESTATION

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Aim: Corticosteroids are employed clinically to improve newborn outcome in cases determined to be at risk of preterm birth. We have previously demonstrated that there is a sex specific response to a change in cortisol concentration in term neonates. We investigated the effect of antenatal glucocorticoids on umbilical cortisol in preterm neonates in relation to birth weight, gestational age and sex.

Methods: Preterm infants (n=61) exposed (n=28) or not exposed to antenatal glucocorticoids within 10 days of delivery, were studied. Umbilical venous (UV) and arterial (UA) cortisol was determined by radioimmunoassay.

Results: Cortisol levels in UA and UV showed a positive relationship (r^2=0.677, p<0.0001). The betamethasone exposed group (A) had a lower gestational age (p=0.0001), birth weight (p=0.001), and UV cortisol (125.58 ± 33 nmol/l vs 251.47 ± 42.9 nmol/l, p=0.09) when compared to the non-betamethasone exposed group (B). In A there was no relationship between UV cortisol and birth weight in male infants but female infants demonstrated a negative correlation (r^2=0.284, p=0.06). In B there was a positive correlation between UV cortisol and gestational age (r^2=0.3316, p=0.0005) and UV cortisol and birth weight (r^2=0.3195, p=0.0004). There was no relationship between fetal sex, birth weight and UV cortisol in B.

Conclusion: Preterm neonates not exposed to betamethasone before delivery had increased umbilical venous cortisol concentrations with advancing gestation and birth weight. The administration of maternal betamethasone resulted in a sex-specific response in relation to birth weight and UV cortisol. Further investigation of sex-specific effects of maternal betamethasone on the fetal HPA and its down-stream actions is warranted.

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DILATED CARDIOMYOPATHY IN A PATIENT WITH CUSHING'S SYNDROME

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A 28-year-old lady was admitted with a 3-week history of dyspnoea. Her medical history was unremarkable except for smoking. Examination revealed signs of biventicular failure. Features of Cushing's syndrome, with mild proximal limb weakness, were also present. She does not drink alcohol and had no family history of cardiomyopathy.

Her initial electrocardiogram showed sinus tachycardia with left atrial enlargement and chest X-ray revealed cardiomegaly and pulmonary oedema. Transthoracic echocardiography showed dilated left ventricle, ejection fraction (EF) of 34% and mild mitral regurgitation, consistent with dilated cardiomyopathy. No auto-immune or infective cause for the cardiomyopathy was found.

Twenty-four hour urinary free cortisol was markedly elevated (964 nmol/24 hours; reference range: 50-350 nmol/24 hours), with suppressed corticotropin (ACTH). Computed tomography of the abdomen showed a 3.8 x 2.3 cm right homogenous adrenal mass with low attenuation. Therefore findings were consistent with ACTH-independent Cushing's syndrome, most likely due to adrenal adenoma.

Her cardiac failure was treated with ramipril, carvedilol, spironolactone and frusemide. However due to her poor cardiac function, adrenalectomy had to be delayed. In the interim, she was treated with ketoconazole to inhibit steroidogenesis. Three months after ketoconazole treatment, urinary free cortisol level was lower (229 nmol/24 hours).

Six months after treatment, her symptoms improved; repeat echocardiography showed reduction in left ventricular size and EF improved to 50%. She then underwent right adrenalectomy. Pathology confirmed a benign adrenal adenoma. She developed postoperative tertiary adrenal insufficiency and required hydrocortisone therapy. Six months after surgery, features of Cushing's syndrome were resolving and repeat echocardiography showed normal left ventricular size and EF (67%).

Reversible dilated cardiomyopathy is a rare complication of Cushing's syndrome, reported only in two previous cases. Although skeletal myopathy is a well recognised complication of Cushing's syndrome, our knowledge of cardiomyopathy in this setting is more limited. A literature review of cardiac function in Cushing's syndrome will be discussed.

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ACROMEGALY AND THYROID SIZE

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AIM: To assess the prevalence of thyroid goitre in individuals with acromegaly and to determine the effect of successful treatment on thyroid size.

Method: A retrospective analysis was conducted of individuals receiving treatment in Westmead Hospital from 1994 to 2005. Each individual had to have at least two thyroid ultrasounds, along with serial measures of IGF-1 and TSH. RESULTS: Results are presented as mean and SD. 9 individuals (7 male, 2 female) were included in the sample. They had a mean duration of disease of 14.67 + 6.25 yrs. While all received Octreotide LAR treatment, they differed in prior treatment modalities. Initial and current results include: TSH: 1.66 +/- 1.96mU/L vs. 1.02 + 0.74mU/L; IGF-1: 70.78 +/- 34.01nmol/L vs. 28.71 +/- 9.64nmol/L. The only significant difference was in IGF-1 levels (t-score=3.57, p<0.01). The initial thyroid ultrasounds showed Thyroid Volume (TV): 23.59 +/- 13.73mls. The current thyroid ultrasounds showed

TV: 25.30 +/- 15.17mls. There were no statistically significant correlation between initial and current TVs with respect to TSH and IGF-1. Disease control was defined as >75% of an individual's recorded IGF-1 being < 45nmol/L. Those with good control (n=6) were compared to those with poor control (n=3). Initial and current TV for the good control group was: 18.97+/- 14.82mls vs. 22.25 +/- 13.60mls. Initial and current TV for the poor control group was: 32.78 +/- 3.85mls vs. 31.42 +/- 19.37mls. There was a significant difference in the initial TVs between good and poor control (t-score=2.14, $p<0.05$).

DISCUSSION: Acromegaly was associated with increased thyroid size. There was no regression of thyroid size despite adequate control of IGF-1. Between those with good and poor control, there was no difference in current TVs. However, those with poor control longitudinally had improved IGF-1 levels which may explain the lack of difference. There is scope for larger cohort studies to follow the progression of the thyroid in adequately treated acromegaly.

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THE USE OF PRE-OPERATIVE ULTRASOUND MAPPING OF CERVICAL LYMPH NODES TO GUIDE SURGERY FOR PERSISTENT AND RECURRENT PAPILLARY THYROID CARCINOMA

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Cervical lymph node (LN) metastases from papillary thyroid carcinoma (PTC) are associated with an increased loco-regional recurrence rate. Surgery remains the therapeutic modality of choice for resectable neck disease in the absence of widespread metastases and in the context of negative whole body scan (WBS). The use of routine prophylactic neck dissection is unpopular with data suggesting that it has no effect on disease-specific mortality. Techniques for LN dissection include “berry-picking” (not advocated for initial surgical treatment of PTC lymphatic metastases), selective LN dissection (a targeted lymphadenectomy more relevant to the nature of the spread of PTC) and modified radical LN dissection (levels I-V are removed). The ideal surgical method to reduce morbidity from recurrent laryngeal nerve neuropraxia and improve surgical outcomes remains uncertain. In recent years several strategies have been used to guide and improve the accuracy and outcome of surgical resection of persistent or recurrent disease including pre-operative neck ultrasound mapping, sentinel node detection using “blue dye”, therapeutic ^{131}I followed by radio-detector probe-guided surgery and intra-operative ultrasound.

We describe our institution's experience with pre-operative ultrasound mapping of loco-regional neck disease in patients with PTC in the context of elevated thyroglobulin and negative WBS. High resolution ultrasound using a 10-14MHz probe is performed and the location of abnormal nodes is marked on the skin using a surgical pen with indelible ink. When the patient is draped at surgery, a sterile clear plastic film is fixed over the operative field and marked with “X”s corresponding with the skin marks. This film can be replaced over the field at any time to guide surgical dissection. Intra-operative ultrasound is used to guide the surgeon to the node under the “X” to ensure all abnormal tissue is removed. This technique may prove to have significant impact on surgical ou tcomes.

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THE DIAGNOSTIC VALUE OF NECK ULTRASOUND AND THYROGLOBULIN MEASUREMENT IN THE FINE NEEDLE ASPIRATE FROM CERVICAL LYMPH NODES IN THE FOLLOW-UP OF PATIENTS WITH PAPILLARY THYROID CARCINOMA

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Papillary thyroid carcinoma (PTC) is the most frequent histological type of differentiated thyroid cancer, with neck lymph node metastases found in up to 70% of cases. TSH-stimulated thyroglobulin (Tg) and whole body scan (WBS) are currently the main surveillance paradigms. However Tg positive, WBS negative disease necessitates further imaging which includes computerised tomography (limited in identifying subcentimetre lymph nodes), ^{18}FDG-PET scan (expensive) and neck ultrasound. Features of metastases on ultrasound include reduced internal echoes, non-homogeneity, a rounded or bulging shape, an absent hilar echogenic line, height : width ratio >0.5 in the transverse view, microcalcification or a cystic component and increased internal vascular signature. The diagnostic usefulness of fine needle aspiration cytology (FNAC) of metastatic neck lymph nodes is limited by the presence of cystic change, which can occur in up to 70% of metastatic PTC (1). Thyroglobulin measurement in the elute from fine needle aspiration (Tg-FNA) is an emerging technique used to diagnose neck metastases and recurrence. Tg-FNA is not affected by the presence of serum Tg antibodies (2). This modality combined with cytology increases the sensitivity of detecting PTC metastases from 76% to 100% (3,4).

We report our institution's experience of Tg-FNA with a small series of eight patients with PTC who have undergone both FNAC and Tg-FNA of suspicious neck lymph nodes detected at follow-up on ultrasound in the setting of elevated serum Tg and negative WBS. Ultrasound in conjunction with Tg-FNA has proved to be an efficient method of surveillance of these patients. Tg-FNA increases the sensitivity and may be superior to FNAC in detecting lymph node metastases from PTC.

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THE PREVALENCE OF THYROID DISEASE AND RELATED RISK FACTORS IN TASMANIA

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Introduction: Recent studies have disclosed re-emergent iodine deficiency (ID) in Tasmania, as well as previously unrecognized endemic ID in mainland Australian states. However, the prevalence of thyroid disease (TD) and the long-term sequelae of ID in the adult Australian population is poorly characterised.

Aim: To evaluate the prevalence of TD in the adult Tasmanian population using a questionnaire and ultrasonographic methodology.

Methods: A random sample of 10 000 adults registered on the Tasmania electoral role in the year 1999. 5774 (57.7%) respondents aged 18-85 years completed a health questionnaire, of whom 463 were randomly selected to undergo thyroid ultrasound.

Results: An history of TD was reported by 3.9% and 15.7% of male and female respondents respectively. The prevalence of goitre was higher for individuals residing in Tasmania since birth* compared to those not born in Tasmania and living greater than 19 years elsewhere** However, ultrasonography revealed thyroid nodules (particularly multinodularity) to be at least as frequent irrespective of birth place and subsequent State of residence (Table)

Characteristics	Born in Tasmania*		Not born in Tasmania**	
	Male %	Female %	Male %	Female %
Questionnaire (n=5774)	24.6	29.9	9.8	9.1
Age (yrs)	51.9±0.5	51.1±0.4	60.7±0.6	58.3±0.7
Goitre	2.5	7.4	0.9	4.2
Thyroid surgery	1.3	4.5	0.7	3.8
Thyroid cancer	0.4	0.9	0.5	0.6
Hyperthyroid	1.1	4.6	1.4	2.9
Hypothyroid	1.2	5.7	0.9	5.5
On thyroxine	0.6	3.9	0.4	3.3
FHx of thyroid disease	11.1	23.6	8.5	13.0
Ultrasonography(n=205)	18.5	34.6	8.3	12.2
Age (yrs)	52.8±2.6	47.7±1.3	64.8±2.6	60.2±2.1
Thyroid volume (ml)	12.7±0.8	8.6±0.6	10.4±1.3	10.1±0.9
No Nodules	71.1	53.5	58.8	40.0
Nodules	28.9	46.5	41.2	60.0
1 nodule	13.2	11.3	11.8	4.0
Multiple nodules	15.7	35.2	29.4	56.0

Conclusion: TD is common both in Tasmania and the broader Australian population. Subclinical thyroid nodularity is frequent and not restricted regions of historical ID .

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THE INFLUENCE OF GESTATION ON URINARY IODINE EXCRETION IN PREGNANCY

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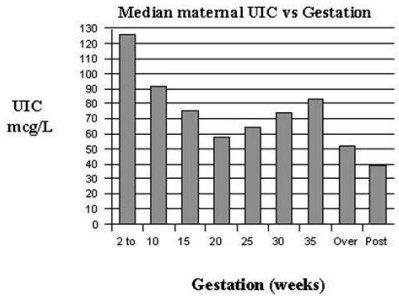
Background: The recommended daily intake of iodine is 200-250 mcg/d during pregnancy, compared to 150mcg/d in non-pregnant adults and 90-120 mcg/d in children. Renal iodine excretion is glomerular filtration rate (GFR) dependant, and GFR increases during pregnancy. Whereas community iodine nutrition is deemed sufficient when median urinary iodine concentration (UIC) in primary school children is ≥100mcg/L, normative ranges for pregnancy have not been established. This study evaluates changes in UIC during pregnancy in a population with documented iodine deficiency (ID).

Methods: 698 urine samples were collected from 461 women attending the antenatal clinic at the Royal Hobart Hospital. The study was conducted between 1998-2001 when median UIC in primary school age children was 84µg/L.

Results: Overall median UIC during pregnancy was 78µg/L at a mean of 22.0 weeks gestation. Stratification by gestation at time of sampling revealed UIC initially increased in early pregnancy, then declined with advancing gestation (Table and Figure).

Gestation (wks)	*n=*	*Median UIC (mcg/L)*	*% women < 50mcg/L*
2 to 9.9	18	126	22.2
10 to 14.9	181	92	24.9
15 to 19.9	168	75	25.0
20 to 24.9	46	58	47.8
25 to 29.9	36	64	27.8
30 to 34.9	59	74	33.9
35 to 39.9	99	83	24.2
over 40 weeks	20	52	46.2
Post partum	71	39	66.2



Conclusion: After an initial GFR related rise in iodine excretion compared to the non-pregnant population, UIC decreases as pregnancy progresses, thus increasing the proportion of women falling into a range indicative of moderately severe ID. These results indicate that assessment of ID in pregnancy requires gestation specific reference ranges. Reporting of non-stratified median UIC in pregnancy is likely to underestimate the severity of ID in pregnant women.

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IMPLEMENTATION AND EVALUATION OF A PROTOCOL FOR REDUCED GLUCOCORTICOID REPLACEMENT IN PITUITARY SURGERY.

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Introduction: For many years the prescription of high doses of glucocorticoids following pituitary surgery has been standard care at RMH and the majority of neurosurgical centres worldwide. This practice may be associated with an unacceptably high incidence of post-operative Cushing's syndrome. A recent review of this practice recommended much lower doses of glucocorticoids, to be given only if there were a high likelihood of post-operative deficiency or biochemical evidence of hypocortisolism.

Aim: To design and implement a protocol of reduced glucocorticoid replacement in pituitary surgery and assess its utility by comparing clinical and biochemical outcomes of pituitary operations performed before (2002-2003; Group A) and after (2004-2005; Group B) its implementation.

Results: 45 and 41 operations were included in Groups A and B, respectively. There were no significant differences in age, gender, underlying pathology or operative approach between the two groups. Frequency of glucocorticoid use was higher in Group A before, during and after surgery. Long-term (beyond eight weeks after surgery) use of glucocorticoids was 79% v 20% in Groups A and B respectively. There was more post-operative diabetes insipidus (29% v 15%) and a similar frequency of adrenal crisis (4% v 5%). The length of stay was higher in Group A (7.2±0.4 v 5.6±0.5 days).

Conclusion: Our protocol of selective glucocorticoid replacement in pituitary surgery is safe and associated with improved clinical outcomes.

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THE EFFECT OF WEIGHT LOSS ON BLOOD PRESSURE, SALT SENSITIVITY AND ADRENOCORTICAL FUNCTION

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Background: The hypothalamic-pituitary-adrenal axis (HPA) and the renin-angiotensin-aldosterone system (RAAS) have been implicated in the pathophysiology of obesity induced hypertension. However, there is little data on the effect of moderate weight loss on the blood pressure response to salt loading and adrenocortical function.

Study design: Twenty five obese subjects (age 39-63 yr, BMI 32.9 ± 4.3 kg/m^2) followed a 12 week weight loss diet before and after which they followed a high (250 mmol/d) or low salt (30 mmol/day) diet for 2 weeks crossed-over and randomised. After each diet, 24-hr ambulatory blood pressure, plasma aldosterone, renin concentrations, 24-hour urinary free cortisol/cortisone, plasma corticosteroid-binding globulin (CBG), low dose (1 mcg IV) ACTH stimulation tests with measures of plasma total and free cortisol concentrations were performed.

Results: Mean arterial pressure fell by 6 mmHg after 7.7 kg weight loss. Salt loading elevated day time blood pressure by 6/3 mmHg which was not altered by weight loss. Plasma aldosterone and renin levels fell with weight loss (aldosterone: 853 ± 156 to 635 ± 73 pmol/L ; renin: 35.4 ± 7 to 24 ± 3 mU/L $P<0.05$). HPA axis measures were not affected by weight loss; there was no change in the plasma total and free cortisol responses to cosyntropin nor did plasma CBG levels change. The 11 beta hydroxysteroid dehydrogenase 1 (11β-HSD1) activity, represented by the ratio of urinary free cortisol to cortisone, and the 24-hour urine free cortisol levels were also unchanged.

Conclusions: Short-term, moderate weight loss was associated with a small reduction in blood pressure and reduced levels of aldosterone and renin. The blood pressure elevating effect of a salt load was not altered. Cortisol secretion or metabolism were unchanged. These findings suggest that aldosterone may have an important role in the BP fall with weight loss via a renin mediated mechanism, perhaps involving renal sympathetic tone.

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PREVALENCE OF VITAMIN D DEFICIENCY IN PATIENTS UNDERGOING PRIMARY ELECTIVE KNEE AND HIP ARTHROPLASTY.

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Vitamin D (25OHD) deficiency remains highly prevalent in western countries including Australia (1). Groups at risk include nursing home residents, darkly-pigmented people and veiled and breast-feeding mothers and their offspring. Osteoarthritis (OA) is the most prevalent form of arthritis causing restricted mobility and hence potentially limits sunlight exposure. This study was undertaken to determine the prevalence of 25OHD deficiency in patients undergoing elective hip and knee arthroplasty for OA. 192 consecutive patients (59M) aged 15-96yr (median 66yr) referred to one surgeon for arthroplasty over 29 months were studied. Those already taking vitamin D supplements and those without OA were excluded. Concentrations of 25OHD were measured by competitive protein binding (Nichols Advantage Specialty System) and classified as no (25OHD > 65 nmol/L), borderline (50.1-65), mild (25.1-50), moderate (12.5-25) or severe (<12.5) deficiency (2). The range was 17-201 nmol/L with moderate deficiency in 5.2% (n=10, all female), mild in 34.9% (52F, 15M), and borderline in 20.3% (25F, 14M). Mild or moderate deficiency was more prevalent in females (F: 62/133, 46.6% vs M: 15/59, 25.4%, chi-square = 7.64, p=0.01). Although 16/27 (59.3%) of females tested in winter had mild or moderate deficiency and only 1/12 (8.3%) of males tested in autumn had mild deficiency, overall there was no significant seasonal variation (chi-square = 6.2, p=NS). Mean (±S.D.) seasonal 25OHD concentrations were: summer 58.7±32.1 (n=43) nmol/L, autumn 64.3±25.8 (54), winter 58.5±21.1 (46), and spring 59.4±25.4 (49). 25OHD concentrations did not correlate with age, height, weight or BMI.

In this relatively high socioeconomic group, patients with severe OA (especially females) are at significant risk of 25OHD deficiency. Since this may cause suboptimal new bone quality (in non-cemented prostheses)(3) and reduced muscle tone during rehabilitation which may contribute to risk of falls, 25OHD deficiency should be excluded prior to surgery.

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ANDROGEN MEDIATED ERYTHROPOIESIS OCCURS VIA CLASSICAL ANDROGEN RECEPTOR SIGNALLING IN MALE MICE

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Androgens are known to increase erythropoiesis however the mechanisms remain unclear. Both the use and abuse of androgens have been shown to increase haematocrit and in some cases to higher than normal levels while anaemia has been reported following treatment with various forms of androgen blockade. Many of the clinical and in vivo and in vitro studies to establish androgen effects on erythropoiesis are old (>20 years) and have reported conflicting data.

We now have improved research tools to investigate the mechanisms of androgen mediated erythropoiesis. We have generated a global androgen receptor knockout mouse (ARKO). In this mouse model classical signalling of the AR is disrupted by deletion of exon 3 which encodes the second zinc finger of the DNA binding domain (Notini, Davey et al. 2005).

Red blood cell (rbc) parameters measured in the blood of 9 to 10 week old male ARKO mice were not significantly different from their male wildtype (wt) littermates. ARKO (n=9): rbc 8.58 (mean) ± 0.14 (SE), Haematocrit (Hct) 45 ± 1.3, Haemoglobin (Hb) 137 ± 1.1; wt (n=3): rbc 8.50 ± 0.11, Hct 44 ± 1.2, Hb 135 ± 1.8. These findings are unsurprising given that there are no reports of anaemia in individuals with androgen insensitivity syndrome.

We have also treated 3 to 4 week old male ARKO mice and their male wt littermates with subcutaneous Silastic implants containing testosterone for a 6 week period. Blood, bone marrow and spleen were collected for differential analysis. Our preliminary results show that the red blood cell count is increased by 6% in response to testosterone in male wt mice and NOT male ARKO mice. Wt (plus testosterone) (n=5): rbc 9.00 ± 0.12 vs wt (no treatment) (n=3): rbc 8.50 ± 0.11, $p<0.05$.

In conclusion, we have demonstrated that the classical signalling pathway of the AR is required for androgen mediated erythropoiesis.

(1) Notini, A. J., R. A. Davey, J. F. McManus et al. (2005). "Genomic actions of the androgen receptor are required for normal male sexual differentiation in a mouse model." J Mol Endocrinol 35(3): 547-55.

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OESTROGEN REGULATION OF THE LIVER RECEPTOR HOMOLOGUE (LRH-1) WITHIN TESTICULAR CELLS.

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The orphan nuclear receptor LRH-1 is expressed in Leydig cells, pachytene spermatocytes and round spermatids, which is similar to the expression of aromatase. We have previously shown that LRH-1 can regulate aromatase expression in these cells by directly binding to the gonadal-type promoter of the CYP19 gene (promoter II) and stimulating transcription. Little is known regarding the regulation of LRH-1; however, recently a role for estrogen receptor alpha (ERalpha) in stimulating LRH-1 expression in breast cancer cells was proposed[(1)]. We therefore hypothesised that aromatase may maintain its own expression in the testis by inducing ERs to activate LRH-1 expression, which in turn increases aromatase promoter II activity. To begin to address this we have used a transgenic mouse that over-expresses human aromatase[(2)] (Arom+). Compared to wild type mice, Arom+ mice displayed a 553% increase in LRH-1 protein expression in the whole testis as determined by Western analysis. Total ER protein levels were also over-expressed in the Arom+ mice by 168%. Immunohistochemical analysis demonstrated that LRH-1 is co-localised with ERalpha in Leydig cells, and with ERbeta in Leydig cells, pachytene spermatocytes and round spermatids in both wild type and Arom+ mice. We are currently assessing whether oestrogen can directly stimulate LRH-1 expression in appropriate cell lines to determine the pathway of this apparent positive feedback mechanism. Our current work therefore indicates that LRH-1 has important roles in estrogen production and regulation in the testis, which may in turn have implications for testicular development, fertility and tumorogenesis.

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'GAIN OF FUNCTION' ANALYSIS IN SKELETAL MUSCLE CELLS SUGGESTS RETINOIC ACID RECEPTOR RELATED ORPHAN RECEPTOR GAMMA CONTROLS METABOLIC GENE EXPRESSION.

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Nuclear Hormone Receptors (NRs) have been demonstrated to regulate metabolism in a cell/organ specific manner. The NR1F (Retinoic acid receptor related Orphan Receptors - RORs) subgroup includes three members: RORα, RORβ and RORγ. The staggerer mice carry a deletion in the RORα gene and display lowered plasma apoA-I/-II, apoC-III, decreased plasma high density lipoprotein cholesterol and triglycerides, develop hypo-α-lipoproteinemia and atherosclerosis. Previously we have shown that, RORα regulates the expression of genes involved in lipid homeostasis in skeletal muscle cells. However, RORγ is also abundantly expressed in skeletal muscle, a major mass peripheral tissue that accounts for 40% of total body mass and energy expenditure. This lean tissue is a primary site of glucose and lipid utilization. Consequently, we utilized gain and loss of function studies in skeletal muscle cells to understand the regulatory role of RORγ in muscle cells. Exogenous dominant negative RORγ expression in skeletal muscle cells specifically represses the endogenous levels of RORγ mRNA, however 'loss of function' did not show a clear phenotype. Interestingly exogenous VP16-RORγ expression (i.e. gain of function) in skeletal muscle cells enhances the endogenous levels of RORα and RORγ mRNA expression. In addition, we observed enhanced expression of many genes involved in lipid homeostasis. This study implicates RORγ in the control of fatty acid utilization in skeletal muscle cells.

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REGULATION OF BODY COMPOSITION DEVELOPMENT BY THE SKI PROTO-ONCOGENE *IN VIVO*

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Introduction: Body composition (i.e. the amount and proportion of body fat, lean tissue and bone) is regulated by a variety of metabolic and hormonal processes and is an important determinant of general health. The Ski proto-oncogene, is a negative regulator of TGF-β signalling. Ski overexpression in a transgenic mouse model leads to a marked increase in muscle and decrease in fat mass. The aim of this study was to investigate the molecular mechanisms of Ski mediated regulation of body composition.

Methods: Growth analysis was conducted on c-Ski Tg mice and their wild-type littermates. At 15 weeks a detailed body composition (BC) analysis was performed using dual-energy X-ray absorptiometry. Gastrocnemius muscles were harvested from 12 month old male mice for gene expression analysis by quantitative real-time PCR.

Results: Ski Tg mice gained more weight between 4 and 15 weeks of age, than their wild-type littermates. At 15 weeks BC analysis of male Tg mice showed both increased lean body mass (26.9±2.2g vs 20.8±1.0g) and reduced total fat mass (2.5±0.5g vs 3.1±0.2g). Expression analysis, relative to wild-type mice, of key metabolic genes revealed a 2-fold decrease in myostatin ($P<0.001$), a negative regulator of muscle growth, and a 10-fold decrease in SREBP-1c ($P<0.001$), a key transcriptional activator of lipogenesis. Transactivation assays revealed that Ski represses activity of the SREBP1c promoter. Expression of downstream targets of SREBP-1c, FAS and SCD-1, were downregulated by 3-fold ($P<0.005$). Attenuation of expression of the glucocorticoid receptor, PPARγ and several orphan NRs involved in metabolism was observed.

Conclusion: The skeletal muscle of c-Ski Tg mice have significant alterations in expression of several muscle and lipogenic regulatory genes. This suggests Ski acts as a major regulator of factors that control body composition and hence risk for obesity and metabolic disease.

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NR3B3 (ERRγ) CONTROLS PATHWAYS THAT REGULATE MUSCLE MASS AND ADIPOSITY IN SKELETAL MUSCLE CELLS.

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Nuclear hormone receptors (NRs) are ligand dependent DNA binding proteins, that translate physiological and nutritional signals into gene regulation. The estrogen receptor-related receptors (ERR) are orphan members of the steroid and NR gene superfamily. The ERR subfamily is comprised of three members ERRα (NR3B1), ERRβ (NR3B2) and ERRγ (NR3B3). ERRα has been demonstrated to control lipid, glucose and energy homeostasis, in an organ specific manner. ERRγ is abundantly expressed in tissues with onerous energy demands such as skeletal muscle (heart, kidney and brown adipose) that depend on mitochondrial fatty acid oxidation for energy. ERRγ is more closely related to ERRβ in its sequence than ERRα, however, its pattern of expression is similar to that of ERRα. PGC-1 is a critical coactivator of ERRα, and has also been demonstrated to interact with ERRγ and serves as a key regulator of mitochondrial biogenesis and mitochondrial oxidative metabolism.

Skeletal muscle, a major mass tissue accounts for ~40% of the total body mass and energy expenditure, and is a major site of fatty acid and glucose oxidation. The obscure role of this orphan NR in this peripheral lean tissue prompted us to investigate the ERRγ function in the regulation of genetic programs that control lipid utilization in skeletal muscle cells. We demonstrate that ERRγ is dramatically induced during in vitro skeletal myogenesis. Moreover, we show that the recently described ERRγ agonist and antagonist, increase and decrease GAL4-ERRγ activity in skeletal muscle cells, respectively, in a dose dependent manner. Furthermore, we demonstrate that ERRγ, a constitutively active orphan NR interacts and recruits coactivators in an agonist independent manner. In the in vitro cell culture model, treatment of cells with ERRγ agonist and antagonists leads to changes in gene expression involved in the regulation of muscle mass and adiposity. In conclusion, NR3B3/ERRγ regulates pathways that control muscle mass, adiposity and lipid utilization in skeletal muscle.

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INCREASED INTRAMYOCELLULAR LIPID CAUSES SKELETAL MUSCLE INSULIN RESISTANCE IN THE YOUNG ADULT GUINEA PIG OF LOW BIRTHWEIGHT

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Restricted fetal growth is characterised by insulin resistance of glucose uptake and utilisation in skeletal muscle in the adult, but the underlying mechanisms responsible are poorly understood. Some known risk factors for insulin resistance act via impaired 5' AMP activated protein kinase (AMPK)/ malonyl CoA fuel sensing and activity, increasing intramyocellular lipids, which impair insulin signalling and its targets. We therefore hypothesized that fetal growth restriction would cause skeletal muscle insulin resistance and increase intramyocellular lipids in the young adult guinea pig, and that activation of AMPK by 5-aminoimidazole 4-carboxamine-riboside (AICAR) to lower intramyocellular lipids would normalise this. Young adult guinea pigs of varying size at birth (n=16) underwent hyperinsulinaemic euglycaemic clamps (HEC) with a bolus of D-2-[1-^{14}C]-deoxyglucose to measure whole body and skeletal muscle insulin sensitivity. Intramyocellular lipid in vastus lateralis and biceps femoris was also quantified. Additional guinea pigs (n=9) were treated with AICAR (60mg/kg sc every 2 days) then HEC carried out. Intramyocellular lipid in the vastus lateralis and biceps femoris generally correlated negatively with birthweight in controls, but positively with birthweight in AICAR treated animals. In particular, low birthweight female guinea pigs exhibit a decrease in intramyocellular lipid in the biceps femoris following AICAR. Insulin sensitivity of glucose uptake and phosphorylation, incorporation into glycogen in skeletal muscle decreased with birthweight in controls, but this was abolished by AICAR. AICAR also increased whole body insulin sensitivity of young adult guinea pigs of low birthweight (+50%) to that seen in high birthweight animals. Therefore AMPK activation by AICAR normalised intramyocellular lipid and insulin sensitivity, suggesting that dysregulated lipid metabolism and accumulation of inhibitory lipid species caused the prenatally induced insulin resistance.

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ANDROGEN REGULATION OF SATELLITE CELL FUNCTION IN VITRO

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Androgens increase the size and strength of muscle in humans. Satellite cells (quiescent myoblasts) are the major source for muscle growth and regeneration. The androgen receptor (AR) has been found in satellite cells. However, the mechanism by which androgens regulate satellite cell function remains unclear. The present study is to investigate the effects of androgen regulation of myoblasts in vitro. Firstly, due to the low level of the AR expression in endogenous C2C12 cells (a mouse myoblast cell line), C2C12 cells overexpressing the AR cDNA driven by the SV40 promoter or control plasmids were generated by stable transfection. Secondly, qualitative determination of the level of the AR protein in C2C12 cells overexpressing the AR cDNA was performed using Western analysis. The cell line demonstrating the highest level of the AR protein compared to the endogenous C2C12 cells was chosen for further experiments. Transfected cells were cultured in charcoal stripped fetal calf serum (CS-FCS) with the addition of either testosterone or dihydrotestosterone (DHT) at concentrations ranging from 10-9 to 10-6 M every 24 hours for up to 3 days. The MTT assay was used to quantitate cell proliferation. No significant effect of androgens on proliferation of transfected C2C12 cells was observed. Differentiation of myoblasts into myotubes is indispensable for myoblast contributing to myofiber formation. The differentiation of C2C12 cells was induced with 3% CS-horse serum with administration of 10-7M DHT or vehicle. Creatine kinase, which is produced by differentiated myoblasts, is being used to quantitate the effect of androgens on myoblast differentiation. Investigation of the change of the AR mRNA signal and other potential androgen responsive target genes in transfected cells will be conducted by using real-time PCR to further explore roles of androgens in modulation of satellite cell function.

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METASTATIC MACROPROLACTINOMA IN MULTIPLE ENDOCRINE NEOPLASIA TYPE 1 (MEN-1) MIMICKING MENINGIOMA IN CERVICAL CORD

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Prolactinomas are common, but metastatic prolactinomas rare. We report a 47 year-old man with MEN-1, and a metastatic prolactinoma masquerading as a cervical meningioma. He has an IVS2-3 mutation (splice site C→G substitution[1]). A 7mm microprolactinoma was diagnosed on computerised tomography (CT) pituitary in 1990. Prolactin was 7355 mU/L. He was lost to follow up. Three years later prolactin was 26,833 mU/L and the tumour was compressing the optic chiasm. On bromocriptine for 1 year prolactin fell to 6545mU/L and the tumour shrank. Prolactin increased to 18,998 mU/L over 2 years, and the tumour grew to the superior aspect of the right internal carotid artery, depressed the floor of the fossa into the right sphenoid sinus, and bowed the optic chiasm. Trans-sphenoidal hypophysectomy was performed. After three years of loss to follow-up, the tumour had grown to 22 mm diameter. Prolactin was 18,311 mU/L. Cabergoline, 2mg / week, did not shrink the tumour, which could not be excised trans-sphenoidally. Incomplete excision was performed by craniotomy and 45Gy radiotherapy given. Magnetic resonance imaging (MRI) showed no apparent residual tumour. Six years later he developed neck pain initially thought to be due to cervical spondylosis, but prolactin was 98,680 mU/L and MRI showed masses in the spinal cord, cerebello-pontine angle and the left side of the foramen magnum. The cervical tumour was initially misdiagnosed as a meningioma but resection confirmed prolactinoma. This spinal lesion was extradural, lobulated, slightly hyperintense to the spinal cord on T1-weighted images and showed moderate and uniform enhancement after intravenous contrast, all commonly seen with meningioma. In the posterior fossa there was enhancement of the tentorium, consistent with a dural tumour tail of meningioma.

In patients with macroprolactinoma, particularly if refractory to dopaminergic therapy, any neurological symptoms or signs may be related, even if extra-cranial.

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ABLATIVE RADIOIODINE THERAPY IN A CASE OF CONCURRENT THYROID CARCINOMA, GRAVES' DISEASE WITH ASSOCIATED OPHTHALMOPATHY AND JUVENILE ONSET GLAUCOMA

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Radioiodine therapy is a well established treatment for differentiated thyroid carcinoma and Graves' hyperthyroidism. However, radioiodine therapy has been associated with exacerbation of Graves' ophthalmopathy, which can be ameliorated by the administration of corticosteroids. We hereby report the case of a 42 year-old man with a background of Juvenile Onset Glaucoma who presented with severe Graves' hyperthyroidism (FT4 >77 pmol/L), Graves' ophthalmopathy and cervical lymphadenopathy, with FNA cytology indicating metastatic papillary carcinoma. He was rendered euthyroid with propylthiouracil and underwent a total thyroidectomy and right modified radical neck dissection. Histopathology confirmed the presence of papillary carcinoma with metastatic disease to ten lymph nodes. Four weeks after his thyroidectomy, he received his initial dose of ablative radioiodine. In light of his Graves' ophthalmopathy, he was administered prophylactic corticosteroids. His treatment was complicated by a worsening of his Graves' ophthalmopathy in addition to acutely rising intra-ocular pressures. After an urgent ophthalmology review, his glaucoma medications were altered, corticosteroids were continued and he had a subsequent recovery from his eye symptoms. The concurrent presence of all of these conditions in a patient is rarely encountered and this case demonstrates the challenges faced by clinicians when weighing up the benefits of radioiodine therapy, the risks of a deterioration of Graves' associated ophthalmopathy and the potential side effects when prophylactic corticosteroid are utilised.

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PITUICYTOMA: AN UNUSUAL PRIMARY TUMOR OF THE PITUITARY WITH CHARACTERISTIC MRI AND HISTOPATHOLOGICAL FINDINGS

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Mr P.W., a 43 yo single male, identical twin, presented with a 2 year history of lethargy and declining physical strength. He had noted 15 months of headaches and occasional visual disturbance on a background of several years of gradual loss of body hair and a reduced libido. Past medical history included recurrent finger infections, amoebic dysentery and surgery for an undescended testicle at age 6. He was on no regular medications. Examination revealed features of hypopituitarism and in particular hypogonadism. A remarkable feature of this case was the stark contrast on both history and examination between Mr. PW and his identical twin (photo to be included). Laboratory investigations confirmed secondary hypogonadism and growth hormone deficiency. TSH 2.10 mIU/L fT4 13.3 pmol/L FSH 2.8 U/L LH 1.5 U/L Prolactin 15.3 mcg/L Testosterone 1.5 nmol/L Cortisol (am) 382 nmol/L HCG < 2 U/L IGF-1 12.4 nmol/L alpha-FP 5 mcg/L MRI of the pituitary showed a "typical" round, lobulated, contrast-enhancing 16mm x 29 mm suprasellar mass . Stereotactic guided needle biopsy of the lesion was consistent with the diagnosis of pituicytoma. This was confirmed after excision of the mass via a craniotomy. Postoperative recovery was uneventful. Mr PW continues on pituitary replacement therapy. A literature review highlighting the histology, imaging features and clinical experience of this rare primary tumor of the pituitary will be presented. Questions for the audience: 1) What is the differential diagnosis? 2) How long has the tumor been present? 3) What is the risk to his identical twin? 4) What are the implications of a suprasellar lesion and its treatment? 5) What is the prognosis - endocrine and tumour related? 6) Should adjuvant treatment be used?

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METABOLIC REHABILITATION: A NEW APPROACH TO MANAGING OBESE PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Aim: We evaluated a novel ambulatory care clinic treating obese adults with type 2 diabetes mellitus (T2DM) using weight management as the primary intervention.

Patients and methods: Patients able to undergo an intensive lifestyle program were eligible. The exercise program consisted of a total of 330 minutes of weekly physical activity that included at least three supervised 1-hour exercise classes. Lifestyle intervention employed dietary, psychological and medical review. Specific obesity therapy

(Sibutramine, Orlistat or meal replacements) was used as indicated and weight neutral therapeutic agents were used in preference to weight inducing anti-diabetic agents.

Results: 37 consecutive adults were eligible; over 12 months of follow-up, 11 dropped out. 26 patients (17 female) with a mean age of 61 (44-83) years and a body mass index of 36.0 (30.0-45.5) kg/m^2 were analysed. The average duration of T2DM was 9.0 years. All patients had the National Cholesterol Education Panel defined metabolic syndrome and 62% were managed by an Endocrinologist prior to clinic referral. In addition, there was a reduction or cessation of at least one metabolic/cardiovascular pharmaceutical agent among all participants.

	Mean ± SD		Change (%)
	Baseline	12 months	
Weight (kg)	96.3 ±15.7	86.9 ± 15.5	- 9.8^
Waist circumference (cm)	112.0 ± 11.3	99.0 ± 11.6	- 11.6^
HbA1c (%)	8.1 ± 1.6	6.9 ± 1.0	- 14.8^
Systolic Blood Pressure (mmHg)	141 ± 15.7	125 ± 9.0	- 11.3^
Diastolic Blood Pressure (mmHg)	81 ± 7.6	73 ± 4.5	- 9.9^
Triglycerides (mmol/L)	1.9 ± 0.9	1.5 ± 0.6	- 21.1^
HDL-Cholesterol (mmol/L)	1.2 ± 0.3	1.4 ± 0.4	+ 16.7*

^p<0.001; *p=0.01

Conclusion: An intensive lifestyle program using a weight management approach as the primary intervention in obese patients with sub-optimally controlled T2DM achieves significant improvements in weight, glycaemic control, blood pressure and lipids while decreasing pharmacotherapy use.

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EVIDENCE FOR FUNCTIONAL EXPRESSION OF VASCULAR AT2 RECEPTORS IN PATIENTS WITH INSULIN RESISTANCE

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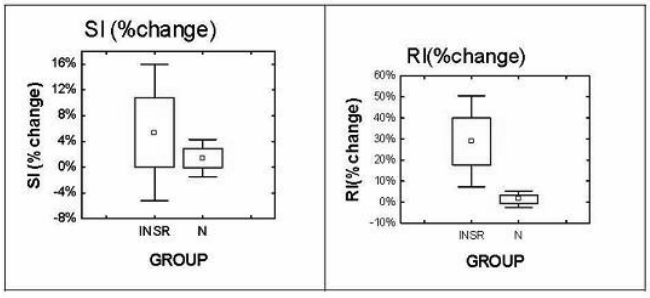
Angiotensin II type 2 receptors (AT2 receptors) are believed to become over-expressed in response to cardiovascular damage and to mediate beneficial effects of vasodilation and suppression of vascular proliferation. However, it is unknown whether AT2 receptors are functionally expressed in patients with insulin resistance (IR). We studied nine subjects with IR (mean age 31±7 yrs, BMI 28.6 ± 4.1 kg/m2, mean cholesterol level 4.7 ± 0.6 mmol/L, mean HOMA-IR mean 2.6 ± 1.0) and nine age- and sex-matched normal subjects (mean age 31±9 yrs, BMI 23.5 ± 2.2 kg/m2, mean cholesterol level 4.1 ± 0.6 mmol/L). All subjects were normotensive, on no medication and were non-smokers. The subjects received a 3 minute infusion of PD123319 (a highly selective AT2 receptor antagonist). Arterial stiffness indices (SI: stiffness index and RI: reflective index), were measured using digital photoplethysmography (Pulse Trace, Micro Medical, Gillingham, Kent, UK) and haemodynamic measurements (CI: cardiac index, SVRI : systemic vascular resistance index and ZI: stroke index) by transthoracic bioimpedance (Cardiodynamics International Corporation, San Diego, CA. BioZ system) at the end of the infusion.



RI increased significantly (p = 0.007) in patients with IR compared to controls (figure-% change RI) which was not accompanied by any significant changes in SI, SBP , DBP, SVRI , CI, ZI.

These results suggest the expression of AT2 receptors in small vessels that determine the inflection of the digital volume pulse wave, and imply the functional expression of vascular AT2 receptors in patients with IR, possibly as an indicator of early vascular damage.

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ELECTRONIC AUDIT IN PRIVATE ENDOCRINE PRACTICE OF EFFICACY & TOLERABILITY OF ROSIGLITAZONE IN TRIPLE ORAL THERAPY

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Background: The pharmacotherapeutic options for patients with Type 2 diabetes and failed dual oral hypoglycaemic therapy have recently been expanded to include addition of rosiglitazone as an alternative to initiation of insulin. The efficacy and tolerability of rosiglitazone in this therapeutic setting has not previously been studied outside of the teaching hospital environment. Modern, low cost clinical software now makes road testing of such drugs feasible in private practice.

Design: Retrospective audit of efficacy and tolerability of rosiglitazone in triple oral therapy – analysis to 6 months.

Method: Audit4 clinical software (S4S Pty Ltd, Australia) was used to identify subjects, and to source and sort their clinical data (demographics, weight, HbA1c, lipids, liver function, drug initiation and cessation dates and cessation reason).

Subjects: Search function identified 101 patients initiated on rosiglitazone in addition to current treatment with metformin and sulfonylurea: 51 male, 50 female, mean age (SD) range 60y (11.1) 32-84, with baseline HbA1c 9.38% (1.26).

Findings: Of the 101 rosiglitazone initiations, 15% patients had ceased within 6 months, 3% because of lack of efficacy, 10% because of adverse reaction, most commonly unacceptable weight gain (2%) and oedema (3%). Of those patients still on rosiglitazone at 6 months, HbA1c fell by mean (s.e) 1.6 % (0.3) and weight increased 2.6 kg (0.5). Compared to baseline, significant increases ($p<0.05$) were found in mean (s.e) values of total cholesterol +0.39mM (0.12) and LDL-cholesterol +0.43mM (0.13), and significant falls in ALP -19.7 U (3.0) and GGT – 13.0 U (2.6).

Conclusions:

1. The findings in community practice support the efficacy and safety of initiation of rosiglitazone as the initial step-up from dual oral therapy, prior to insulin therapy.

2. Appropriate clinical audit software enables clinicians in private practice to analyse their own data and provide a rational basis for management decisions.

(Acknowledgement: author thanks GSK for financial support for this study)

HbA1c (%) Change	count	% patients		Weight (kg) change	count	percentage
< 0.5	9	23.7%		< 2kg	18	46.2%
0.5 - 1.0	4	10.5%		2kg - 5kg	13	33.3%
1.0 - 1.5	4	10.5%		5kg - 10kg	8	20.5%
1.5 - 2.0	9	23.7%		> 10kg	0	0.0%
> 2.0	12	31.6%				

Table 1: Distribution of change in HbA1c and weight at 6 months post initiation rosiglitazone

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CLINICAL PROFILE OF PATIENTS WITH MICROALBUMINURIA

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The appearance of microalbuminuria in a diabetic patient predicts the development of diabetic nephropathy and cardiovascular disease. In patients with type 2 diabetes and microalbuminuria, the Steno-2 study has shown 50% reduction in the risk of cardiovascular and microvascular events with intensified intervention aimed at multiple risk factors.

We compared the clinical profile of our patients with microalbuminuria with patients in the Steno standard and Steno intensive sub groups. We then used the UKPDS risk engine to calculated the 10 year cardiovascular risk profile of our patients.

One thousand three hundred and thirty four patients with type 2 diabetes attended the diabetes assessment clinic at The Queen Elizabeth Hospital between January 2004 and March 2006. Two hundred and thirteen were detected to have microalbuminuria. This group comprised of 60% males vs 70% and 79% in the Steno standard and the Steno intensive subgroups. Our patients were older (61+/-13 vs 55+/-7.2 yrs), had shorter duration of diabetes (1.3 vs 6 and 5.5 yrs), had lower HbA1c (7+/-1.5 vs 8.8+/-1.7 and 8.4+/-1.6 %) and lower serum cholesterol levels (4.6+/-1 vs 5.8 +/-1.3 and 5.4 +/-1.1 mmol/L), their mean systolic blood pressure was (136+/-22 vs 149+/-19 and 146+/-20 mmHg). Our patients comprised of 20% smokers vs 34% and 40% in the other two groups. There was a higher prevalence of stroke (9% vs 3% and 2%) and peripheral vascular disease (7% vs 2.5% and 1%) in our patients. The prevalence of ischaemic heart disease was lower (10% vs 29% and 23%) and that of retinopathy was similar between the three groups (26%).

By lowering HbA1c to < 7%, total cholesterol to <4 mmol/L and systolic blood pressure to 130 mmHg, the absolute 10 years risk of coronary heart disease in our patients can be reduced from 17.7% to 11.2%.

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SCREENING FOR PHAEOCHROMOCYTOMA IN RENAL FAILURE

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35 male was referred to the Endocrinology outpatient clinic for further investigation of severe hypertension after a review by his GP in Dec 2005 when he presented with the predominant symptom of fatigue. Investigations included 24hr urinary catecholamine collection and renal function tests. His had a past history of a solitary left kidney with reflux and evidence of proteinuria since 1990, but had been lost to follow up.

He was found to have significant renal impairment with a Creatinine of 615umol/L, the patient had not noticed a decreased urine output. 24 hour urinary catecholamines were positive with a noradrenaline level of 1729nmol/day and dopamine of 6.1

Further investigations by the renal unit at a tertiary hospital included plasma metanephrines; these were elevated with a normetadrenaline level of 910pmol/L (<780) and metadrenaline of 320pmol/L (<300). Imaging, including a CT abdomen and a MIBG scan were negative.

The patient subsequently commenced haemodialysis and once stabilised had his first Endocrinology outpatient visit, querying the possibility of phaeochromocytoma accountingin part for his hypertension.

BP readings have remained around 210/100 despite therapy with four agents.

Further plasma metanephrines pre and post dialysis are being processed.

Questions raised include:

How to interpret screening investigations for phaeochromocytoma (1,2) in the setting of renal failure, in particular given the less reliable method of 24 hour urine collection?

If plasma metanephrines and imaging are used in patients with renal failure (3), how should the plasma metanephrines in a patient on haemodialysis be interpreted and what impact does haemodialysis have on these results and thus is the timing of collection relative to haemodialysis important?

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GRAVES' DISEASE IN PREGNANCY: WHEN MORE THAN JOY ALONE CAN SET YOUR HEART RACING

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Management of hyperthyroidism in pregnancy is challenging as one has to consider both mother and baby.

Case 1

A 35 year old woman with a prior history of Graves' disease developed a post-partum exacerbation of thyrotoxicosis after her first pregnancy and required antithyroid medication. Whilst on carbimazole 10mg daily, she fell pregnant again with thyroid function showing thyroid stimulating hormone (TSH) 0.01mU/L, free T4 39.1pmol/L, free T3 8.9pmol/L with positive thyroid receptor antibodies (TRAb) 8.7U/L (reference range 0-1). She was thyrotoxic with heat intolerance, palpitations and anxiety. Thyroid hormone levels increased during the first trimester (fT4 36.7pmol/L, fT3 17.1pmol/L) and carbimazole was increased to 10mg bd. During antenatal surveillance, a major congenital heart disease was detected in the fetus. She is currently in the third trimester, clinically stable but remains mildly thyrotoxic (TSH

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MULTIPLE INSUFFICIENCY FRACTURES OF THE PELVIS AND FEMORA IN A POST-MENOPAUSAL WOMAN ON ALENDRONATE THERAPY

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Insufficiency fractures are increasingly recognized as a clinical entity in the aging population. Likely to be the result of a discrepancy between physiological stress and skeletal strength, they occur most commonly in the pelvic girdle, the sacrum, the tibia and the femoral neck. Insufficiency fractures of the femoral diaphyses are rare, with only few reported cases. The strongest associations exist with untreated osteoporosis. We describe an unusual case of multiple insufficiency fractures in a 73-year-old Chinese woman who presented with a 12 months history of bilateral groin pain and difficulty with walking in the absence of trauma, diagnosed 18 months [Fig. 1.1 and 1.2] following the commencement of alendronate, highlighting the challenge in the management of this condition. Presentation is non-specific; plain radiographs are commonly normal initially, with fracture lines, fracture callus or osteocondensation visible in only about 60% of cases. Scintigraphy is more sensitive in the detection of such fractures. Pathogenesis of insufficiency fractures is poorly understood. Possible mechanisms may not be accountable solely by low bone mineral density, and may be attributable to over-suppression of bone turnover during long-term use of bisphosphonates, microcracks accumulation and microarchitectural distortion. This case aims to increase the recognition of insufficiency fractures, especially in patients with risk factors and atypical pain not accountable by changes on plain radiography, and to promote utilization of more sensitive imaging modalities for investigation. Prompt referral to physiotherapy to rectify an abnormal gait may be a crucial yet neglected step in the prevention of insufficiency fractures.

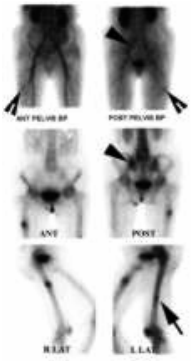

Figure 1.1 Hyperemia in the lateral cortex of the diaphysis of the right femur (open arrowhead), and the left sacroiliac joint (solid arrowhead). Delayed images show uptake in the lateral cortex of the right femoral diaphysis (open arrowhead).

Figure 1.2 A transverse breach of the thickened lateral cortex of the right femur (arrowhead) corresponding to the scintigraphic site of fracture. A thickened lateral cortex is evident in the left femur (arrow), with no discrete fracture line.

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THE PROSTATE-SPECIFIC ANTIGEN (*PSA*) POLYMORPHISM (G-158A) ALTERS INTERACTION OF ANDROGEN RESPONSE ELEMENT 1 WITH THE ANDROGEN RECEPTOR

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Prostate cancer accounts for approximately 10% of all male cancers and is the fourth leading cause of cancer deaths in men. Although not well understood, it is well established that androgens play an important role in the earlier stages of prostate cancer aetiology. The prostate specific antigen or kallikrein 3 (*PSA/KLK3*) gene is primarily expressed in the prostate and has been shown to process various factors that are important in prostate (patho)-physiology, suggesting an active role for PSA in prostate cancer progression. In the prostate, *PSA* is regulated by androgens with three well-described androgen response elements (AREs) identified in the *PSA* promoter. Interestingly, a polymorphism that results in a G to A transition (G-158A) is located within the second canonical half-site of *PSA* AREI. Some epidemiological studies suggest that G-158A is associated with risk of developing prostate cancer. We have therefore, investigated the functional significance of the G-158A polymorphism in prostate cancer using *in silico* and *in vitro* analyses. We have found that the *PSA* AREI alleles bind with a two-fold difference with the AR-DBD, which was further confirmed in limited proteolysis experiments. Molecular modelling for binding of the variant AREI alleles with the AR-DBD suggest that these differences may be conferred by the introduction of two extra hydrogen bonds for the A allele at the -160 position of AREI with Arg_{568} of the androgen receptor. Furthermore, reporter assays using three-tandem copies for each *PSA* AREI allele demonstrated that the G-158A polymorphism also alters androgen induced transactivation of the *PSA* promoter in two different prostate cancer cells. In conclusion we have shown that the G-158A polymorphism alters interaction of the AREI with the androgen receptor, which may account for the observed differential expression of PSA in prostate cancer, as well as the association of this polymorphism with prostate cancer risk.

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IGF-I:IGFBP:VN COMPLEX ENHANCED BREAST CANCER CELL MIGRATION INVOLVES BOTH VN-BINDING INTEGRINS AND THE IGF-1R THROUGH ACTIVATION OF THE AKT/PI3-K SIGNALLING PATHWAY.

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We previously reported that IGF-I can bind to the extra-cellular matrix protein vitronectin (VN), via the involvement of select IGFBPs. As both IGF-I and VN have established roles in promoting tumour cell dissemination we wished to investigate the functional consequences of the interaction of IGF-I, IGFBPs and VN in the model MCF-7 breast cancer cell line. Substrate-bound IGF-I:IGFBP:VN complexes stimulated synergistic increases in cellular migration above that induced by the individual components alone. This effect was also seen in non-tumourgenic MCF-10A mammary epithelial cells. Furthermore, studies using IGF-I analogs determined this stimulation to be dependent upon both ternary complex formation and the IGF-1R. The synergistic effects on cellular migration were abolished upon incubation of cells with function blocking antibodies directed at VN-binding integrins, in particular αvβ5, and the IGF-1R. IGF-I:IGFBP:VN complexes stimulated transient activation of the ERK/MAPK signalling pathway, while also producing a sustained activation of the AKT/PI3-K pathway. However, the AKT/PI3-K pathway was only activated when all the components of the complex were present. Experiments using pharmacological inhibitors of the PI3-K/AKT pathway, have shown that activation of this pathway is vital for IGF-I:IGFBP:VN complex stimulated cell migration, whereas the ERK/MAPK pathway was not. To confirm these results we are currently over-expressing wild-type, dominant negative and constitutively active-AKT to determine the effects on IGF-I:IGFBP:VN stimulated migration. Furthermore, microarray experiments have also been undertaken to identify candidate genes involved in enhanced cell migration. Results from this project will contribute valuable information regarding tumour cell metastasis and result in the development of improved therapeutics for the treatment of breast cancer and other carcinomas.

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EXPRESSION OF COMPONENTS OF THE GHRELIN/GROWTH HORMONE SECRETAGOGUE RECEPTOR AXIS IN BREAST CANCER CELL LINES AND IN BREAST CANCER HISTOPATHOLOGICAL SPECIMENS

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Breast cancer is the most common malignancy in females and is a major cause of death in Western women. While oestrogen and progesterone are clearly implicated in the development of breast cancer, there is increasing evidence that the ghrelin/growth hormone secretagogue receptor (GHSR) axis could be involved. We have previously demonstrated that ghrelin stimulates the growth of prostate cancer cells, which is also a hormone dependent cancer. Ghrelin, a 28 amino acid peptide hormone, mediates numerous physiological functions, including growth hormone release, through the GHSR. In this study, we have demonstrated the expression of ghrelin and its two receptor isoforms (GHSR1a and 1b) in the MDA-MB231, MDA-MB435, T47D, MCF7 and MCF10A breast cell lines *in vitro* and in histopathological breast cancer tissues. We have also demonstrated that breast cell lines and normal breast tissue express an exon 3 deleted isoform of proghrelin. This novel ghrelin isoform, which we first described in prostate cancer tissue, encodes a novel C terminal peptide. Through real time PCR a 12 fold increase in this exon 3 deleted mRNA isoform in the oestrogen receptor negative breast cancer cell line, MDA-MB-435 has been demonstrated. We have also shown that oestrogen receptor negative breast cancer cells, MDA-MB435 and MDA-MB231 proliferate (up to 40% and 20% above control respectively) in response to ghrelin treatment. Ghrelin may therefore play a role in the autocrine/paracrine stimulation of proliferation in oestrogen receptor negative breast cancers. Through further analysis of this axis, novel therapeutics for these oestrogen receptor negative cancers may be developed.

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NEUROBLASTOMA CELL DIFFERENTIATION BY FIBROBLAST GROWTH FACTOR-2 (FGF-2) INVOLVES REGULATION OF INHIBITOR OF DIFFERENTIATION (ID) GENES AND SUPPRESSOR OF CYTOKINE SIGNALING-2 (SOCS-2)

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An early event in the pathogenesis of neuroblastoma (NB) is the arrested differentiation of neuroblasts. We demonstrated that FGF-2 promotes NB cell differentiation and over-rides the mitogenic action of insulin-like growth factor-I (IGF-I). A number of genes have been implicated in the regulation of neuronal cell proliferation and differentiation, including the transcriptional regulator ID genes and signaling molecules including the SOCS genes. Whether these genes mediate FGF-2 differentiation of NB cells and/or inhibition of IGF-I action is unknown. Therefore we analysed the expression of ID1-3 and SOCS1-3 in SK-N-MC cells cultured in the presence or absence of FGF-2 (50ng/ml) and/or IGF-I (100ng/ml). The TUJ1 and GAP43 markers confirmed neuronal differentiation.

ID1 expression was detected by RT-PCR in untreated and IGF-I induced SK-N-MC cells, but it was decreased by FGF-2. In contrast ID2 was strongly induced by FGF-2, but not in untreated or IGF-I stimulated cells. ID3 mRNA was not affected by treatments.

The FGF-2 signaling modulator SOCS-2, but not SOCS-1/3, was up-regulated by FGF-2. SOCS1-3 were detectable but not regulated in the untreated or IGF-I stimulated cells.

The balance between expression of various ID isoforms is required to maintain normal cellular homeostasis, such that ID1 regulates proliferation and ID2 regulates differentiation. Deviation from this balance might lead to cell transformation and malignancy. Our data provide evidence for unbalanced expression of ID genes in arrested neuroblast differentiation. For the first time we show that FGF-2 reverses this "balance" towards growth arrest (inhibition of ID1) and differentiation (induction of ID2) of NB cells. The induction of SOCS-2 expression by FGF-2 is associated with reduced mitogenic action of IGF-I suggesting inhibition of IGF-IR signaling by SOCS-2, a member of the SOCS family not previously associated with regulation of IGF receptor signaling.

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NEUROBLASTOMA CELL ADAPTATION TO HYPOXIA IS ACHIEVED VIA COMPLEX MODULATION OF GENES ENHANCING CELL SURVIVAL AND METASTASIS.

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Solid tumors, including neuroblastoma (NB), adapt to oxygen and nutrients deprivation by increasing their intra-tumor neovascularisation, leading to invasive/metastatic behaviour. Hypoxia inducible factor-1ά (HIF-1ά), a key regulator of oxygen homeostasis, and its target genes modulate cell survival and growth in cells exposed to hypoxia. In this study SH-SY5Y cells were employed to determine the mechanisms regulating adaptation to hypoxia in NB.

NB cells were cultured in serum free medium in the presence or absence of CoCl2 (100μM, hypoxia mimic) for up to 48 hours.

SH-SY5Y cell number was not affected by CoCl2 treatment, while mitochondrial activity was reduced by about 50%. HIF-1ά protein was detected as early as 30 min post-hypoxia, followed by increases of mRNA for hypoxia responsive genes (ie. erythropoietin, VEGF) at 4 hour post-hypoxia. In order to determine whether the NB cells response to hypoxia also involves modulation of genes enhancing survival migration and invasion we utilise real-time PCR and analysed the expression of genes involved in these processes. In hypoxic SH-SY5Y NB cells, genes involved in maintenance of cell-cell and cell-matrix interaction (ie. APC, E-cadherin, catenin, EphB2, fibronectin-1, TIP30, TIMP4) were down-regulated by up to 90%. Under the same conditions, genes involved in enhancement of metastatic behaviour (integrin a7b1, HGF, TGFB1, VEGF, KiSS1, IL1B) were dramatically up-regulated above 200%. We have demonstrated that NB cells are able to adapt to low oxygen/hypoxia via mechanisms involving modulation of HIF-1ά expression and hypoxia responsive genes including VEGF. In addition we have for the first time also determined regulation of key genes promoting NB cell transition from an adherent phenotype to a potential highly migrating, invasive and aggressive NB cell type. Similar mechanisms might exist and contribute *in vivo* to enhancing metastatic behaviour of solid tumors, including neuroblastoma.

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CD151 GENE KNOCK-DOWN SUPPRESSES THE MOTILITY OF PROSTATE CANCER CELL LINE PC3: A LINK TO PROSTATE CANCER METASTASIS?

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Introduction: In previous work from our laboratory, we found that the tetraspanin family member CD151 has prognostic value in prostate cancer; patients with lower tumour content have a more favourable prognosis compared to patients with higher amounts of expression. Furthermore, we showed that CD151 gene over-expression promotes the motility and invasion properties of the prostate cancer cell line LNcap. To confirm the role of CD151 in prostate cancer metastasis, we set up a CD151 gene knock-down model in PC3 cells using SiRNA method.

Methods : A mixture of four different CD151 oligo SiRNAs were obtained from Dharmacon, USA. The CD151 SiRNAs were transiently transfected into the prostate cancer cell line PC3 using Oligofectamine (Invitrogen). The knock-down effect was confirmed by Western blot at 24, 48 and 72 hours time points. The cell knock-down at the 48 hour time point were chosen for migration and invasion studies using an Invasion Chamber (Invitrogen). The wild type and control SiRNA transfected PC3 cells were used as controls. We also created 2 pairs of pBabe/U6/CD151 SiRNA constructions, which can be used in permanent knock-down.

Results: The CD151 gene knock-down PC3 cells were less invasive than the wild type and control SiRNA transfected cell lines ($P<0.01$, $P<0.01$). The same trend was also found in migration ($P<0.01$, $P<0.01$). However, proliferation was not changed by CD151 gene knock-down.

Conclusion: Knocking down of CD151 gene suppresses the migration and invasion properties of the prostate cancer cell line PC3. These data suggest that CD151 plays a specific role in promoting prostate cancer cell motility and invasion.

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EVIDENCE FOR CROSS TALK BETWEEN MELANOCORTIN-1 RECEPTOR AND NR4A NUCLEAR RECEPTOR SIGNALLING IN MELANOCYTIC CELLS.

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The melanocortin-1 receptor (MC1R) is a G-protein coupled receptor that is a key regulator of melanocyte function and is known to play a significant role in the determination of diverse pigmentation phenotypes observed in mammals including humans. Peptide ligands α-MSH and ACTH binds with high affinity to MC1R promoting a functional switch to the synthesis of darker melanins primarily via a positive coupling to adenylate cyclase. This switch is most notably observed as part of the UV induced tanning response mediated by increased release of α-MSH and ACTH (among other bio-active peptides) by both keratinocytes and melanocytes. The MC1R locus has been shown to be highly polymorphic in humans with a number of functional variants being strongly associated with red hair and fair skin phenotypes. Individuals with such phenotypes have a significantly greater incidence of melanoma and non-melanoma skin cancer highlighting the importance of MC1R in the co-ordination of melanocyte photo-protective function. Delayed responses to α-MSH stimulation of melanocytes such as increased melanin content and activity of pigmentation genes are well established, however the regulatory mechanisms initiated beyond the immediate cAMP response remain poorly understood. Accordingly, we aimed to identify transcriptional control points that function within the immediate/short term following activation of MC1R. One potential candidate group is the NR4A sub-family of nuclear receptors that includes Nur77 (NR4A1), Nurr1 (NR4A2) and NOR-1 (NR4A3), genes that are known to be rapidly induced by a range of inflammatory and mitogenic stimuli in other cell types. Preliminary evidence obtained using human and mouse melanoma cell lines and primary human melanocytes has demonstrated a striking and transient immediate-early response of these genes in response to MC1R activation by NDP-MSH. Current investigations are aimed at further characterising this response and determining the functional role the NR4A genes play in melanocytic signalling.

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MALIGNANT INSULINOMAS WITH HEPATIC METASTASES SUCCESSFULLY TREATED WITH SELECTIVE INTERNAL RADIATION THERAPY (SIRT).

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Insulinomas are rare pancreatic islet cell tumours with an incidence of 4 cases per million per year. About 10% of all insulinomas are malignant. The prognosis for malignant insulinoma is poor, with 10-year survival estimated at 29%. Individuals suffer from severe debilitating and life threatening hypoglycaemia. Such episodes are worse during periods of fasting. Malignant insulinomas are poorly responsive to conventional therapy. Current therapy is therefore palliative. Surgical cure is possible if detected early enough before unresectable disease occurs. Selective Internal Radiation Therapy (SIRT) is an emerging area in radiological oncology that may improve the prognosis. SIRT in combination with intrahepatic 5-floxuridine chemotherapy has been effective in treating hepatic metastases from colorectal cancer. The therapy relies on delivering 32 micrometre spheres impregnated with yttrium-90 (90Y) injected through a femoral artery catheter, which has been positioned into the hepatic artery supplying the tumour. The beads become lodged within the tumour vessels where they exert their local radiation effects causing tumour cell death. We report 2 cases with extensive hepatic metastases that had been very difficult to manage despite aggressive treatment but had achieved a sustained clinical remission only when treated with SIRT. We believe this is the first time such a therapy has been successfully used in malignant insulinomas with hepatic metastases. Use of SIRT should be considered in other similar patients.

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CD151 – A NOVEL CLINICAL PROGNOSTIC TUMOUR MARKER IN PROSTATE CANCER.

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Introduction: Prostate cancer represents the highest incidence of cancer amongst Australian men with a lifetime risk of 1 in 11 and the second highest cause of cancer death amongst men. [1] Treatment options for intermediate Gleason grade carcinomas (Gleason 5-7) often provide a management dilemma for the treating clinician since disease progression is poorly understood and the course of the disease is often unpredictable. A new prognostic tumour marker may help change that. CD151 is a cell membrane protein from the tetraspanin family and has been shown to be over-expressed in a variety of malignancies including lung, colon, melanoma, pancreatic and prostate. [2, 3] Recent research suggests CD151 over-expression acts to increase cell motility and alters intracellular signalling pathways, both important factors in the metastatic cascade. [3-5] Furthermore, CD151 has now been shown to be a better prognostic indicator than Gleason score.[6] The present study aims to corroborate the relationship between CD151 expression in human prostate cancer with survival and to determine if a correlation exists between clinical and biochemical parameters.

Methods & Results: 190 patients with primary prostate cancer diagnosed between 1984 and 1998 were recruited retrospectively. Paraffin blocked tissue was Gleason graded then immuno-histochemically stained with anti-CD151 antibody. Quantitative measurements by MCID of staining intensity and subsequent statistical analysis will be performed on the clinical subgroups. However, preliminary immunohistochemistry suggests a correlation between CD151 over expression and poorer prognosis.

Discussion: A correlation between expression and outcome suggests the importance of CD151 as a prognostic tumour marker. In the diagnostic phase of patient work-up, CD151 may therefore prove to be a useful tool in the clinician's arsenal in determining appropriate treatment.

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MELATONIN AS AN IMMUNOENHANCING VACCINE ADJUVANT

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Melatonin has immunoenhancing effects. In particular, it enhances T helper cell activity and IL-2 production. This raises the possibility that melatonin could potentially be used as a vaccine adjuvant to enhance the immune response.

Recent attention has focused on the need for a suitable adjuvant to enhance the efficacy of both human and avian influenza vaccines. We sought to test whether oral melatonin enhanced the vaccine response to a formalin inactivated influenza vaccine (Fluvax©, CSL). To compare the adjuvant effect of melatonin, a control group was immunised with Fluvax© plus microparticulate inulin (Advax©, Vaxine Pty Ltd) which is a known effective influenza adjuvant. Mice in groups of seven were injected intramuscularly twice, two weeks apart with Fluvax© with or without Advax©. Melatonin was added in the drinking water 5 days prior to immunisation and continued 5 days post immunisation. Mice were bled 14 days after the 2nd vaccination (day 28) and serum total IgG, IgG1, IgG2a were measured by ELISA. After sacrifice spleen cells were extracted and lymphoproliferation was measured using CSFE technique. Splenocyte culture supernatants were also assayed for γ-IFN. This study provides information regarding the possibility of melatonin's role as a vaccine adjuvant to enhance the efficacy of influenza vaccine.

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IS DEFECTIVE IMMUNITY A FEATURE OF ALSTROM SYNDROME?

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Alström syndrome is a rare autosomal recessive disorder cause by mutations in *ALMS1*, a gene whose function remains unclear. This syndrome affects multiple organs and results in development of childhood obesity, metabolic disturbances (insulin resistance, type 2 diabetes, hypercholesterolaemia, hepatosteatosis), sensorineural hearing loss and infertility. The syndrome has variable clinical expression and members bearing the same mutation often present with different symptoms and at variable ages. The Fat Aussie (FATs) is an obese mouse strain with a spontaneous inactivating mutation in *ALMS1*. FATs develop similar clinical features of Alström syndrome with hyperphagic obesity, diabetes, hepatosteatosis, dyslipidemia and impair spermatogenesis. We were interested to test whether the gene, *ALMS1*, which is widely expressed in tissues throughout the body, has a role in immune function. To evaluate this, four groups of seven mice; FATs and wildtype littermates, were immunised twice (two weeks apart) with Fluvax (CSL) with or without Advax (Vaxine). Fourteen days after the 2nd injection (Day 28), the mice were bled and serum collected to measure total IgG, IgG1, 2a, 2b by ELISA. This study provides data regarding the potential immunological actions of *ALMS1*, offering further insight into its role in Alström syndrome.

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THE RELATIONSHIP BETWEEN BODY COMPOSITION AND PHYSICAL PERFORMANCE: A STUDY IN YOUNG RECREATIONAL ATHLETES.

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To understand the relationship between body composition and physical performance we studied 84 recreational athletes (60 m, 24 f) aged 18-40, exercising ≥ 2 times/week for ≥ one year. Performance was assessed by 4 parameters: a) sub-maximal cycle test for VO_2max, b) dead lift dynamometry for maximal strength, c) single vertical jump for maximal power, and d) cycle Wingate test for anaerobic sprint capacity. Lean body mass (LBM) and fat mass (FM) were measured by DEXA and data analysed by simple and multiple linear regression. In the entire cohort, LBM correlated positively with all performance measures (r^2=0.50-0.83, $p<0.001$). FM correlated negatively with jump height only (r^2=0.10, p=0.002). All performance measures and LBM were higher in men. In men, LBM positively correlated with all measures (r^2=0.15-0.55, $p<0.001$) except jump height, while FM correlated negatively with jump height only (r^2=0.16, $p<0.001$). In women, LBM positively correlated to all performance measures (r^2=0.23-0.73, $p<0.001$) whereas FM was not related to any.

	VO_2max [L/min]	Dead lift [kg]	Jump Height [cm]	Total Work [kJ]	LBM [kg]	FM [kg]
Male	3.8±0.8	191.4±35.2	55.1±8.1	22.8±3.8	63.9±8.3	16.4±8.1
Female	2.3±0.6*	123.1±24.6*	35.4±7.5*	12.7±3.2*	40.3±5.2*	18.9±5.4
Mean±SD; *: p<0.001 vs male						

In a multiple regression LBM accounted for >50 % (r^2=0.50-0.83, p<0.001) of the total variance for all measures while FM negatively predicted dead lift (r^2=0.05, p<0.02) and jump height (r^2=0.15, p<0.001) only. Gender exerted a minor but significant effect on jump height only (r^2=0.03, p<0.02)

In summary, LBM had a positive correlation and stronger relationship with all performance measures than FM, which negatively correlated with dead lift and jump height. The influence of gender was minor after accounting for differences in body composition. In conclusion, in recreational athletes, body composition and physical performance are closely interrelated and gender differences in most performance parameters could be attributed to differences in body composition. Prospective studies are required to address whether growth hormone-induced changes in body composition alter performance. (Supported by the World Anti-Doping Agency and Australian Government Anti-Doping Research Program.)

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LEGUMAIN IN EARLY BOVINE PLACENTATION

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Reproduction, Agresearch, Hamilton, New Zealand

We examined the maternal-embryo interactions during the early endometrial apposition of the trophoblast by comparing the proteins present at Day 17 in uterine luminal fluid (ULF) from pregnant and non-pregnant cows using 2D-gel electrophoresis. Three protein spots that were presentat reduced levels in pregnant ULF were identified by Mass spectrophotomtry (MALDI-TOF) as post translational isoforms of legumain.1 Legumain, a lysosomal cysteine endopeptidase that specifically cleaves on the carboxyl side of asparagine residues is particularly abundant in mouse kidney and placenta. 2 Legumain throughout the estrous cycle and in early pregnancy was examined using a beef tissue library of endometrial samples collected throughout the estrous cycle and Days 1-31 of pregnancy (n=2 per day).

Legumain mRNA was present in low amountsin endometrial tissue early in the estrous cycle, increasing three-fold on Days 13 and 14 then dropping, from Day 16, to low levels by Day 20. The pregnant endometrium followed the same expression pattern until Day 18 when expression of legumain was 1.5-2 times greater than that of non-pregnant uteri, returning to low levels between Days 26-31 of pregnancy.

Western blotting of proteins from Day 18 endometrial tissue revealed the presence of the 56kDa proform of legumain as well as three higher molecular weight bands. Day 18 ULF proteins contained the 56kDa proform and the 46kDa active form of legumain. More legumain was present in non-pregnant than in pregnant ULF from empty horns than in the gravid horns.

A function of legumain is to activate other protease zymogens by proteolytic cleavage of an asparaginal bond, therefore, its up-regulation in expression during the peri-implantation period may suggest an involvement in the modulation of protease activity necessary for successful invasion of the endometrium.

(1) Chen JM et al. 1998. J Biochem 335:111-117.

(2) Peterson AJ and Lee RS 2003. Therio 59:687-697.