Gene regulatory elements as breast cancer susceptibility loci and biomarkers

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Aberrant expression of cancer-associated genes contributes to initiation and progression of the tumourigenic process. Such changes in gene expression result from defects in transcriptional control elements, including promoters and enhancers, and in post-transcriptional control elements, including those found in the 3'UTR. Epigenetic defects include methylation of transcriptional regulatory elements and altered targeting of post-transcriptional regulatory elements by trans-acting factors including microRNAs. Our group has a long-standing interest in elucidating the genetic and epigenetic regulation of breast cancer associated genes, including BRCA1. Our studies have led to the identification of regulatory sequences mapping to promoter, intronic, 3'UTR and exonic sequences of BRCA1, and the promoter of AR and a number of miRNAs, including miR-200b. We have also identified proteins and miRNAs that target these sequences, including the RNA binding protein HuR, which targets the BRCA1 3'UTR. We have shown that genetic and epigenetic changes in these sequences, some of which are associated with breast cancer susceptibility and progression, affect gene expression and are sometimes associated with altered targeting by proteins or miRNAs. We have also identified a number of miRNAs that are differentially expressed in the pre-malignant mammary glands of a BRCA1-associated breast cancer mouse model. Current studies involve elucidating the mechanism of this regulation and the role of these events in mammary tumourigenesis and the clinical utility of these regulatory elements and events as biomarkers of cancer susceptibility and progression.

Long-term persistence of hormonal adaptations to weight loss

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Background: Following weight loss, changes occur in circulating levels of several peripheral hormones involved in the homeostatic regulation of body weight. It is not known whether these changes are transient, or persist over time.

Methods: Fifty non-diabetic overweight or obese subjects started a 10-week weight loss program using a very-low-energy diet (VLED), followed by 1 year of attempted weight loss maintenance. Circulating concentrations of leptin, ghrelin, peptide YY (PYY), gastric inhibitory polypeptide (GIP), glucagon-like peptide 1 (GLP-1), amylin, pancreatic polypeptide (PP), cholecystokinin (CCK) and insulin, and subjective ratings of appetite were examined at baseline, after the VLED, and at 1 year.

Results: Mean initial weight loss of 13.5 ± 0.5 kg led to significant reductions in leptin, ghrelin, PYY, and glucagon-like peptide 1 (GLP-1), amylin, pancreatic polypeptide (PP), cholecystokinin (CCK) and insulin, and subjective ratings of appetite were examined at baseline, after the VLED, and at 1 year.

Conclusion: Compensatory changes in circulating mediators of appetite which encourage weight regain after diet-induced weight loss persist 12 months after initial weight reduction.

Silencing of Ghrelin Receptor Expression Inhibits Endometrial Cancer Cell Growth in vitro and in vivo

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Ghrelin is a peptide hormone produced in a range of organs and cancers derived from them, including adenocarcinomas. It has endocrine, paracrine, and autocrine roles in both normal and disease states. Ghrelin is expressed in endometrial cancers, while its receptor, the growth hormone secretagogue receptor 1a (GHSR1a) is expressed at various levels in normal endometrium and cancer tissues. We previously showed that ghrelin has proliferative and anti-apoptotic functions in endometrial cancers, suggesting its potential role in promoting tumour growth. To further investigate ghrelin-GHSR1a in endometrial cancer cell progression, we examined the effect of knockdown of GHS-R1a expression in the Ishikawa endometrial cancer cell line by using RNA interference (RNAi) both in vitro and in the NOD/SCID xenograft mouse model. The lentiviral short hairpin RNA targeting GHSR1a(GHSR1a-shRNA) resulted in a stable reduction of GHS-R1a mRNA and protein. GHSR1a-shRNA Ishikawa cells showed less non-stimulated cell proliferation compared to the scrambled controls and proliferated less in response to 100nM ghrelin than with controls (100% vs 118% of control, P<0.05). Tumour volumes of GHSR1a-shRNA Ishikawa xenograft tumours were significantly reduced compared with scrambled control tumours.
immunohistochemistry demonstrated decreased GHS-R1a protein levels in SDHA, SDHB, SDHC, SDHD and non-SDHc subunits. In summary, our results indicate that decreasing the GHS-R1a protein level by RNAi significantly inhibits endometrial cancer cell line and xenograft tumour growth, hence ghrelin-GHSR1a signalling may have an important role in the development of endometrial cancer. Demonstration of a functional role for ghrelin in endometrial growth and the detection of its receptor in endometrial cancers suggest that blocking GHS-R1a may be therapeutic in this cancer.

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The Decline in Pulsatile Growth Hormone Secretion throughout Early Adulthood in Mice is Exacerbated by Dietary Induced Weight Gain

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The pathological decline in growth hormone (GH) secretion in early adulthood is detrimental to attainment of peak lean muscle and bone mass, and promotes adiposity and susceptibility for the development of obesity. It is thus essential that we identify factors that may advance the age-associated decline in GH secretion throughout early adulthood.

To characterize the impact of dietary induced weight gain on the decline in GH secretion throughout early adulthood, we first characterized pulsatile GH secretion in C57BL/6J mice maintained on a standard diet at 12 and 16 weeks of age. We then assessed GH secretion in mice following 4 and 8 weeks of high fat feeding. In addition, circulating levels of IGF-1 and hepatic triglycerides were assessed.

We observed a significant decline in pulsatile GH secretion in mice from 12 to 16 weeks of age. This was characterized by a significant decline in total (725±136 vs 365±68.3ng/ml per 6h, p=0.014), pulsatile (594±129 vs 326±68.5ng/ml per 6h, p=0.022), and the mass of GH secreted per burst (143±140 vs 414±38.8ng/ml per 6h, p=0.005). Alongside an increase in body weight, we observed a suppression of total (873±160 vs 442±36.3ng/ml per 6h, p=0.015), pulsatile (732±140 vs 414±38.8ng/ml per 6h, p=0.040) and basal GH secretion (141±32.4 vs 26.7±6.02ng/ml per 6h, p=0.002) following 4 weeks of high fat feeding and a further decline in hepatic GH secretion by 8 weeks of dietary intervention. In addition, impaired GH secretion coincided with an elevation in hepatic triglycerides and an eventual reduction in circulating levels of IGF-1.

Observations suggest that dietary induced weight gain advances the age-associated decline in pulsatile GH secretion throughout early adulthood in mice. Whether the advanced decline in GH secretion following dietary induced weight gain will have long-term ramifications on adult health awaits further investigation.

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Interaction between hypoxia and mutations in SDH subunit genes associated with phaeochromocytoma/paragangliomas

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Introduction
Phaeochromocytomas (PC) and paragangliomas occur in the context of inherited syndromes in 30% of cases. Of 11 loci that have been defined to date, 4 encode succinate dehydrogenase genes (SDHA, SDHB, SDHC, and SDHD, collectively SDHx). Mechanisms by which germline SDHx mutations stimulate tumour formation are poorly understood. Clinical data implicate an interaction with environmental hypoxia. This study investigated effects of hypoxia on cellular function in the presence or absence of SDHx mutation.

Methods
1. Wild type and mutant SDHx cDNAs tagged with GFP were expressed in HEK293 cells, and cultured in either normoxic or hypoxic conditions. Cellular localisation was visualised by confocal microscopy and confirmed by western blotting.
2. Mitochondrial function was studied in a novel model of SDHx dysfunction using neural stem cells obtained from patients with SDHx mutations.
3. The hypoxic-responsive mR210 was previously measured by qPCR in PC/PGLs containing mutations in SDHB and non SDHB PC/PGLs. MrR210 was also measured in neurospheres.
Results
Wild-type SDHx localised to mitochondria under normoxic conditions, but less so under hypoxia. In contrast, some SDHx mutants constitutively failed to localise to mitochondria whereas other mutants retained mitochondrial localisation in normoxic but not hypoxic conditions. Neurosphere cells containing SDHB mutations exhibited reduced SDHB expression, reduced complex II function, and increased membrane potential. MiR210 levels were increased in SDHB-mutated tumours compared with non-SDHB-mutated tumours or normal samples. MiR210 was increased in neurospheres containing SDHB mutations.

Conclusions
1. SDHx mutations impaired SDHx mitochondrial localisation, either constitutively or in conjunction with hypoxia.
2. The neurosphere model confirmed that some complex II dysfunction is evident with heterozygous SDHx mutation.
3. Elevated miR210 was evident in PC/PGLs associated with SDHB mutations, consistent with known pseudohypoxic gene signature of these tumours. Our study suggests an interaction between SDHx mutation and hypoxia in the pathogenesis of PC/PGL.

SLIRP represses retinoic acid and Notch signalling in colorectal cancer and is a good prognostic factor

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Colorectal cancer (CRC) is the second highest cause of cancer death and the third most common malignancy. Aberrant Notch signalling in CRC promotes epithelial to mesenchymal transition, chemoresistance and metastasis. Its effects are mediated through transcription factors (TFs), in particular HES1 and HEY1, which influence COX2, NF-B and Wnt signaling. In CRC, Notch signalling can be activated by retinoic acid, through the nuclear receptor (NR) RARs and the early developmental TF SOX9, which activates HES1. Using CRC tissue microarrays (TMAs), we found that SLIRP, a nuclear receptor (NR) corepressor, is a good prognostic factor in CRC, with a tumor suppressor phenotype. High tumor SLIRP expression in a TMA cohort of 967 patients correlated with improved 5 year survival (p<0.01) and inversely with tumor stage and lymph node invasion. In two CRC cDNA microarray sets, high SLIRP mRNA expression correlated with improved disease free survival over three years following surgery (p<0.05). Here we investigated the mechanism for this clinical advantage and the hypothesis that SLIRP is a repressor Notch signalling. In human CRC cell transfection studies using luciferase (Luc) reporters, siRNA mediated depletion of SLIRP increased the activity of ligand-stimulated RAR, HES1 and SOX9 signaling, resulting in increased expression of downstream targets, including NOTCH2, NOTCH3, NFKB1, NFKB2, LMO2, HES1 and SOX9. In ChIP studies, SLIRP was recruited (with RARα) to the HES1 and SOX9 promoters. When SLIRP was depleted from the cells with siRNA, ChIP studies revealed an increase in RARα recruitment to the HES1 and SOX9 promoters, along with increased binding of SOX9 to the HES1 promoter, and a decrease in binding of HES1 to its own promoter. Further, we found that siRNA mediated SLIRP depleted CRC cells were more invasive in matrigel assays and more resistant to the standard CRC chemotherapeutic agents, 5-Fluouracil and irinotecan. Taken together, these data suggest that SLIRP is a potent suppressor of Notch and RARs signalling in CRC and provide insight into the mechanisms by which SLIRP functions as a tumor suppressor to reduce invasion and enhance sensitivity to chemotherapy in this disease.

Role of androgens via AR in PTEN inactivation induced uterine pathology

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Inactivating mutations in the phosphatase and tensin homolog (PTEN) gene cause Cowden syndrome (CS), an autosomal dominant genetic disorder which increases the risk of hormone dependent reproductive cancers including breast and uterus. We have demonstrated experimentally that androgen action via androgen receptor (AR) inhibits hormone dependent growth
and carcinogenesis of the mouse mammary gland. Therefore, we hypothesized that androgens via AR may also reduce PTEN inactivation induced uterine endometriosis.

Our objective was to determine the effect of AR inactivation on PTEN inactivation induced uterine pathology. To test our hypothesis, development of uterine pathology was compared between wild-type (WT), heterozygous PTEN knockout (PTENKO) and combined heterozygous PTEN and complete AR knockout (PTENARKO) female mice (Cre/LoxP system; global deletion). Serum and ovarian steroids were analyzed using liquid chromatography tandem mass spectrometry.

Against our hypothesis PTEN inactivation induced uterine pathology was significantly reduced by AR inactivation. While 37% (19 out of 52) of PTENKO mice developed macroscopic uterine abnormalities, only 9% (2 out of 23) of PTENARKO had normal uteri as detected at termination with median age of 45 weeks (Pearson Chi-square, p=0.013). No uterine abnormalities were found in WT females (n=32). The macroscopic uterine abnormalities were manifest as significantly increased uterine weight in PTENKO [1690±334mg (means±SE); n=52] females compared to WT (156±23mg; n=32) and PTENARKO (516±163mg; p=0.007).

Unexpectedly, serum progesterone (P4) was significantly (p=0.001) increased in PTENKO (17±2.2ng/ml; n=28) females compared to WT (4.4±0.7ng/ml; n=18) and PTENARKO (5.4±1.2ng/ml; n=16). Increased circulating P4 in PTENKO could arise from ovarian secretions, as intra-ovarian P4 was non-significantly increased in PTENKO (10.8±4.7ng/ml; n=4) females compared to WT (2.8±0.3ng/ml; n=4) and PTENARKO (3.1±1ng/ml; n=5) (p=0.453).

In conclusion, the increased circulating P4 in PTENKO was not able to protect against uterine pathology. However, AR inactivation protected against PTEN inactivation induced uterine pathology a well as increases in circulating P4. Therefore, further analysis on mechanism(s) involved as well as uterine specific effects of AR are warranted.

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Hypoxia inducible factor-1α increases promoter II-driven aromatase expression in postmenopausal breast cancer

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Introduction: The majority of postmenopausal breast cancers are oestrogen-dependent. Tumour-derived factors such as prostaglandin E2 (PGE2) stimulate CREB binding to cAMP response elements (CREs) on aromatase promoter II (PII), leading to increased expression of aromatase and biosynthesis of oestrogens within breast adipose stromal cells (hASCs). Hypoxia inducible factor-1α (HIF-1α) is a key mediator of hypoxic responses in tumours. PGE2 has been shown to stabilise HIF-1α independent of oxygen availability in colon and prostate cancer cells. We have identified a consensus HIF-1α binding motif overlapping with the proximal CRE of PII. However, the regulation of aromatase expression by HIF-1α in breast cancer has not been characterised. We aimed to characterise the role of HIF-1α in regulating aromatase PII. Methods: HIF-1α expression and localisation were examined in primary hASCs using qPCR, western blotting, immunofluorescence and high content screening. Reporter assays and ChIP were performed to assess the effect of HIF-1α on PII activity and binding. Treatments included PGE2, forskolin/phorbol-ester (FSK/PMA; PGE2 mimic) or DMOG (HIF-1α stabiliser). Double immunohistochemistry for HIF-1α and aromatase was performed on formalin-fixed, paraffin-embedded tissues from breast cancer and cancer-free patients.

Results: PGE2 increases HIF-1α transcript and protein expression, nuclear localisation and binding to aromatase PII in hASCs. Reporter assays demonstrate that HIF-1α significantly increases PII activity in the presence of DMOG and/or FSK/PMA, and HIF-1α and CREB1 act co-operatively to increase PII activity. There is a significant increase in HIF-1α positive ASCs in breast cancer patients compared to cancer-free women. HIF-1α and aromatase double-positive, and single positive ASCs for HIF-1α or aromatase, are significantly increased in tumour patients compared to normal. Moreover, double negative ASCs are significantly decreased in tumour cases compared to normal. Conclusion: This study identifies HIF-1α as a modulator of PII-driven aromatase expression in postmenopausal breast cancer. Together with our on-going studies on the role of AMP Kinase in the regulation of breast aromatase, this work provides another link between disregulated metabolism and breast cancer.

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AR-Cell Cycle Crosstalk: impact on cancer progression & therapeutic response

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Prostate cancers of all stages are exclusively dependent on the activity of the androgen receptor (AR). Ablation of AR activity is the first line of treatment for non-organ confined tumors; however, recurrent, castration resistant tumors (CRPC) ultimately emerge for which no effective therapy has been identified. CRPC arises as a result of resurgent, often ligand-independent AR
activity and resumption of AR-dependent cell cycle progression despite sustained castration therapy. Strikingly, there is currently no durable mechanism to treat CRPC. Thus, it is imperative to discern the mechanisms by which AR is controlled and promotes aggressive tumor phenotypes in advanced disease. Recent findings suggest that crosstalk between AR and the cell cycle machinery plays a major role in disease progression:

First, genome-wide analyses revealed that AR exhibits cell cycle stage specific binding events and concomitant transcriptional regulatory functions, consistent with the findings that a subset of cell cycle regulators serve as effectors of AR activity. The underlying basis for and consequence of cell-cycle specific AR activity is under investigation, and preliminary findings will be presented.

Second, selected tumor-associated perturbations of the cell cycle machinery appear to differentially reprogram AR activity. Mediated by the ability of RB to suppress AR expression, loss of RB function and/or gain of E2F1 promotes enhanced AR expression that is sufficient to drive expression of CRPC-specific AR activity in vivo. Conversely, alterations in the cyclin D1 pathway promote AR-dependent metastatic phenotypes. Alternative splicing of the CCND1 pre-mRNA in prostate cancer results in upregulation of a highly oncogenic variant, cyclin D1b, that promotes alternative AR signaling. Modeling cyclin D1b and investigation of the consequence for AR activity strikingly revealed that cyclin D1b induces AR-dependent expression of genes associated with epithelial-to-mesenchymal transition (EMT) and metastasis. Mechanistic investigation revealed that cyclin D1b facilitates binding of AR to the SNAI2 regulatory locus and resultant upregulation of Slug expression. These cyclin D1b-dependent AR functions resulted in acquisition of anchorage-independent growth and in vitro migratory phenotypes.

Third, concordant with in vitro analyses, perturbations in AR-cell cycle crosstalk were found to result in aggressive tumor phenotypes in vivo, and clinical investigation supports alterations in the RB and D-cyclin pathways as promoting transition to castration-resistance and metastasis, respectively. Combined, these findings identify perturbations cancer-associated alterations of the cell cycle as major effectors of aberrant AR expression and activity that promote aggressive tumor phenotypes and progression to advanced disease. Ongoing studies are directed at achievable means to target the AR-cell cycle in prostate cancer.

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**ESA-Antibiotic Resistance, Guidelines and Stewardship**

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Sixty Years ago, antibiotics were hailed as miracle drugs. No other medical discovery has saved more lives in that time. In recent years, the miracle has been seriously threatened and the rise of resistance has meant that many of the advances in modern medicine may become seriously compromised. The WHO has listed the rise of resistance as one of the 3 greatest threats to human health. This has been compounded by the marked decline in new antibiotics entering the pipeline, with fewer than 10 being registered over the last decade. Infections with antibiotic resistant bacteria used to be thought to be a part of hospital practice, but are now widespread in the community. About 25% of severe Staph aureus infections from the community are MRSA, and multi drug resistant Gram negatives such as E coll and Klebsiella are not uncommon. The solution to this problem is not obvious. Increased efforts towards new drug discovery are being made and in Australia, the NH&MRC has prioritised research on infections and resistance. Maintaining the utility of our current antibiotic armamentarium is a priority, and this means reducing the selective pressure of antibiotics in general. Ensuring that only those who truly need antimicrobial therapy get it, and that narrow spectrum antibiotics are used whenever possible has led to the development of antibiotic stewardship programs which will be a mandatory part of accreditation of all healthcare facilities in Australia. Essential components of stewardship programs include guidelines, formularies and restrictions, audit and feedback. Every specialty has been asked to include antibiotic prescribing and the principles of antibiotic stewardship in their curriculum. This strategy is hoped to contribute to prolonging the utility of current agents to allow time for new strategies such as vaccines and new agents to be developed.

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**Iron Chelation Prevents Obesity by Increasing Hypothalamic Hypoxia-Inducible Factor-1α, Metabolic Rate and Adipose Tissue Browning**

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**Background:** Increased body iron status has been linked to an increased risk of obesity, metabolic syndrome and diabetes. In contrast, lower iron status associates with a decreased risk of obesity and associated diseases. In animal studies, iron deficient diets improved diabetes. Although a mechanistic link between high iron levels and metabolic activity is yet to be identified, one possibility is the regulation of the transcription factors Hypoxia Inducible Factors 1α (HIF-1α) and 2α (HIF-2α), both of which are known to be stabilised by iron depletion. We examined the effects of decreasing body iron stores upon development of obesity in mice.

**Methods:** Mice were fed a high-fat diet (HFD) with or without the oral iron chelator deferasirox (DFS). We have previously shown that DFS can increase HIF-1α protein levels both in vitro and in vivo (Cheng et al., JCI, 2010).

**Results:** Iron chelation completely prevented excess weight gain in mice on a HFD, without rendering mice anemic. HFD+DFS fed mice ate slightly more, had increased metabolic rate and body temperature. In addition, DFS treatment led to a 3-6 fold increase in ‘browning’ of white adipose tissue and increased expression of genes involved in brown fat programming, including...
Prdm16, Ppargc1a (encoding PGC1α) and Ucp1. The hypothalamus regulates food intake, weight and energy expenditure. To investigate whether the effects of DFS were centrally mediated, hypothalamic arcuate nucleus specific HIF-1α-null mice were created. These mice were resistant to the beneficial effects of DFS on weight and white adipose tissue ‘browning’.

Discussion: Our data demonstrate that 1) iron chelation prevents obesity in mice on a HFD by increasing energy expenditure, 2) iron chelation induces white adipose tissue ‘browning’ and 3) hypothalamic HIF-1α is a novel and important regulator of weight and energy expenditure. This is a potential new therapeutic strategy for treatment of human obesity.

Intrauterine growth restriction induces central fat in the adult sheep

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Background: In humans, low birthweight and accelerated neonatal growth, predict later obesity and metabolic disorders. Whilst central adiposity (a predictor of later metabolic disease) develops by 4 years of age in intrauterine growth restricted (IUGR) children1, there are limited and conflicting findings regarding obesity and fat distribution in the IUGR adult2,3. Similarly, IUGR sheep exhibit catch-up growth and increased visceral fat mass as juveniles4,5. Whether this persists and alters adult fat mass distribution is undetermined.

Methods: Placental restriction (PR) was induced by removing most uterine implantation sites of ewes before mating. Weight and size were measured at birth and throughout life in control (CON; n=29), and PR (n=16) offspring. Fat and lean tissue masses were assessed at ~43 weeks by dual x-ray absorptiometry for total body, upper (predominantly omental) and lower abdominal (includes perirenal and retroperitoneal depots) regions (Figure).

Results: PR reduced birthweight (23%; CON: 6.09±0.34 kg; PR: 4.72±0.32 kg; p=0.02) but not adult weight. In PR offspring, absolute (AGR) and fractional (FGR) neonatal growth rates (0 – 16 d) correlated with birthweight (AGR: r=0.603, p=0.013; FGR; r=-0.744, p=0.001; n=16). In CON offspring, only AGR correlated with birthweight (AGR: r=0.603, p=0.013; FGR; r=-0.356, p=0.058; n=29). Total body fat (% body weight) was not correlated with birthweight (r=0.076, p=0.620, n=45), but, both upper abdominal fat (% total body fat; r=-0.401, p=0.006, n=45) and total body lean mass (r=0.446, p=0.002, n=45) correlated with birthweight.

Conclusions: IUGR is associated with reduced lean tissue mass and unchanged overall adiposity in adult sheep, similar to that reported in humans, but with redistribution of fat centrally, possibly contributing to IUGR-associated adverse cardiovascular and metabolic health outcomes.

Vitamin D Exerts Anti-proliferative and Modulatory effects in the Differentiation of Skeletal Muscle Cells

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Background: An association between vitamin D deficiency and muscle weakness has been reported for decades. However, the mechanisms are unclear. Whether vitamin D exerts direct effects or indirect effects (e.g., via calcium and phosphate homeostasis) remains unclear. Central to this question is the debate about whether vitamin D receptor (VDR) is expressed in muscle and regulates transcription at this site.

Methods: C2C12 cells, an established cell culture model of skeletal muscle and VDR knockout (VDRKO) mice were studied.

Results: Using a highly-specific VDR antibody, we detected VDR protein in contractile C2C12 myotubes. Following 1,25(OH)₂D treatment, VDR mRNA increased in a dose-dependent fashion. The maximum increase was 10-fold following 24 hrs of 10⁻¹⁵ M 1,25(OH)₂D, p<0.005. We examined the differentiation of C2C12 cells treated ±1,25(OH)₂D or vehicle. At 72 hours, 1,25(OH)₂D dose-dependently reduced myoblast number due to an anti-proliferative effect. Flow cytometric analysis of cell proliferation (BrdU and 7AAD) showed 82% vs. 72% of cells in resting phase of the cell cycle, p<0.005. Continued treatment with 1,25(OH)₂D delayed transformation of C2C12 myoblasts to myotubes and reduced their number. This coincided with down-regulation of myogenic transcription factors including myf5, myogenin, desmin and myoD. However, despite delayed proliferation and lower absolute number of myotubes, by day 10, cells treated with 1,25(OH)₂D were markedly larger (1.8-fold increase in cross-sectional area, p<0.005). Myostatin, a negative regulator of muscle mass displayed pronounced down-regulation (>10-fold, p<0.005).

In vivo, vitamin D receptor knockout mice (VDRKO) display lighter muscles, whether expressed as weight or as proportion of body weight (gastrocnemius: 0.50% vs. 0.57%, p=0.01), smaller muscle fibres and reduced grip strength vs. wild-types (1.8-fold decrease, p<0.005).

Conclusions: VDR was present in C2C12 cells, and responded to 1,25(OH)₂D. Treatment increased muscle fibre size. These results suggest that vitamin D is an important regulator of muscle differentiation and size.

Establishing regularity: Assessing pulsatile GH secretion in early-pubertal versus early-adult mice.

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The secretion of Growth Hormone (GH) is characterized by distinct periods of elevated secretion dispersed with periods of low secretion. This pulsatile GH secretion pattern is thought to change relative to growth and ageing, with the most pronounced change seen between puberty and adulthood. It is thought that peaks in GH secretion reach maximal levels at puberty, and decline following the completion of linear growth. While the change in GH secretion in humans throughout puberty is well characterised, the underlying mechanisms that account for this remain largely unknown. To address this, we assessed pulsatile GH secretion in early-pubertal (5-week old) and early-adult (10-week old) wild-type C57BL/6J mice (between 0700h and 1300h). Raw values were processed via deconvolution and approximate entropy (ApEn) analysis as described previously [1]. Basal and peak GH secretion per pulse, and total GH secreted over the sampling period did not change between early-pubertal and early-adult mice. Compared to early-pubertal mice, an increase in the regularity of the secretory pattern of GH was observed in early-adult mice. This was reflected by an increase in the mode of pulsatile GH release, and a decrease in the number and ApEn of GH pulses. Observations suggest that while peak levels of GH secretion are similar between early-pubertal and early-adult mice, the regular pattern of pulsatile GH secretion only becomes established once mice transition into early-adulthood. Compared to early-adult mice, we observed higher levels of IGF-1 within the muscle of early-pubertal mice. This coincides with peak periods of linear growth. Our observations imply that the synchronous pulsatile release pattern of GH is established by early-adulthood, and that an increase in pulse frequency of GH secretion may underlie the promotion of rapid pubertal growth in the mouse.

This work is supported by the National Health and Medical Research Council (NHMRC) and University of Queensland. Kevin Lee is a recipient of the Australian Postgraduate Awards (APA) scholarship. Dr Shyuan Ngo is a Motor Neurone Disease Research Institute of Australia (MNDRIA) Bill Gole Postdoctoral Fellow.

The decline in overall spontaneous and stimulated growth hormone (GH) secretion in overweight and obese individuals is well established1. While impaired GH secretion is usually observed alongside increased adiposity, some debate exists as to whether impaired GH secretion contributes to or is a consequence of increased adiposity. To this extend, mouse models may provide essential information to elucidate the relationship between adiposity and GH secretion. To further assess the relationship between adiposity and GH secretion in the mouse, we first characterized the impact of obesity on pulsatile GH secretion in C57BL/6J mice following 8 weeks of high fat feeding. Second to this we assessed the correlation between body weight, adiposity, circulating levels of leptin and free fatty acids (FFA) in healthy control C57Bl/6J mice. Subsequently, the correlation between overall pulsatile GH secretion and adiposity was evaluated. For measures of pulsatile GH secretion, tail-tip whole blood samples (4μl) were collected over a 6hr period at 10min intervals starting at 0700h and assayed for GH2. Pulsatile GH secretion was analyzed by deconvolution analysis following guidelines established previously3. Our observations confirm a significant reduction in overall GH secretion in mice following dietary induced weight gain. This occurred alongside an increase in adiposity and the corresponding increase in circulating levels of leptin. Assessment of the relationship between pulsatile GH secretion and adiposity in healthy control mice demonstrate a significant negative correlation between GH parameters (total, pulsatile GH secretion and mass of GH secreted per burst (MPP)) and adiposity, as well as circulating levels of leptin. Observations further demonstrate the close inverse relationship between adiposity and pulsatile GH secretion in mammals. This work is supported by NHMRC and The University of Queensland.


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RF-amide Related Peptide-3 (RFRP-3) Neurons do not Mediate Leptin Actions
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Leptin, an adipose-derived anorectic hormone, has several hypothalamic actions including a permissive regulation of fertility. Leptin sends information to the hypothalamic gonadotrophin-releasing hormone (GnRH) neuronal system regarding the body’s metabolic status. Our lab has shown that leptin does not directly act on GnRH neurons (Endocrinology 150:2805), and the indirect mechanisms by which leptin acts upon GnRH remain unclear. When the hypothalamic leptin receptor is stimulated, signal transducer and activator of transcription-3 (STAT-3) is phosphorylated mainly in the regions of the arcuate nucleus, ventral premamillary nucleus, medial preoptic area, and to a lesser extent the dorsal medial hypothalamus (DMH). RFRP-3 neurons are located in the DMH, and have been proven to directly act on GnRH neurons to inhibit reproduction. We tested whether leptin could indirectly act via RFRP-3 neurons to regulate GnRH activity. To examine this, we used two approaches. First, the presence of leptin-induced STAT-3 in RFRP-3 neurons was examined in female and male wild type C57BL/6J mice. Mice were given an acute leptin challenge (1 mg/kg) two hours prior to perfusion, followed by dual label immunohistochemistry for RFRP-3 and pSTAT-3. Results showed that although being present and in close proximity in the DMH, there was no colocalization between RFRP-3 neurons and pSTAT-3. Lastly, qPCR was used to compare RFRP mRNA levels between leptin-deficient ob/ob mice and wild type mice of both sexes. There was no significant difference between the levels of RFRP mRNA in wild type versus ob/ob males and females. Collectively, these results show that although it is able to act in the same region, leptin does not act on RFRP-3 neurons to modulate fertility.

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Circadian rhythms and the regulation of mood disorders.
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Circadian rhythms pervade all aspects of our physiology, from sleep/wake cycles, body temperature, hormone levels, and even cognition, attention and mood. It has long been recognised that mood disorders are associated with altered circadian function, with sufferers of depression often displaying blunted or abnormal rhythms of temperature, cortisol, noradrenaline, thyroid stimulating hormone, blood pressure and melatonin. We have found that animal models of depression also display disrupted behavioural, hormonal and gene expression rhythms. Current therapies do not address these symptoms. The atypical antidepressant agomelatine, which is an agonist at melatonin receptors (MT1 and MT2) and an antagonist at the 5-HT2c receptor, can phase advance and synchronise circadian rhythms when administered at the appropriate time of day. Furthermore, clinical trials have demonstrated agomelatine treatment significantly reduces HAM-D scores and increases the rate of response compared to placebo for sufferers of major depressive disorder. In this presentation, we review the relationships between circadian rhythms and depression, and discuss recent studies on potential targets of agomelatine.
Fat and the brain - interactions between obesity, appetite and mood

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Overweight and obesity have reached epidemic proportions in Australia. Less than one third of the Australian adult population has normal weight. Obesity is a known risk factor for decreased brain tissue volume, cognitive impairment and Alzheimer’s disease. It is also associated with depression, decreased quality of life, and decreased executive function. These alterations could be attributed to the excess fat, or to the metabolic and cardiovascular changes that are seen in obese individuals, such as hypercortisolaemia, poor physical fitness, impaired respiratory function, pro-inflammatory state, dyslipidaemia, hyperglycaemia, insulin resistance and hypertension. Leptin is an adipokine produced mainly by the white adipose tissue, and it is increased in obesity. Leptin plays key roles not only in regulating food intake, but also in mood and cognition by affecting neurogenesis, neural growth, and neural survival in brain areas other than the hypothalamus.

The regulation of human body weight, appetite and mood relies on sophisticated interactions between the central nervous system and the periphery. Food intake and energy expenditure are regulated by central and peripheral signals from the adipose tissue, gastrointestinal tract, and pancreas. Changes in adiposity, in turn, might also be associated with changes in mood and cognition, at least partially limited by leptin.

The effects of excess fat mass and of the components of metabolic syndrome on mood, quality of life and executive function need to be better characterised, and correlated with the hormonal alterations that are seen in obesity and with the genetic polymorphisms known to predispose to mood and cognitive disorders. Ongoing studies will allow the development of prophylactic and therapeutic targets against obesity-associated mood and cognitive disorders.


Low prolactin during early pregnancy impairs maternal behaviour postpartum, and delays the onset of puberty in female offspring

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From early pregnancy levels of prolactin increase dramatically, which leads to an increase in maternal gestational neurogenesis in the subventricular zone of the lateral ventricle (SVZ). As SVZ neurogenesis generates new olfactory neurons, which may contribute to perception of offspring, we hypothesized that the prolactin-induced increase in neurogenesis might be important for normal maternal behaviors. When prolactin secretion was suppressed early in pregnancy in mice, to prevent the normal increase in maternal neurogenesis, maternal anxiety was significantly increased postpartum, and maternal behavior markedly impaired. Injections of the mitotic inhibitor, methylazoxymethanol, to specifically suppress maternal neurogenesis without affecting prolactin secretion, also caused postpartum anxiety and impaired maternal behavior. These data demonstrated that the prolactin-induced increase in maternal SVZ neurogenesis during pregnancy is required for normal expression of postpartum maternal behaviors. In this model of postpartum anxiety we observed that daughters of anxious mothers had delayed onset of puberty. Correct levels of reproductive neurons are essential for normal reproductive physiology. Hence, we hypothesized that decreased levels of prolactin in the mother during early pregnancy alters neuronal development in offspring. Female fetuses of low prolactin (anxious) mothers had an increase in DNA methylation on day 9, but not day 7 of gestation, compared to control fetuses, demonstrating a change in the pattern of gene expression. Levels of neuronal apoptosis were increased on postnatal day 4 in both first and third generation daughters of anxious mothers, and this was associated with reduced kisspeptin expression in the hypothalamus of immature and adult female offspring. These results indicate that low levels of prolactin during early pregnancy not only significantly affect maternal behaviors, but also have a sustained and persistent effect on the reproductive physiology of female offspring.

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Prenatal programming of stress dysregulation: epigenetic and placental contributions

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Neurodevelopmental disorders including autism and schizophrenia show strong sex biases in presentation, onset and treatment. Such disorders have been associated with fetal antecedents including maternal stress. The programming mechanisms through which stress contributes to disease development are not well understood; though likely involve a complex interaction between the maternal environment and effects on the placenta. We have identified a sensitive period of early gestation where maternal stress has sex-dependent epigenetic programming effects on offspring stress pathway neurodevelopment. Male offspring show increased stress sensitivity as adults in behavioral and physiological measures including tests assessing cognitive performance and stress coping strategies. These males also demonstrate physiological features of dysmasculinization including reduced testosterone levels, smaller testes, and a shorter anogenital distance supporting a disruption in normal perinatal masculinization. Mechanistically, we have found dramatic changes in the neonatal brain miRNA environment in response to early prenatal stress that is programmed by the neonatal testosterone surge. From these large bioinformatics data mining analyses, families of miRNA genes specific to the developing hypothalamus were identified. In addition to the brain, maternal stress so early in pregnancy likely influences placental programming. As the placenta is a sex-specific tissue, we identified a limited set of genes that consistently differ between the sexes across pregnancy, all of which are located on the sex chromosomes. Utilizing a proteomics approach, we have found a candidate placental glycosylation enzyme and its biochemical target proteins that are significantly altered by maternal stress in male tissue. Chromatin immunoprecipitation analyses support a direct mechanism whereby stress experience decreases the transcriptional activator mark histone H3K4me3 association with this gene, decreasing its expression. As protein glycosylation typically competes with phosphorylation events, such broad changes are likely to yield important outcomes in placenta function and nutrient support of the developing fetus following stress. These results may provide critical insight into the mechanisms contributing to sex biased disease vulnerability to prenatal stress during early pregnancy. Further, as many neurodevelopmental disorders have a sex bias in presentation, these studies may provide novel biomarkers predictive of at-risk pregnancies.

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Acute effect of calcium supplementation on cardiovascular function

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Calcium supplements have recently been reported to be associated with an increased risk of cardiovascular events1. However, the validity of these findings has been questioned. A major concern is that the mechanism underlying an increase in cardiovascular events has not been demonstrated2. Calcium initiates cardiac and vascular contraction following influx of calcium into cardiac and smooth muscle from extracellular fluid. We have investigated whether the acute rise in serum calcium following supplement administration is associated with changes in cardiovascular function. In an open interventional study, we recruited 25 volunteers (16 female, age 60.3±6.5 years, BMI 25.7±2.7 kg/m2) from the community who were not taking calcium supplements. Participants were studied before and 3 hours after a single oral dose of 1000 mg calcium citrate. We assessed well-validated markers of arterial stiffness (pulse wave velocity, PWV), arterial wave reflection (augmentation index, AIx) and myocardial perfusion (subendocardial viability ratio, SEVR) by pulse wave analysis and endothelial function (reactive hyperaemia index, RHI) by peripheral arterial tonometry. Total and ionized serum calcium were acutely increased by 0.10±0.07 and 0.06±0.03 mmol/l respectively 3 hours after calcium citrate administration (p=0.001 for both comparisons). Following administration of calcium citrate there was a fall in AIx from a median of 29.7% (23.8-34.0) to 26.4% (22.7-34.0, p=0.03) and increase in SEVR from 163% (148-174) to 170% (149-185, p=0.007). PWV and RHI were not significantly altered. The change in total calcium was negatively correlated with the change in AIx (r=-0.48, p=0.02).

In summary, the acute increase in serum calcium following calcium supplement administration is associated with reduced arterial wave reflection and increased myocardial perfusion. If maintained long-term, these changes would be expected to reduce cardiovascular risk. Acute serum calcium-mediated changes in these parameters of cardiovascular function are unlikely to underlie an association between calcium supplementation and cardiovascular events.

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Trimester Specific Reference Intervals for Thyroid Function

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Methods

A central tenet of thyroid physiology is the inverse, log-linear relationship between serum thyroid stimulating hormone (TSH) and free T4 (fT4). Studies of the TSH-fT4 relationship have however, mostly been limited to cross-sectional analysis of small datasets. The aim of this study was to evaluate the TSH-fT4 relationship by cross-sectional analysis of a large population and examine intra-individual changes in TSH and fT4. Utilizing these approaches we hope to improve understanding of the hypothalamic-pituitary-thyroid feedback loop and how this relationship may be influenced by gender, age and thyroxine therapy.

Methods

We examined records with concurrent TSH and fT4 measurements performed over a 12 year period by a single, predominantly community-based, pathology provider using the Siemens Centaur assay. After applying exclusions, 445,918 records from 152,261 patients were available for further analysis. The TSH-fT4 relationship was examined in a cross-sectional analysis of these 152,261 patients. In 29,858 subjects with three or more sets of results, we calculated individual median TSH and fT4 concentrations and examined the relationship between change in TSH for any given change in fT4 (delta TSH and delta fT4).

Results

In the cross-sectional analysis, the relationship between TSH and fT4 was not log-linear but a combination of sigmoidal curves. The rate of change of TSH with fT4 below the reference range was steep, less so with fT4 within the reference range and almost flat for elevated fT4 levels. Within the fT4 reference range the curves for patients treated or not treated with thyroxine did not significantly differ. Over the whole fT4 range the median TSH in thyroxine treated individuals was 0.3 mU/L lower than untreated individuals however, for an elevated fT4, thyroxine treated patients had a higher median TSH than untreated patients. In subjects with high-normal fT4, the relationship differed between genders such that women had lower TSH concentrations than men at given high-normal fT4. In older people, TSH was less elevated in response to a low fT4 than in younger
people. Within the reference range however, median TSH was higher for older individuals compared with younger individuals. In the intra-individual analysis, the relationship between delta TSH and delta FT4 showed distinct curves depending on whether delta FT4 was positive or negative. A gender difference was again apparent and in older patients, TSH appeared less responsive to delta FT4 than in younger patients.

Conclusion
The relationship between TSH and FT4 is not log-linear but rather a complex combination of curves which differs between genders and changes with aging. This suggests that there are gender-specific and age-related differences in hypothalamic-pituitary-thyroid function.

Nadolol impairs pancreatic glucose sensitivity in patients with liver cirrhosis

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Objective: Glucose intolerance in liver cirrhosis is associated with hepatic decompensation and increased mortality. Nadolol, a beta-blocker used for the treatment of portal hypertension, worsens glucose intolerance; however the mechanism is unclear. We examined the impact of nadolol on BCF in patients with liver cirrhosis. Methods: Twenty cirrhotic patients participated in a double-blind randomised controlled cross-over trial of nadolol versus placebo for 3 months and, after a 1-month washout period, 3 months on the alternative. The present study includes 15 patients (10 male, 5 female, age range 45–61 years) who had measurements of insulin resistance and BCF, including five time point sampling for c-peptide, insulin and glucose during a 2 hour 75g OGTT performed at the end of each 3-month period. Mathematically-modelled components of BCF included beta-cell glucose sensitivity, beta-cell sensitivity to the rate of change of glucose (rate sensitivity) and potentiation (relative increase in insulin secretion from beginning to end of OGTT). Results: Six cirrhotic patients had impaired glucose tolerance (IGT) and 2 had diabetes at recruitment. Three normoglycaemic patients developed IGT on nadolol (p=0.15). Following 3 months treatment with nadolol, glucose sensitivity was reduced (97±16 vs 81±13pmol.min-1m-2, p=0.03) without change in rate sensitivity or potentiation; with similar basal and total insulin secretion during the OGTT. Conclusion: We show for the first time that nadolol specifically impaired pancreatic beta-cell glucose sensing ability. Further work is required to confirm whether the detrimental effect of nadolol on glucose tolerance is due to down-regulation of peroxisome proliferator-activated receptor gamma.

Higher free thyroxine levels predict increased incidence of dementia in older men. The Health In Men Study.

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Context
Both hypothyroidism and subclinical hyperthyroidism hinder cognitive function. However, it is unclear whether differences in thyroid hormone levels within the normal range might modulate risk of dementia particularly in older adults.

Objective
We aimed to determine whether more subtle alterations of thyroid hormone levels predict increased incidence of dementia in ageing men.

Design
Prospective longitudinal study.

Participants
Community-dwelling men aged 70-89 years.

Main outcome measures
The Standardised Mini-mental State Examination (SMMSE) was performed at baseline (2001-2004), and circulating thyrotropin (TSH) and free thyroxine (FT4) were assayed. Men with known thyroid disease or dementia, or SMMSE<24 were excluded from follow-up. New-onset dementia, defined by ICD codes, was ascertained using data linkage (2001-2009).

Results
During follow-up 147 of 3,408 men (4.3%) were diagnosed for the first time with dementia. Men who developed dementia had higher baseline FT4 (16.6±2.5 vs 16.0±2.3 pmol/L, p<0.001), but similar TSH (2.2±1.4 vs 2.3±1.6 mIU/L, p=0.22) compared with men who did not receive this diagnosis. After adjusting for age, body mass index, smoking, education, baseline SMMSE, medical comorbidities, social support and sensorial impairment, higher FT4 predicted new-onset dementia (quartiles, Q2-Q4 vs Q1: adjusted HR=1.79, 95%CI=1.05-3.05). There was no association between TSH quartiles and incident dementia. When the analysis was restricted to euthyroid men (excluding those with subclinical hyper- or hypo-thyroidism), higher FT4 remained associated with incident dementia (two-fold increase in adjusted HR for Q2-Q4, p=0.001 for trend).

Conclusions
Organ reperfusion results in an ischaemia reperfusion injury (IRI) that can compromise subsequent transplant function. IRI causes an inflammatory response activated via the Toll-like Receptor-4 (TLR4) pathway. Since the proinflammatory cytokine activin A is stimulated via the TLR4 pathway in mouse models of sepsis, we undertook studies to determine if activin A was a regulator of inflammation in IRI during lung transplantation. Activin A and its binding protein follistatin were measured by a specific ELISA and radioimmunoassay respectively, in serum samples collected from 48 patients undergoing lung transplantation just prior to the induction of anaesthesia, at about 2 hrs into the surgical procedure representing a time-point about 30 mins before lung reperfusion and also at 15 mins, 2hrs, 8hrs and 24 hrs after initiation of transplant reperfusion.

Basal serum levels of activin A (236 +/- 36 pg/ml) were elevated above normal levels in men and women (~160 pg/ml), probably related to the lung pathology requiring a transplant (eg cystic fibrosis, pulmonary hypertension). Serum activin A levels increased between basal and peak levels in the 2hr samples (844 +/- 100 pg/ml), consistent with the acute phase response to surgery. The activin A levels remain elevated at the start of the reperfusion phase and decline to normal levels at 24 hrs. The substantial activin A increase associated with transplant surgery is a likely driver of inflammation that may cause primary graft dysfunction. While follistatin increases from basal 12.6 +/- 1.0 ng/ml to 30.0 +/- 7.9 ng/ml in the 2hr sample, further elevation of follistatin levels during this period, through its capacity to bind and neutralise the bioactivity of activin A, may diminish the impact of IRI thereby improving graft function and organ survival.

Nevertheless, it is unknown if there are differences between lean and overweight/obese men in the activity of the sympatho-adrenal system in response to psychological stress. The activity of the sympatho-adrenal medullary system can be ascertained by measuring salivary alpha amylase (sAA) response to psychological stress in lean and overweight/obese men. We tested the hypothesis that overweight/obese men will have a greater sAA response to psychological stress compared to lean men. Lean (BMI=20-25 kgm^-2; n=19) and overweight/obese (BMI>27-35kgm^-2; n=17) men (50-70 years) were subjected to a well-characterised psychological stress (Trier Social Stress Test; TSST) at 3pm. Saliva samples were collected every 7-15min from 2pm-5pm. Concentrations of sAA were determined by a kinetic assay and were compared within and between groups using repeated measures ANOVA.

Salivary alpha amylase (sAA) response to psychological stress in lean and overweight/obese men

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We have shown in humans that overweight/obesity does not affect salivary cortisol responses to psychological stress1. Nevertheless, it is unknown if there are differences between lean and overweight/obese men in the activity of the sympatho-adrenal medullary system in response to psychological stress. The activity of the sympatho-adrenal medullary system can be ascertained by measuring salivary alpha amylase (sAA)2,3,4. We tested the hypothesis that overweight/obese men will have a greater sAA response to psychological stress compared to lean men. Lean (BMI=20-25 kgm^-2; n=19) and overweight/obese (BMI>27-35kgm^-2; n=17) men (50-70 years) were subjected to a well-characterised psychological stress (Trier Social Stress Test; TSST) at 3pm. Saliva samples were collected every 7-15min from 2pm-5pm. Concentrations of sAA were determined by a kinetic assay and were compared within and between groups using repeated measures ANOVA.

BMI, body weight, percentage body fat, resting systolic and diastolic blood pressures were significantly (p<0.05) higher in overweight/obese men compared to lean men. Both groups responded to the TSST with a significant(p<0.05) elevation of sAA (overweight/obese=111% increase, lean=138% increase) but this response did not differ significantly between lean and overweight/obese men (time*treatment, p=0.281). There were no significant differences between lean and overweight/obese men in pre-treatment sAA concentrations (112.1±16.1 vs 140.8±16.5 U/ml, respectively), delta sAA (155.1±51.2 vs 156.9±31.0 U/ml, respectively), peak sAA concentration during stress (267.3±55.5 vs 297.7±41.0 U/ml, respectively) or area under the curve (5220.5±2735.1 vs 3130.7±1525.4 U/ml/min, respectively) (p>0.05 for all).

Both groups had a substantially but similar sAA response to psychological stress. The results did not support the hypothesis that overweight/obese men will have a greater sAA response to psychological stress compared to lean men. Our data suggest that elevated sympatho-adrenal medullary system responses to acute psychological stress (measured by sAA) may not be a major mechanism that increases the risk of overweight/obese men developing stress-related disease. Further research is required to verify this.1,2

A subset of tumour cells in primary localized prostate cancers are resistant to androgen deprivation (castrate resistant): Implications for treatment strategies.

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Publish consent withheld

PGE2 downregulates tumour suppressor p53 and enhances aromatase expression in human breast adipose stromal cells

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Background: The majority of postmenopausal breast cancers are dependent on locally produced oestrogens for their proliferation. Oestrogens are converted from androgens by aromatase and aromatase expression in breast adipose stromal cells (ASCs) adjacent to a tumour is increased in response to tumour-derived factors such as PGE2 via the activation of its proximal promoter II. p53 is a tumour suppressor and women with breast cancer often carry sporadic mutations in the gene that encodes p53. However, mutations in p53 in ASCs are infrequent. We have identified three putative p53 response elements on PI. This study aimed to determine the role of p53 in regulating aromatase expression and the effect of PGE2 on p53 expression and activity in human ASCs in the context of postmenopausal breast cancer.

Methods: Primary ASCs, isolated from breast reduction surgery, were treated with PGE2 or FSK/PMA (PGE2 mimetic) and/or RITA/Nutlin-3 (to stabilise p53). Aromatase, p53 and 18s or beta-actin (housekeeping gene) transcript expression was examined by real-time PCR. Reporter assays were performed to determine the effect of different treatments on PI and p53 activities in HEK293 cells. Immunofluorescence was performed to determine effect of PGE2 on p53 subcellular localisation in ASCs and compare the expression of p53 in tumour-free and tumour-bearing breast tissue.

Results: RITA-stabilised p53 significantly reduced the PGE2 or FSK/PMA-induced aromatase expression and PII activity. FSK/PMA treatment significantly decreased p53 expression and transcriptional activity. Immunofluorescence showed that FSK/PMA and PGE2 treatment decreased p53 nuclear expression in hASC and enhanced perinuclear p53 expression was found in tumour-bearing tissue. ChIP demonstrated that p53 interacts with PII under basal conditions and that this interaction is decreased with FSK/PMA. In conclusion, p53 is a negative regulator of aromatase and its inhibition by PGE2 provides a novel mechanism for aromatase regulation in breast cancer.

The use of Long Terminal Repeats as Androgen-Responsive Enhancers in the PSA-Kallikrein Locus

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The androgen receptor (AR) signalling pathway is a common therapeutic target for prostate cancer because it is critical for the survival of both hormone-responsive and castrate-resistant tumour cells. Most of the detailed understanding we have of AR transcriptional activation has been gained by studying classical target genes. For more than two decades, Kallikrein 3 (KLK3, Prostate-Specific Antigen, PSA) has been used as a prototypical AR target gene because it is highly androgen-responsive in prostate cancer cells. Three regions upstream of the KLK3 gene, including the distal enhancer, are known to contain consensus androgen responsive elements required for AR-mediated transcriptional activation. Here we show that KLK3 is one of a specific cluster of androgen-regulated genes at the centromeric end of the kallikrein locus with enhancers that evolved from the long terminal repeat (LTR40a) of an endogenous retrovirus. Ligand-dependent recruitment of the AR to individual LTR-derived enhancers results in concurrent up-regulation of endogenous KLK2, KLK3, and KLKP1 expression in LNCaP prostate cancer cells. At the molecular level, a kallikrein-specific duplication within the LTR is required for maximal androgen responsiveness. Therefore, KLK3 represents a subset of target genes regulated by repetitive elements, but is not typical of the whole spectrum of androgen-responsive transcripts. These data provide a novel and more detailed understanding of AR transcriptional activation and emphasise the importance of repetitive elements as functional regulatory units.
Prostate epithelial AR inactivation in mice prevents experimental prostate cancer progression induced reduction in intraprostatic DHT

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In men androgens, testosterone (T) and its more potent 5α-reduced metabolite dihydroT (DHT) acting via the androgen receptor (AR), are the main sex hormones in the circulation and necessary for the development of prostate cancer. Intracrine androgen signalling involving AR, which regulates 5α-reductases (enzymes converting T to DHT) are involved in the origins and progression of prostate cancer. In the present work, we investigated the effect of PTEN tumor suppressor inactivation-induced prostate pathology on intracrine androgens and how this is regulated by intraprostatic AR.

Prostate histopathology as well as serum and intraprostatic androgens (liquid chromatography tandem mass spectrometry) and prostate 5α reductase expression (real-time RT-PCR) were compared between wild-type (WT) mice and prostate epithelia specific PTEN (PTENKO) and combined PTEN and AR knockout (PTENARKO) mice (Cre/loxP system).

Prostate specific PTEN inactivation (PTENKO males) featured increased prostate weight and severe epithelial pathology. In PTENARKO males prostate epithelial pathology was still present but the weight increase was not observed when AR inactivation was superimposed on PTEN inactivation. Surprisingly, intraprostatic DHT content was significantly reduced in PTENKO compared to WT (2.2±0.5 vs 8.8±1.1ng/mg; n=≥5; p=0.026), whereas AR inactivation (in PTENARKO males) significantly increased the intraprostatic DHT (14.8±3.5ng/mg) compared to both WT (p=0.048) and PTENKO (P<0.001). Prostatic expression of 5α-reductase2 mRNA was significantly increased in PTENARKO compared to WT (2.8-fold; p=0.04) and PTENKO (11.7-fold; p=0.018), whereas 5α-reductase1 was very low in all prostates. Serum T and DHT and intraprostatic T were not significantly (p>0.05) different between genotypes.

In conclusion, we demonstrate that prostate epithelium-specific AR inactivation does not prevent PTEN inactivation-induced severe prostate epithelial hyperplasia. However, the results suggest that during progression of experimental cancer, prostate cells may be sensitized to low levels of the potent androgen DHT, a feature that is prevented by epithelial AR inactivation.

A novel steroid hormone receptor coactivator associated with tamoxifen resistance

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Resistance to tamoxifen therapy is a major determinant of survivorship in oestrogen receptor (ERα)-positive breast cancer patients. Many patients exhibit de novo resistance, despite the presence of ERα in their tumours, whilst others who initially respond acquire resistance at a later stage. Understanding the mechanisms underlying this resistance will help in developing strategies to combat it, and aid identification of those patients most likely to relapse. Recently, the human homolog of Timeless (a Drosophila gene involved in circadian rhythm) was shown to be (i) positively correlated with increased tumour grade in breast cancer patients, (ii) a strong predictor of tamoxifen relapse and (iii) aberrantly increased in tamoxifen-resistant breast cancer cells in vitro. The function of Timeless in humans is unknown, but it is clearly not involved in circadian rhythm or clock function, as it is in Drosophila.

Recently, we isolated a Timeless-encoding cDNA clone during a protein:protein interaction screen using the orphan nuclear receptor LH1 (NRS2A) as bait. Given recent interest in Timeless and its associations with tamoxifen resistance, we hypothesized that Timeless might act as a steroid receptor coactivator. We transfected HEK293 cells with cDNAs encoding various steroid receptors, appropriate steroid receptor-responsive reporter genes, full-length Timeless (or vector control) in the presence or absence of appropriate ligands. Timeless enhanced ligand-induced activity of ERα, AR and GR by 2-3 fold, but inhibited aldosterone-induced activity of the MR. In MCF-7 ERα-positive breast cancer cells, 17β-oestradiol treatment increased expression of the ERα target genes pS2 and c-myc by 2-3-fold, but failed to increase expression of these genes when endogenous Timeless expression was inhibited using shRNA.

Our data suggest that Timeless could represent a novel steroid hormone receptor coactivator. We are currently seeking to confirm this hypothesis, and to understand the potential involvement of Timeless in tamoxifen resistance.


Androgens Modify Skin Formation and DMBA-Induced Pathology in Mice

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Epidemiological studies show significantly more non-melanoma skin cancers in men than in women, suggesting a role for sex steroid hormones. Androgen receptor (AR) is widely distributed at different layers of the skin suggesting tissue specific effects of androgens via AR in the skin. This is consistent with diverse modifications of skin morphology by endogenous or exogenous
androgens. Yet, the role of androgens acting via AR in skin structural development as well as in experimental skin carcinogenesis is not well understood.

We used the androgen insensitive, AR knockout (ARKO; Cre/loxP global inactivation) mouse model to determine the role of AR in 7,12-dimethylbenz[a]anthracene (DMBA)-induced experimental skin cancer. Male and female wild-type (WT) and ARKO mice were treated with 1mg DMBA/week (in 100μl sesame oil) for 6 weeks from 8 weeks of age. We demonstrate that males were significantly (p<0.05) more susceptible to DMBA-induced experimental skin cancer than females and AR inactivation significantly delayed detection of skin tumors in both male (median time to palpable tumor 21 vs 30 weeks, p=0.0014) and female mice (27 vs >35 weeks, p=0.009).

To determine if the AR inactivation modified skin structure at the time of DMBA exposure, skin from 8 week old intact male and female WT and ARKO mice was analysed for epidermis and dermis thickness and thickness of collagen layer. Compared to females, males had thicker dermis (337±35 vs 229±21μm in WT [mean±SE; n=3; p=0.003]) and 243±11 vs 185±9μm in ARKO [n=5; p=0.031]) and collagen layer (323±33 vs 203±14μm in WT [n=3; p=0.004] and 230±22 vs 146±8μm in ARKO [n=3; p=0.023]). AR inactivation significantly delayed detection of skin tumors in both male and female WT and ARKO mice (27 vs >35 weeks, p=0.009). These observations suggest a role for AR in modulating skin structure at the time of DMBA exposure. In conclusion, we demonstrate that androgen action operating via AR accelerate progression of experimental skin cancer and have a significant role in skin structural development.

Generation of a doxycycline-inducible mammary specific Liver Receptor Homolog-1 knock-in mouse

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**Background:** Breast cancer is one of the leading causes of cancer-related death in Australian women. Almost 70% of postmenopausal women are diagnosed with breast tumours receptive to estrogen receptor-a (ERa). Treatment for these tumours is successful; however treatments for tumours that are not receptive to ERa are less successful. New therapeutic targets need to be developed for treatment for tumours that don't respond to current treatments. Liver Receptor Homologue-1 (LRH-1) is an orphan nuclear receptor that plays vital roles in embryogenesis, cholesterol homeostasis, steroidogenesis and certain diseases. LRH-1 is aberrantly expressed in 43% of invasive breast cancers and promotes proliferation, migration and invasion of breast cancer cells. To understand the effects of LRH-1 in the development of breast cancer, we have generated a doxycycline inducible mouse model, in which the MMTV promoter drives expression of the reverse tetracycline transactivator (rTA) allowing for selective induction of the human LRH-1 transgene in mammary epithelium. **Methods:** Real Time-PCR and immunohistochemistry were employed using whole mammary glands. **Results:** Doxycycline induced homogenous transgene LRH-1 expression specifically throughout the mammary luminal epithelium on short (3 weeks) and long term (3 months) treatment. In addition, doxycycline increased transcript levels of LRH-1 at both treatment time points. Increased LRH-1 expression was associated with an increase in expression of the proliferation marker Ki-67, and decreased expression of the apoptosis marker Caspase 3 protein in epithelial cells at both treatment times. Furthermore, LRH-1 expression altered mammary gland morphogenesis as evident by a reduction in lateral bud number both stages of treatment (p=0.05 vs. control). These observations suggest a role for LRH-1 in mammary epithelial cell proliferation, ductal morphogenesis and branching. **Conclusion:** We have developed a mammary-specific doxycycline-inducible mouse model, which will be used to further explore the role LRH-1 plays in mammary development and breast cancer.

Identification of novel LRH-1 target genes in breast cancer cells

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The orphan receptor Liver Receptor Homologue-1 (LRH-1) has roles in development, bile-acid homeostasis and steroidogenesis. It also promotes tumourigenesis in gastric, colon, pancreatic and breast cancer. In breast cancer epithelial cells LRH-1 stimulates cell proliferation while in the tumour associated stroma it is critical in increasing local estrogen production by activating expression of the aromatase (CYP19A1) gene. Our previous expression profiling of breast cancer cells identified the heterogeneous ribonucleoprotein hnRNPs as potential LRH-1 target genes. Here, we aimed to determine the effects of LRH-1 and hnRNPs on breast cancer cell proliferation and invasiveness. Expression of LRH-1 and/or hnRNPA1 was knocked down in MDA-MB-231 breast cancer cells. Cy-Quant assay and wound healing assay were performed to assess the cell proliferation and invasiveness. To understand the potential mechanism of LRH-1 and hnRNPs on cell proliferation, mRNA levels of pyruvate kinase isoform I and II were measured using real time PCR. Silencing LRH-1 resulted in a 50% (p=0.01) decrease in cell proliferation and a 20% reduction in cell invasiveness, compared to cells transfected with control shRNA. Silencing hnRNPA1 led to a 25% (p=0.01) decrease cell proliferation. When both LRH-1 and hnRNPA1 were knocked down, cell proliferation was reduced by 30% (p<0.01). We also detected a shift in pyruvate kinase isoform expression from type II to type I in LRH-1 and hnRNPA1 knockdown cells (60% and 30% reduced respectively, p<0.01). The actions of LRH-1 in breast cancer progression are poorly understood. Here we identified hnRNPA1 and hnRNPA2/B1 as novel LRH-1 target genes. Inhibition of LRH-1 and hnRNPA1 expression decreases cell proliferation and invasiveness in breast cancer cells. Although the underlying mechanism for this regulation is not clear, our data suggest that a metabolic pathway switch through pyruvate kinase isoform expression may be involved.
Obesity in Pregnancy; A Legacy for the Next Generation?

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Publish consent withheld

The clinical utility of genetic testing: lessons from inherited endocrine disorders.

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There are 3 broad groups of genetic tests commonly used to diagnose inherited genetic disorders; those that detect gain or loss of large segments of DNA sequence (copy number variations or CNVs); those that detect change in the sequence of a single gene; and those that indirectly test gene expression. This paper will outline the technologies commonly used to detect CNVs (karyotype, FISH and array-CGH), detect DNA sequence changes (MLPA and related technologies, Sanger sequencing and massively parallel sequencing) and indirectly test gene expression (diagnostic immunohistochemistry). Clinical examples of families with inherited endocrine disorders will be used to illustrate issues related to the clinical utility of these technologies, as well as important pitfalls and limitations of genetic testing.

Genetic testing for CAH: A complex biosynthetic pathway playing a key part of a defining life event 'Did you have a boy or a girl?'

I McGown, J Wu, Y Jeske, C Choong, M Harris, Andrew Cotterill, D Cowley

Publish consent withheld

Monogenic obesity in humans

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Obesity has reached epidemic proportions worldwide. Genetic and environmental factors, individually and through gene-environment and gene-gene interactions, play a major role in determining fat mass in humans. Genome-wide association studies have identified common gene variants associated with moderate alterations in body weight. In contrast, single-gene or monogenic obesity disorders result in severe early-onset obesity. Mutations in genes encoding key regulators of appetite in the hypothalamus, including leptin, the leptin receptor, pro-opiomelanocortin (POMC), prohormone convertase 1/3 and the melanocortin 4 receptor (MC4R), have been identified in humans with severe, early-onset obesity. Most of these disorders are rare and inherited recessively, with the exception of MC4R deficiency, an autosomal dominant disorder accounting for 3-6% of severe obesity cases. A striking feature of all monogenic disorders is marked hyperphagia, with little, if any measurable defect in energy expenditure. Humans with mutations in leptin and leptin receptor are also characterised by T-cell hyporesponsiveness and neuroendocrine dysfunction. POMC deficiency results in early-onset obesity, red hair and adrenal insufficiency. MC4R deficiency is characterised by increased fat and lean body mass, disproportionate hyperinsulinaemia (up to age 20 years), tall stature, increased bone density, lower-than-expected pulse rate and relative protection from the development of hypertension. The study of humans with these disorders will shed light on the importance of the leptin-melanocortin pathway in determining human adiposity and will also allow the development of more specific and better-targeted treatment options. Indeed, treatment of leptin-deficient humans with subcutaneous rh-leptin results in significant weight loss, due entirely to loss of fat (as opposed to obligatory loss of both fat and muscle in diet-induced weight loss), a consequence of a significant reduction in hyperphagia. MC4R agonists are currently in development, but potential adverse cardiovascular effects, such as tachycardia and hypertension, need to be addressed.

Macrophage MR signalling regulates systolic blood pressure and cardiovascular remodelling

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Background: Mineralocorticoid receptor (MR) activation in the presence of high salt promotes vascular and cardiac inflammation, remodeling and fibrosis. A critical step in the development of fibrosis and tissue injury in this model is macrophage recruitment and vascular inflammation. A recent study showed mice in which the macrophage MR is selectively deleted, are protected from hypertension and cardiac inflammation and fibrosis without apparent change in macrophage recruitment.

Methods: We seek to verify this important role of macrophages by using mice in which monocyte chemoattractant protein 1 (MCP-1) is selectively deleted, thus impairing macrophage recruitment. This will independently interrogate the role of macrophages in mediating cardiac inflammation and fibrosis. Male mice from each genotype (wild type and MCP-1−/−) were uninephrectomised, given 0.9% NaCl to drink and treated for 8 days or 8 weeks with either vehicle (n=6-8) or deoxycorticosterone (DOC) (n=6-10).

Results: At 8 weeks, a significant reduction in cardiac macrophage (>50%, p<0.005) and CD3+ T cells (>50%, p<0.01) density was observed in the MCP-1−/− compared to wild type regardless of treatment. MCP-1−/− mice given DOC showed no increase in systolic blood pressure or cardiac fibrosis at 8 weeks, in contrast to wild type mice (11% reduction in SBP and 30% reduction in cardiac collagen area, p<0.005). Cardiac hypertrophy was similar for each genotype. Expression of pro-oxidative and inflammatory genes was significantly reduced in the MCP-1−/− mice in response to DOC. Expression of profibrotic markers (TGF-β1 and CTGF) showed a significant treatment effect with DOC in both genotypes which may be attributed to expression in other cell types in the myocardium.

Conclusion: Macrophage recruitment and activation play a significant role in the regulation of systolic blood pressure and cardiac remodeling and fibrosis.

3. Young, M.J. Mechanisms of mineralocorticoid receptor-mediated cardiac fibrosis and vascular inflammation. Curr Opin Nephrol Hypertens 17, 174-180

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Restricted growth before birth enhances allergenic immune responses in adolescent sheep

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Background: Prenatal exposures including maternal diet can alter immune function after birth. Preterm birth is associated with impaired response to vaccination in IUGR humans, suggests IUGR may shift immune responses from Type 1 to Type 2.

Methods: Intrauterine growth was restricted by surgical removal of most placental implantation sites from the uterus of sheep prior to mating. Spontaneous restriction also occurred due to twinning. Lambs from unoperated ewes (CON, 26 twins, 9 singletons) a priori were collected at baseline and 14 days after the last injection; cellular immune responses were assessed at 28 weeks by cutaneous allergen challenge.

Results: Placental restriction reduced birth weight (Figure A) by 11% overall (P=0.032) and by 23% in singleton lambs (P=0.005). HDM-specific IgE responses were greater (P=0.010) and OVA-specific total Ig responses tended to be greater (P=0.061) in PR than CON lambs (Figure B). Overall, late-phase cutaneous reactions to HDM tended to occur less frequently in light birth-weight lambs than those of heavier birth-weights (P=0.067), and in singletons, late phase cutaneous reactions to OVA occurred less frequently in PR than CON lambs (Figure C, P=0.008).

Conclusions: Increased IgE responses to HDM and decreased late-phase cutaneous reactivity in PR and/or low birth-weight lambs, together with impaired response to vaccination in IUGR humans, suggests IUGR may shift immune responses from Type 1 to Type 2.

Sex specific alterations in the renal renin-angiotensin system during kidney development may contribute to sexual dimorphism in adult cardiovascular and renal pathophysiology

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Sexually dimorphic expression of renin-angiotensin system (RAS) may account for differences in cardiovascular and renal disease progression. Perturbations during kidney development can result in reduced nephron endowment and hypertension in offspring in adulthood, with many models showing an effect on renal RAS. In our ovine model of reduced nephron number (due to fetal uninephrectomy at 100 days of gestation, term=150 days) we have shown uni-x male offspring have low plasma renin and reduced renal expression of angiotensin II type 1 and 2 receptors (AT1R and AT2R). This is associated with elevated blood pressure (BP) and reduced renal blood flow (RBF) at 6 months and 4 years of age (1,2).

Aim: The present study determined expression of the renal RAS in male and female fetuses 14 days post-uni-x (114d) and in the adult female. In addition, plasma levels of the RAS were examined in adult female sheep.

Results: Fetal uni-x did not alter renin mRNA levels in the fetus. Fetal uni-x had no effect on AT1R or AT2R expression in female fetuses however both were significantly lower in uni-x male compared to sham counterparts. In adulthood, female uni-x sheep had reduced expression of AT1R, however, AT2R levels were not different between groups. Plasma and tissues levels of renin and angiotensin II were reduced in the uni-x females. In contrast to male sheep, uni-x female sheep had similar BP and RBF to control animals prior to one year of age, however BP was elevated and RBF reduced at 4 years of age.

Conclusion: This study shows the uni-x offspring have reduced circulating renin and angiotensin II associated with sex specific alterations in renal AT receptor expression during both fetal and adult life. An activated RAS is required for appropriate transition of renal function from fetal to extrauterine environment. It is speculated that the onset of renal dysfunction in males may in-part be associated with differential response of RAS to perturbation in kidney development between the sexes incurring an inadequate adaptation of postnatal renal function in males compared to females.


Vitamin D Receptor Attenuates Hepatic Fibrosis in Mice

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Vitamin D signalling plays an important role in inflammation and fibrosis in a number of tissues. Recent studies have demonstrated that the vitamin D receptor (VDR) is expressed in liver cells, especially hepatic stellate cells responsible for fibrogenesis. In chronic liver disease, vitamin D deficiency is associated with more severe fibrosis progression, but whether this is causal, or an effect of impaired hepatic 25-hydroxylation of vitamin D is unknown.

Methods: We tested the effect of VDR deletion on liver fibrosis in response to a known liver toxin (thioacetamide). VDR knockout, heterozygous and wild type mice received oral thioacetamide ad 0.3g/L in drinking water for 10-12 weeks.

Results: Livers of VDR knockout mice displayed the most fibrosis (average 2.65 on METAVIR score). Quantitation of collagen content was also increased by ~2.5-fold in VDR-null mice.

Real-time PCR (table A and B) showed that VDR knockout livers displayed greater expression of pro-fibrotic mRNAs, such as: Collagen-1α, α-SMA (α-Smooth Muscle Actin), TIMP-1 (Tissue Inhibitor of Metalloproteinases-1) and MMP-9 (Matrix Metalloproteinase-9).

Conclusions: These studies strongly indicated that VDR inhibits liver pro-fibrotic gene expression and fibrosis. These findings are clinically relevant, as VDR is a potential therapeutic target for the resolution of hepatic fibrosis, and a high proportion of people with liver disease are vitamin D deficient.


Effects of castration on activin and follistatin levels during skin repair in an adult mouse model

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Activin and follistatin play an important role in the development and function of the skin. In particular they influence various cutaneous processes including inflammation, angiogenesis and wound healing. Androgens also have an important influence on wound healing. Although previous studies provide evidence for an interaction between testosterone and activin/follistatin
signals during cutaneous wound healing, no data has been done to confirm this interrelationship. The aim of this study was to examine the relationship between testosterone and the actions of activin/follistatin in the skin during wound healing. In the study, intact and castrated male mice received two linear full-thickness incisions. Serum and skin levels of activin and follistatin were measured at 0, 3, 5, 7 and 14 days post-wounding. Macroscopic and histological analysis showed that intact male mice had a significant delayed in wound healing compared to castrated males. Following injury, intact males had a decrease in serum follistatin, whereas in castrated males, levels remained constant. Castration affected unwounded skin, with a decrease in follistatin (p<0.001) and an increase in activin (p<0.001) skin levels. When wounded, castrated males displayed a delay in the peak of activin with reduced IL-6 levels, compared to the intact males. This study provides the first evidence that the actions of androgens on wound healing are associated with changes in cutaneous levels of activin and follistatin. The male genotype is considered to be a risk factor for impaired healing, with chronic ulcers commonly found in the elderly population. The finding that activin and follistatin are participating in the effects of sex hormones during wound healing processes are of great interest since new targets for treatments of wound healing using follistatin as a regulator of activin could be considered as strategies to solve these kind of problems.

### Disrupted GH-IGF-1 throughout disease progression in the hSOD1G93A mouse model of Amyotrophic Lateral Sclerosis (ALS)

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Pre-symptomatic hSOD1G93A mice have similar pulsatile GH secretion profiles when compared to wild-type age-matched controls. When compared to age-matched wild-type mice, hSOD1G93A mice at the onset of disease have a dramatic increase in GH secreted per burst, pulsatile GH secretion rate, and total GH secretion rate (p<0.05, n=9 wild-type, n=7 hSOD1G93A, t-test). As observed previously1, hSOD1G93A mice at the end-stage of disease present with a significant decrease in the amount of GH secreted per burst, pulsatile GH secretion rate, and total GH secretion rate (p<0.05, n=11 wild-type, n=8 hSOD1G93A, t-test). Increased GH at the onset of disease in hSOD1G93A mice coincided with an increase in muscle IGF-1 (p=0.0171, n=6, t-test), and neuromuscular denervation (p=0.0002, n=6, t-test). Diminished GH at the end-stage of disease in hSOD1G93A mice occurred alongside a decrease in circulating IGF-1 (p=0.0407, n=6, t-test) and extensive neuromuscular denervation (p<0.0001, n=6, t-test). Analysis of IGF-1R in skeletal muscle reveals a significant decrease in IGF-1R in hSOD1G93A mice at the end-stage of disease (p=0.0082, n=6, t-test). Disruptions to GH and IGF-1 occur throughout ALS disease progression in hSOD1G93A mice. Whether these disruptions contribute to, or exacerbate the disease process remains to be determined.

### Sex and stress steroids:- endocrine regulators of thermogenesis

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Adaptive thermogenesis is the dissipation of energy through heat production and occurs in specialised tissues including brown adipose tissue and skeletal muscle. Our work aims to characterise endocrine factors that regulate thermogenesis in these two tissues. In order to address this, we developed an ovine model. We demonstrate that post-prandial thermogenesis is evoked in sheep by meal feeding, where food is restricted to a set daily meal time. With this, we have characterised sex differences and the effects of sex and stress steroids on thermogenesis in muscle and fat. We show that testosterone reduces thermogenesis in both tissues in males but not females. On the other hand, estrogen increases heat production in both tissues in females. Thus the control of thermogenesis is sexually dimorphic and affected by sex steroids. In addition, we determined whether differences in thermogenesis alter predisposition to obesity. To address this we developed a model that used cortisol responsiveness as a marker for predisposition to obesity. Using a synacthen (ACTH) challenge, animals were characterised as either high or low cortisol responders (HR and LR). Animals characterised as HR have greater propensity to become obese when placed on a high energy diet, but this is not due to a difference in food intake. LR animals have a greater thermogenic capacity than HR animals and this confers relative protection against obesity. Interestingly, the...
Early Life Programming Of Brown Adipose Tissue Thermogenesis

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Non-shivering thermogenesis in brown adipose tissue (BAT) is a crucial physiological adaptation in many newborn mammals (including humans), which assists in the maintenance of core body temperature before insulatory white fat stores are deposited. Whilst the amount of BAT in adipose depots decreases with advancing age, it can still be recruited in adults in response to physiological challenges including cold (cold-induced thermogenesis) and excess caloric intake (diet-induced thermogenesis). The capacity of individuals for diet-induced thermogenesis has an important impact on their ability to resist weight gain in response to increased energy intake – and therefore their risk of overweight and obesity. In this presentation, I will discuss the evidence that an individual’s capacity for BAT thermogenesis can have its origins in the nutrient environment experienced before birth and/or in early infancy – and that being exposed to an inappropriately high or inappropriately low nutrient supply during this perinatal period can result in a life-long reduction in an individual’s capacity for diet-induced thermogenesis, and a consequent increase in their susceptibility to diet-induced obesity. This is of particular importance given the recent studies which have shown that BAT is present in significant amounts in adult humans, and not just in rodents and hibernating mammals as was previously thought, and that the programming of BAT thermogenic function is therefore likely to play a role in the early life origins of human obesity.

Stand Up, Sit Less, Move More, More Often - A New Exercise Prescription and Its Relevance to Menopausal Health

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In contemporary society, prolonged sitting has been engineered into our lives across many settings, including transportation, the workplace, and the home. There is new evidence that too much sitting (also known as sedentary behavior – which involves very low energy expenditure, such as television viewing and desk-bound work) is adversely associated with health outcomes, including cardio-metabolic risk biomarkers, type 2 diabetes, some cancers and premature mortality. Besides the decreased energy metabolism of sitting compared with light-intensity activity, sitting may also be harmful because of the prolonged absence of muscle contractile activity in the lower limbs. Importantly, these detrimental associations remain even after accounting for time spent in leisure time physical activity. This presentation will provide an overview of recent evidence from epidemiological and experimental studies. This new evidence is beginning to make a persuasive case that too much sitting should now be considered as a potential new element of physical activity and health recommendations – particularly for reducing the risk of type 2 diabetes and cardiovascular disease. Findings from the Australian Diabetes, Obesity and Lifestyle Study (AusDiab) have shown prolonged TV viewing time to be related to biological markers of diabetes and cardiovascular disease risk, which were much stronger for women than for men. New AusDiab findings specifically in the context of menopausal health will be highlighted, showing relationships of sedentary time with risk biomarkers across the menopause transition. Future directions for this research and the practical implications of focusing on too much sitting as a modifiable health risk in mid-age and older women will be outlined.

Adult growth hormone deficiency Outcome and Responsiveness, from clinic to pharmacogenomics

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Hypopituitarism is a complex endocrine deficiency due to a hypothalamic-pituitary disease. It is associated with increased morbidity and mortality, mainly from cardiovascular diseases. These data derives from patients who have received conventional replacement therapy, but not GH. GH replacement in adult hypopituitary patients has demonstrated reduced abdominal obesity, diastolic blood pressure, improved serum lipid profile, reduction in systemic inflammatory markers and improved quality of life. More recently published studies have suggested that the doubled standardised mortality rate seen in previous studies is near normalised in large cohort of patients who have received long-term GH replacement therapy.

The individual treatment response to GH is adults vary considerable. This is to some extent explained by the interaction between GH receptor signalling and sex steroid actions. Oestrogen increases the expression of SOCS2, which attenuates the phosphorylation of JAK2 and thereby attenuates the action of GH. This is most evident at the hepatic level during oral oestrogen replacement therapy that reduces the serum IGF-I response to GH. Testosterone on the other hand augments some of the action of GH such as the protein anabolic response and the increase in extracellular water, but the true mechanisms behind these interactions is not known. Other clinical predictors that have less impact are body mass index and serum insulin concentrations.

Acute and chronic effects of low-dose prednisolone on carbohydrate metabolism in subjects with inflammatory rheumatologic disease.

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High-dose glucocorticoids reduce hepatic and peripheral insulin sensitivity and insulin secretion. However, the metabolic consequences of typical therapeutic glucocorticoid doses (e.g. prednisolone <10 mg/day) are poorly characterised. The aim was to determine the acute effect of low-dose prednisolone on carbohydrate metabolism and whether chronic prednisolone increased visceral adiposity, amplifying carbohydrate metabolism perturbations. Nine subjects (4 female, age 58±11 years, BMI 27.5±5.8 kg/m²) with inflammatory rheumatologic disease not taking oral glucocorticoids were studied before and after prednisolone 6 mg/day for 7 days. Data were compared with 12 matched subjects (6 female, age 61±8 years, BMI 27.4±3.3 kg/m²) taking chronic (>6 months) prednisolone (6.3±2.2 mg/day). Hepatic glucose output was calculated from the change in isotopic enrichment of glucose after primed (5 mg/kg), continuous (3 mg/kg/hr) infusion of 6,6-2H₂ glucose. Peripheral insulin sensitivity was assessed by hyperinsulaemic-euglycaemic clamp (80 mU/m²/min for 120 minutes). Insulin secretion was determined by 60-minute frequently sampled intravenous glucose tolerance test (300 mg/kg glucose). Visceral adiposity was quantified by abdominal computed tomography.

Acute prednisolone administration increased hepatic glucose output (p=0.01) and reduced peripheral insulin sensitivity (p=0.02) and first- (p=0.01) and second-phase (p=0.02) insulin secretion (Table). There was no significant difference in visceral fat mass between subjects not on prednisolone and chronic prednisolone users (108±27 vs 97±11 cm², p=1.00). In subjects taking chronic prednisolone, hepatic glucose output was significantly greater (p=0.03) while insulin secretion was not significantly different from subjects not on prednisolone (Table). Peripheral insulin sensitivity was similar in chronic prednisolone users to following acute prednisolone administration (p=0.83) (Table).

In summary, acute low-dose prednisolone adversely affects all aspects of carbohydrate metabolism. However, in subjects taking chronic low-dose prednisolone increased hepatic glucose output is the major perturbation. Treatment of diabetes in patients on chronic low-dose prednisolone should primarily target a reduction in hepatic glucose output and also increase peripheral insulin sensitivity.
Acknowledgments:
The study was supported by grants from the Diabetes Australia Research Trust and Foundation Daw Park. Dr Carolyn Petersons is supported by an Australian Postgraduate Award and Flinders Centre for Clinical Change and Health Care Research Scholarship. Dr Morton Burt is supported by a South Australian Health Practitioner Fellowship.

<table>
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<th>Pre-prednisolone</th>
<th>Acute prednisolone</th>
<th>Chronic prednisolone</th>
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<tr>
<td>Hepatic Glucose Output</td>
<td>2.2±0.1</td>
<td>2.5±0.1*</td>
<td>2.9±0.3*</td>
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<td>(mg/min/kg)</td>
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<td>Peripheral insulin sensitivity</td>
<td>14.2±1.1</td>
<td>12.3±0.9*</td>
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<td>MIT (mg/min/kg=FFM/mUL)</td>
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<td>1st phase insulin secretion</td>
<td>5.9±0.7</td>
<td>3.9±0.5*</td>
<td>5.7±1.0</td>
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<td>(mU/mmol)</td>
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<td>2nd phase insulin secretion</td>
<td>4.6±0.8</td>
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<td>(mU/mmol)</td>
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Values are mean±SEM, *p<0.05 vs controls pre-prednisolone

Plasma, salivary and urinary cortisol levels following physiological and stress doses of hydrocortisone

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Objective, Design, Subjects: Optimal method for monitoring oral hydrocortisone replacement therapy has not been established, and there are few data on cortisol levels following intravenous hydrocortisone given at “stress” doses. Cortisol profiles were measured in plasma, saliva and urine following physiological (20 mg oral) or stress (50 mg intravenous) doses of hydrocortisone in dexamethasone-suppressed healthy subjects (8 in each group), compared to their endogenous cortisol levels.

Measurements: Plasma cortisol was measured half-hourly, and salivary cortisol and urinary cortisol:creatinine ratio were measured hourly from time 0 (between 0830 and 0900) to 5h. Endogenous plasma corticosteroid-binding globulin (CBG) levels were measured at time 0 and 5h, and hourly from time 0 to 5h following administration of oral or intravenous hydrocortisone.

Results: After oral hydrocortisone administration, the measurement of plasma, salivary or urine cortisol at 2h post-dose gave a good indication of peak cortisol concentrations, which were supraphysiological. The correlation between plasma and salivary cortisol concentrations after oral hydrocortisone (R = 0.83) was stronger than that using endogenous concentrations (R = 0.62), whereas plasma-urine cortisol relationship was similar (R = 0.61, compared to endogenous R = 0.56). Intravenous hydrocortisone administration achieved very high peak cortisol levels and strong correlations between plasma and saliva (R = 0.94) and urine cortisol (R = 0.82) levels were observed. Cortisol clearance was significantly higher following intravenous compared to oral administration. There was no difference in CBG levels during the sampling period.

Conclusion: Based on the cortisol levels achieved, an oral dose of hydrocortisone 20 mg is excessive for routine maintenance, while stress doses above 50 mg 6-hourly are rarely necessary in acute cortisol deficiency. Salivary cortisol and urinary cortisol:creatinine ratio may provide useful alternatives to plasma cortisol measurements to monitor replacement doses in hypoadrenal patients.

Taking the Skeleton out of the Closet: Performance and Cost-effectiveness of a ‘Fracture Capture’ Service

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A 2009 hospital orthopaedic unit audit identified that amongst patients over 50 years of age who were discharged directly from the emergency department after sustaining a fragility fracture, only 2% subsequently had a DXA scan and 6% were treated with an osteoporosis agent. One year later, a follow-up audit demonstrated that the introduction of an osteoporosis policy that guided investigation and referral significantly improved investigation rates, however did not alter treatment rates. Therefore in 2010 with funding from the pharmaceutical industry, a ‘Fracture Capture’ service was established to improve osteoporosis detection and management in outpatients over 50 years of age who had sustained a fragility fracture. A nurse-coordinator (0.3 EFT) screened patients attending orthopaedic clinics and arranged pathology and DXA testing before physician review (0.1 EFT).

This study aimed to assess the performance and cost-effectiveness of the ‘Fracture Capture’ Service over the inaugural two-years from April 2010 to April 2012.

Performance analysis included a clinic database audit and a patient quality assurance questionnaire. Cost-effectiveness analysis assumed a 5-year treatment duration with no mortality. Medication and investigations costs were taken from the Pharmaceutical Benefits Scheme and Medicare Benefits Schedule respectively. Staffing costs were derived from employment contracts while the direct medical costs associated with fractures were taken from published Australian data. Five-year fracture risk was calculated using the Garvan Fracture Risk Calculator and the fracture risk reduction assigned to each osteoporosis agent was the published non-vertebral fracture risk reduction. Published utility values for osteoporotic fractures were used to calculate Quality-Adjusted Life Years (QALYs) lived. The study was approved by the Melbourne Health Research Ethics Committee.

Osteoporosis agents were prescribed to 124 patients (61%): 44 risedronate, 32 alendronate, 27 strontium ranelate, 16 zoledronic acid, 2 teriparatide, 1 denosumab, 1 pamidronate, 1 testosterone. Ninety questionnaires were returned: 94% very satisfied/satisfied with the service; 74% reviewed by the physician within 2 months, 84% maintained medication compliance. We estimated that with treatment as above for 5 years, ‘Fracture Capture’ would reduce the number of refractures from 59 to 50, improving QALYs by 0.056 with net cost $1500 per patient. This equates to an incremental cost-effectiveness ratio of $26806/QALY gained, within a reasonable Australian cost per QALY threshold of <$50000. Although excluded from the cost-effectiveness analysis, ‘Fracture Capture’ also led to the identification and treatment of 6 cases of primary hyperparathyroidism, 3 cases of thyrotoxicosis, 4 cases of hypogonadism and 2 cases of monoclonal gammopathy of unknown significance.

‘Fracture Capture’ is a popular, cost-effective model to improve outpatient osteoporosis management.


The link between nephrolithiasis, bone density and fractures in transfusion-dependent thalassaemia

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3. Endocrinology, Monash Medical Centre, Melbourne, Australia
4. Thalassaemia Unit, Monash Medical Centre, Melbourne, Australia

Introduction: Thalassaemia is a disorder of haemoglobin synthesis due to mutations in the globin chains (a or b). Transfusion-dependent thalassaemia is associated with reduced bone mineral density (BMD) and fractures. Many causes are implicated
including hypogonadism, growth hormone deficiency, marrow expansion and iron overload. However, the relationship of nephrolithiasis to BMD and fractures has not previously been studied.

**Method:** A retrospective cohort study of 166 patients with transfusion-dependent thalassaemia was undertaken to determine the prevalence of nephrolithiasis, and its association with BMD and fractures. Logistic regression analysis using age and gender matches was employed to account for potential confounding factors.

**Results:** There were 73 (44%) male and 93 (56%) female participants aged between 4 to 66 years with a median age of 34 years. Fractures occurred in 19.9% of study participants and were more common in males than females (27.4% vs 14%). The overall prevalence of nephrolithiasis was 18.1%, occurring in 28.7% of males and 9.7% of females. Reduced femoral neck $Z$ scores were associated with an increased risk of nephrolithiasis (OR=1.63; 95% CI: 1.10-2.42). Furthermore, subgroup analysis showed nephrolithiasis in males was associated with an increased risk of fracture after adjusting for femoral neck or lumbar spine $Z$ score (OR=5.59; 95% CI:1.16-27.03, OR=5.21; 95% CI:1.06-25.64, respectively).

**Conclusion:** Nephrolithiasis is common and significantly associated with reduced BMD and fractures after adjusting for potential confounders. These findings demonstrate the need for ongoing surveillance of BMD, fractures and nephrolithiasis in the management of transfusion-dependent thalassaemia.

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**Cushing’s syndrome hypertension and virilisation from a malignant adrenal cortical tumour in a 5 months old baby with Li Fraumeni syndrome**

**Ganesh Chockalingam, Kim Tuck-Lee, Tony Lafferty**

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OB presented to a paediatrician at 5 months of age with a 2 month history of virilisation, linear growth failure and slowing head growth. His weight gain was maintained but he had delayed gross motor milestones. He was noted to have had recent pubic hair and penile growth, significant acne on face and trunk with body odour. His blood pressure was elevated. The provisional diagnosis was Cushing’s syndrome due to adrenal tumour with mixed androgen and glucocorticoid secretion was made and subsequent investigation confirmed the clinical findings and identified a 3cm left adrenal mass on ultrasound. He was referred to Sydney Children Hospital for staging and surgical management.

He was born by LUSCS at 41 weeks weighing 4.9kg pregnancy complicated by maternal pregnancy induced hypertension and was treated for neonatal hypoglycaemia with formula feeding. His father had been diagnosed with Non Hodgkin’s lymphoma at 2-3 years earlier and mother had PCOS, duplex kidney with scarring and hypertension from 22 years of age.

The initial blood tests showed normal renal function and hypokalaemia of 3.3 mmol/L. Endocrine evaluation showed an elevated midnight cortisol with low ACTH. The testosterone and DHEAS were elevated with normal renin and aldosterone. The CT abdomen confirmed the complex left adrenal mass with no metastases seen in lungs or on bone scan.

He underwent laparoscopic left adrenalectomy to removal of a 20.8 gram tumour, indeterminate, stage 1 (virilisation alone) adrenal cortical carcinoma. He required stress dose of hydrocortisone and antihypertensive peri-operatively. The tumour had favourable prognostic features and no chemotherapy was required. Postoperative surveillance included regular MRI imaging and biochemistry.

At 9 months the Cushingoid features and virilisation had resolved, cortisol levels were normal, testosterone and DHEAS levels and quarterly MRI scans remain normal. Short synacthen test demonstrates HPA axis recovery. Literature review revealed that a 5 year survival rate of 91% with stage 1-2 disease. Higher grade tumours show a poorer response to chemotherapy.

Genotyping identified a *de novo* TP53 germline mutation in OB (with both parents being normal) confirming suspicion of Li Fraumeni Syndrome, a rare autosomal dominant cancer syndrome. Li Fraumeni syndrome is associated with increased susceptibility to various cancers in particular, breast, brain (esp. choroid plexus), bony and soft tissue sarcomas, adrenal cortical carcinoma and acute leukemias.
Oestrogen excess phenotype associated with loss of heterozygosity of the STK11 gene in the testes of two boys with Peutz Jeghers Syndrome

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Background: Peutz Jeghers syndrome (PJS) is an autosomal dominant disorder characterised by the association of gastrointestinal hamartomatous polyps and mucocutaneous pigmentation which is due to mutations in the STK11 gene that encodes the LKB1 protein. PJS males may have oestrogen excess manifesting as gynaecomastia and an advanced bone age. We and others have previously described an increase in testicular aromatase expression in PJS patients. However, the underlying mechanism has not yet been explored. The aim of this study was to characterise the role of LKB1 in regulating the expression of aromatase in the testes of two boys with PJS via signaling pathways involving pAMPK, CRTC1, CRTC2 and CRTC3.

Methods: We studied testicular biopsies from two boys with STK11 mutations; a 13-year-old boy and an unrelated 4-year-old boy with prepubertal gynaecomastia and advanced bone age.

Results: Loss of heterozygosity of STK11 in Sertoli cells of abnormal cords of PJS testis samples, measured by the absence of LKB1 immunofluorescent staining, was associated with loss of p21 expression and decreased phosphorylation of AMPK, known downstream targets of LKB1, as well as the increased expression of aromatase. The cytoplasmic retention of the potent stimulator of aromatase CRTC3, which arises as a consequence of phosphorylation by AMPK, was decreased in cells where aromatase was detected.

Conclusions: Loss of heterozygosity of the STK11 gene leads to an increase in aromatase expression associated with loss of CRTC3 cytoplasmic retention, thereby providing a mechanism whereby PJS is associated with oestrogen excess.

Special K: A rare case of Gitelman’s Syndrome and Diabetes Mellitus

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1. Department of Diabetes and Endocrinology, Royal Melbourne Hospital, Melbourne, Victoria, Australia

A 28 year old man of Chinese ethnicity presented to hospital in 2008 with a three week history of generalised and progressive muscle weakness and eventual conscious collapse. This occurred on a background of increased intake of simple carbohydrate foods over the preceding few weeks. Initial arterial blood gas demonstrated severe hypokalemia and mild metabolic alkalosis (K 1.3 mmol/L pH 7.45 HCO3 28.7 mmol/L) which was confirmed on venous testing. Electrolytes (mmol/L) - K 1.4, Mg 0.63, Na 135, CI 90, HCO3 24, Cr 110, Ca (cor) 2.48, PO4 0.72. Spot urine Na 74, K 25. ECG revealed biphasic T waves and prolonged QTc (513ms). TFT and thyroid antibodies were normal. Diuretic and laxative screens were negative. FBG was 14.6 mmol/L despite no history of diabetes.

Aggressive potassium (780 mmol) and magnesium (70 mmol) supplementation was administered with complete symptom resolution despite persistent hypokalemia (K 2.8 mmol/L). After potassium replacement, hyper-reninemic hyperaldosteronism was evident (renin 161ng/ml pH 7.45 HCO3 28.7 mmol/L) which was confirmed on venous testing. Electrolytes (mmol/L) - K 1.4, Mg 0.63, Na 135, CI 90, HCO3 24, Cr 110, Ca (cor) 2.48, PO4 0.72. Spot urine Na 74, K 25. ECG revealed biphasic T waves and prolonged QTc (513ms). TFT and thyroid antibodies were normal. Diuretic and laxative screens were negative. FBG was 14.6 mmol/L despite no history of diabetes.

More recently in December 2011, the patient was admitted to hospital in China with recurrent severe hypokalemia (K 1.5 mmol/L) and hyperglycaemia (38.9 mmol/L). Despite long-term non-compliance with potassium supplementation, he denied interval symptoms until a change in dietary pattern similar to that which preceded his previous presentation. Potassium, magnesium and insulin were recommenced with rapid resolution of muscle weakness. Despite self ceasing insulin post-discharge, he maintained reasonable glycaemic control (HbA1c 7.2%) while on potassium supplementation and normal diet.

Glucagon stimulation test was performed to determine beta cell function in the hypokalemic and normokalemic states. In the hypokalemic state (K 3.0 mmol/L), C-peptide increased from 0.55 to 1.10 nmol/L (50% rise) post 1mg of glucagon, indicative of relative insulin deficiency. Despite administration of 240 mmol of potassium over 24 hours, a normokalemic state could not be achieved. Difficulty correcting hypokalemia has been demonstrated in previous small studies involving patients with Gitelman’s Syndrome.

There are no published case studies that demonstrate an association between Gitelman’s Syndrome and diabetes mellitus. However, Gitelman’s Syndrome affects the same element of the distal convoluted tubule as thiazide diuretics, which are strongly linked to new-onset diabetes. We propose that hyperglycaemia causes osmotic diuresis and hence exacerbates renal potassium wasting. Progressive hyperglycaemia may decrease beta-cell insulin secretion and exacerbate hyperglycaemia although the mechanism is not well understood.


Skin lightening cream: An Emerging Medical Challenge

Rebecca F Goldstein, Helena J Teede, Carolyn A Allan

A 24-year-old Sudanese woman presented with one month of fatigue, dizziness, abdominal distension and facial oedema. Hypocortisolaemia was present: morning cortisol 13 nmol/L (RR 240-620nmol/L), afternoon cortisol 9 nmol/L (RR 100 - 280nmol/L).

She appeared Cushingoid (moon facies, central obesity, skin fragility, striae) with skin depigmentation on face, back and hands. Blood pressure was 130/80 mmHg, fasting glucose 7.3 mmol/L (RR 4-6 mmol/L) and HbA1c 7.0% (RR<6.0%). ACTH was < 2pmol/L (RR 0–10pmol/L), with inadequate cortisol response to ACTH (169nmol/L at 60 minutes). Screening for exogenous glucocorticoid exposure revealed prolonged use of three skin lightening creams containing hydroquinone, 0.05% clobetasol propionate and 0.05% betamethasone respectively. She was advised to cease the creams, and weaning treatment with short-term glucocorticoid replacement and hypoglycaemic therapy was initiated.

Skin lightening product use in African women is common (~25%). These may contain potent corticosteroids, eg. clobetasol propionate and betamethasone dipropionate (super potent; class 1), and fluticasone propionate (potent; class 3). Corticosteroids decrease propriocortin (precursor for melanocyte signalling hormone) and cause epidermal cytostasis; prolonged use may reduce epidermal turnover, with fewer and less pigmented melanocytes.

Severity of complications depends on potency, quantity, duration and extent of application. Cutaneous complications include atrophy, telangiectasia, dermatitis, acne, striae, purpura, hypopigmentation and skin addiction syndrome. Endocrine complications include Cushing syndrome, diabetes mellitus, hypertension, oedema, menstrual irregularities and osteoporosis. Prolonged inhibition of HPA-axis may result in hypoadrenal crisis if the product is withdrawn abruptly. Application of 50g/week of clobetasol propionate (3.5g applied twice daily) may cause secondary adrenal failure; this equates to 500g/wk of 1% hydrocortisone.

Lightening creams are exported to, and manufactured locally within, African countries (eg Nigeria) with high usage. In Australia, prescription of highly potent topical steroids is limited to specialist prescribers however internet availability bypasses these regulations; ingredients may not be disclosed and counterfeit versions are available.


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Obesity in pregnancy – maternal diet and neonatal outcomes

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Obesity is a significant health issue for women during pregnancy and childbirth, with estimates suggesting that 35% of women aged between 25 and 35 years are overweight or obese. More recent data suggests that approaching 50% of women enter pregnancy with a body mass index greater than 25kg/m². There are well documented risks associated with obesity during pregnancy and childbirth, maternal complications including hypertensive conditions and pre-eclampsia, gestational diabetes, infection, thromboembolic events, need for induction of labour, caesarean section and perinatal death. Infants of mothers who are overweight or obese are more likely to be macrosomic, require admission to the neonatal intensive care unit, be born preterm, be identified with a congenital anomaly, and require treatment for jaundice or hypoglycaemia. While there is an extensive body of literature related to defining the problems and potential complications associated with obesity during pregnancy and childbirth, there is more limited information available related to effective interventions that may be implemented to improve maternal and infant health outcomes.

This presentation will focus on maternal diet during pregnancy and the impact on neonatal outcomes.

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Improving prediction and diagnosis of pre-eclampsia; better outcomes for mother and infant?

Lucilla Poston

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Pre-eclampsia remains a major cause of maternal and neonatal mortality and morbidity worldwide. Despite some improvement in understanding of the aetiology, there is no cure other than delivery. Since pre-eclampsia may occur from the late second trimester onwards, and may be associated with fetal growth restriction, the disorder is associated with a high incidence of premature birth and small for gestational age deliveries, with risk of immediate and longer term problems for the health of the child. There are, nonetheless, benefits of accurate prediction of pre-eclampsia, including prophylactic treatment with low dose aspirin which confers some protection against development of the disorder, and stratification of high risk women to heightened antenatal surveillance.

Despite enormous effort there is at present inadequate evidence for at test that can recommended for routine screening for risk of pre-eclampsia in pregnancy. Risk assessment based on clinical history alone is currently advised but is at best a modest predictor of outcome, although recent studies in both high and low risk women suggest that current estimates of risk could be improved upon (Chappell et al, 2008; Bramham et al, 2011; North et al 2011). An accurate test for prediction is likely to develop from a combination of clinical risk factors and biomarkers and a first trimester screening test based on clinical risk, uterine artery Doppler and biomarkers of placental origin has been reported, but leads to a high false positive rate which may lead to unnecessary anxiety for the mother for the rest of her pregnancy. Hitherto the biomarker approaches have focussed on the more obvious candidate biomarkers, predominantly of placental origin, but 'omics technol (metabolomics, proteomics) have identified new molecules with predictive potential, including some characterised in our laboratory from studies of the urinary proteome.

Accurate diagnosis of pre-eclampsia which is at present based on measurement of the blood pressure and proteinuria can also be troublesome, particularly in women with underlying disorders such as renal disease where incorrect diagnosis may lead to inappropriate treatment. Here, new tests based on the measurement of placental placental growth factor [PlGF], the vascular endothelial factor [VEGF], binding protein, sFlt-1, are showing promise and are currently being evaluated.

New methods for prediction and diagnosis of pre-eclampsia in development could improve antenatal care, and thereby the potential for better health in mother and infant.


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What does the Calcitonin Receptor do?

Rachel A Davey

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Calcitonin acting via the calcitonin receptor (CTR), is a potent inhibitor of osteoclasts, the bone resorbing cells. Accordingly, calcitonin has been used to treat disorders characterised by increased bone resorption, including Paget’s disease and hypercalcæmia. The lack of any obvious pathophysiology in individuals either deficient in, or with excess levels of serum calcitonin, however, has led to much debate as to whether calcitonin, and/or the CTR, have a physiological role. Recent
advances in the study of calcitonin and the CTR have been made using genetically modified mouse models, providing evidence for a physiological role of calcitonin and the CTR in regulating bone formation and in protecting the skeleton in times of calcium stress. Global knockout mouse models for either calcitonin and its related peptide (CGRP) or the CTR have identified a surprising physiological role for calcitonin and the CTR in inhibiting bone formation. CT/CGRP KO and global-CTRKo mice have increased bone mass due to increased bone formation. The inhibitory action of the CTR does not appear to be mediated via its expression on osteoclasts as osteoclast-specific CTRKos have normal bone mass. The mechanism by which the CTR exerts its inhibitory effects on bone formation remains to be elucidated, but may be via its actions on osteocytes and/or the central nervous system. Further evidence from these CT/CGRP and CTRKO mouse models also suggest that calcitonin, acting via the CTR, plays an important role in regulating and conserving bone during times of increased bone resorption such as in calcitriol induced-hypercalcemia, and in high states of calcium demand, such as pregnancy and lactation. Studies utilising global and tissue-specific knockout mouse models of calcitonin or the CTR are continuing to provide further insight into the physiological role of calcitonin and its receptor in regulating bone and calcium homeostasis.

Ephrin signalling modifies the bone-building action of parathyroid hormone

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Parathyroid hormone (PTH) is the only current anabolic therapy available for patients with a high risk of bone fracture (osteoporosis). Although effective, its mechanism of action remains unclear, particularly since continual high levels of circulating PTH lead to bone destruction. We have reported that EphrinB2 expression by osteoblasts (bone forming cells) is stimulated rapidly by parathyroid hormone (PTH) and inhibition of the interaction of ephrinB2 with one of its receptors EphB4 impaired late stages of osteoblast differentiation in vitro.

To study ephrinB2/EphB4 signaling in PTH anabolic action, we treated mice with PTH in the presence and absence of soluble EphB4 (sEphB4) to inhibit ephrinB2/EphB4 signaling. This treatment, with or without PTH, increased osteoblast formation, but production of osteoid and formation of mineralized bone was not increased, suggesting a functional impairment. Strikingly, sEphB4 treatment converted PTH anabolic effect to a resorptive response because formation of osteoclasts (bone destroying cells) was increased and trabecular number reduced. This influence of sEphB4 was confirmed in vitro; osteoclast formation, and RANKL production by osteoblasts, in response to PTH, 1,25-dihydroxyvitaminD3 or oncostatin M was increased with addition of sEphB4.

In addition genetically altered mice lacking the ephrinB2 reverse signaling domain showed increased osteoblast formation, but the level of bone formation was not increased, as observed with sEphB4 treatment. In contrast to the effects of systemic ephrinB2/EphB4 inhibition, osteoclast numbers were reduced.

These results indicate that ephrinB2/EphB4 signaling enhances late stages of osteoblast differentiation, and communicates with the hemopoietic lineage to restrict osteoclast formation, and that this is required for the effective anabolic action of PTH.

Steroid action in bone development

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4. Centre for Urological Research, Monash University, Clayton, Victoria, Australia

The sex steroids play a crucial role in many aspects of bone development, such as bone mass accrual, skeletal modelling and growth plate regulation, including epiphyseal fusion. However, the mechanisms underlying their effects are yet to be fully defined.

The recognition of males with oestrogen receptor mutations or aromatase deficiency highlighted the importance of oestrogen in both genders. Subsequent studies have demonstrated differential roles of the two main oestrogen receptor subtypes, ER alpha and beta, in rodent receptor knockout models, but translating these findings to human models has proved problematic. We found that selectively blocking ER alpha signaling in an in vitro model of growth plate chondrogenesis inhibited both proliferation and differentiation, while blocking ER beta had the opposite effect(1). These data support the rodent findings, where unopposed ER beta activity causes slower growth and epiphyseal fusion. The findings of osteoporosis in the male with ER alpha mutation coupled with rodent studies suggests that bone mass may also be influenced variably by the two ER subtypes.

Interfering with the action of oestrogen on the growth plate can delay epiphyseal fusion and potentially increase final height. However, the physiological actions of oestrogen at this crucial stage of development in both genders means this strategy may cause harm. Aromatase inhibitors have been trialled to increase final height in males, with some modest improvements in height outcomes. However, we demonstrated that rodent models exposed to aromatase inhibitors showed reduction in bone strength, alteration in skeletal geometry, lowering of IGF-I levels and focal prostatic hyperplasia(2). Human subjects treated with aromatase inhibitors have shown increased rates of vertebral deformities(3). Therefore, there is a need for the development of a selective strategy that inhibits oestrogen action at the growth plate without causing unwanted non-skeletal effects. Greater understanding of ER subtypes will greatly assist in this process.


Neuroregulation of bone mass

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The last decade has seen a marked increase in our understanding of the extent and importance of neural processes upon the regulation of bone mass, revealing that neural inputs are fundamental to bone homeostasis. Moreover, novel relationships have demonstrated between bone and other systems, namely energy and glucose homeostasis and the endocrine system. Our group has examined the relationship between bone homeostasis and the neuropeptide Y (NPY) system, a fundamental regulator of energy homeostasis and endocrine function. We have shown that signals processed in the hypothalamus of the brain, and travelling through the sympathetic nervous system exert powerful regulatory influences upon the osteoblast, and that by inhibiting these signals we can stimulate the production of bone by these cells. Our studies of NPY control of bone mass revealed actions within the hypothalamus and osteoblast to inhibit bone formation. Moreover, central actions of NPY provide a mechanism whereby responses to energy homeostasis are coordinated. Bone homeostasis is amongst the processes regulated by these central NPY-mediated energy homeostatic processes. The hypothalamus is best known for regulating endocrine pathways through the pituitary, suggesting the possibility of interaction between the neural and hormonal pathways form the hypothalamus. Indeed we have identified marked co-regulation by these two pathways. NPY signalling has been shown to interact with 2 major hormonal axis affecting bone mass: sex steroids and glucocorticoids, with influences upon bone mass. Our most recent work demonstrates NPY’s involvement in unique endocrine actions of the osteoblast: Regulating control of glucose homeostasis through specific signalling within the osteoblast, and regulating bone mass through central signalling of osteocalcin, thereby closing the brain/bone circuit. This work illustrates the importance of neural signals to bone homeostasis and the interrelation of this signalling pathway with known skeletal modulators.

Gene mapping in the 21st century: GWAS and beyond

Emma Duncan¹

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Obesity, diabetes & atherosclerosis: role of novel metabolic hormones

Karen SL Lam¹

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Research in recent decades has extended the realm of the endocrine system beyond the classical endocrine glands. Endocrine cells have been found in almost all organs or tissues within the body, with hormones being secreted from the gut, kidney, liver, heart and lung, and from the skin, bone, muscle and fat tissues. Indeed the adipose tissue, previously considered as an inert energy store, is probably the largest endocrine organ in the body. Since the identification of leptin as a fat-derived hormone or adipokine, a large number of adipokines have been identified through genomic and proteomics based strategies, and shown to impact on glucose and lipid metabolism, energy homeostasis and inflammation. Dysregulated secretion of various adipokines has been demonstrated in the obese state, leading to insulin resistance and chronic, low-grade, systemic inflammation which, at least in part, explain why obesity is responsible for 58% of the cases of diabetes and 21% of ischemic heart disease worldwide. Of the known adipokines, adiponectin and adipocyte fatty acid binding protein (A-FABP) have the highest circulating levels in humans and are biomarkers predictive of atherosclerotic diseases and/or type 2 diabetes, with increased risk being conferred by low adiponectin or high A-FABP levels. On the other hand, high levels of fibroblast growth factor 21 (FGF21), a liver-derived hormone with beneficial metabolic effects in animal studies, are found in obesity, suggesting the presence of FGF21 resistance. High FGF21 levels are also found in subjects with diabetes, dyslipidaemia, carotid and coronary atherosclerosis. Interestingly, FGF21 resembles some of the classical hormones in exhibiting a circadian rhythm and interacts with growth hormone as an endogenous regulator of lipolysis. Research on these metabolic hormones have improved our understanding of the pathogenesis of obesity-related pathologies and provided increasing evidence for their role as biomarkers and therapeutic targets for obesity-related cardiometabolic diseases.

Reproduction, Fertility & Development: New developments in 2012 and beyond

Tony Flint¹

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Interventions to prevent diabetes: Exercise

Glenn McConell

1. Victoria University, Melbourne, Australia

Obesity is associated with an increased risk of several diseases and premature mortality. Although we are all aware that obesity increases the risk of type 2 diabetes and that losing weight by exercising is protective, what is less understood is that a single bout of exercise increases insulin sensitivity for 24-48 hours. The major site of insulin resistance in type 2 diabetes is skeletal muscle. This talk will discuss the regulation of skeletal muscle glucose uptake into skeletal muscle and compare and contrast the effects of exercise and insulin. Although people with type 2 diabetes have reduced insulin-stimulated glucose uptake into skeletal muscle, importantly, during exercise their skeletal muscle glucose uptake is normal. Therefore, if we can understand what regulates skeletal muscle glucose uptake during exercise agents may be able to be developed to mimic the exercise pathway in those that can not or will not exercise. Although there are distinct pathways regulating skeletal muscle glucose uptake in response to exercise and insulin, exercise is able to increase insulin sensitivity in skeletal muscle. The potential regulators of this beneficial effect of exercise will be discussed including recent work we have conducted examining the role of nitric oxide. In addition, there will be discussion of the evidence that exercise early in life can attenuate the increased diabetes risk of being born small.

Nutrition interventions to prevent diabetes

Kerin O'Dea

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The prevalence of type 2 diabetes is increasing sharply in developed and developing countries as a consequence of the increasing prevalence of overweight and obesity. Nutrition interventions should ideally address both individual-level and population-level change. The major challenge is to prevent excessive weight gain, and ideally this should commence early in life. At the individual level the most important intervention is to prevent excessive energy intake, and to ensure a sufficient intake of protein and nutrients. This can be achieved through a diet that restricts the intake of energy-dense, nutrient-poor processed foods high in saturated fats, added sugars and salt. Such a diet would contain minimally processed foods of low energy-density and high nutrient-density: fresh fruit and vegetables, nuts, pulses, lean meats, fish, eggs, some dairy products. Liquid calories and large portion sizes should be minimized. The traditional Cretan Mediterranean diet of the 1950s provides one model, but there are many variations that are highly palatable. The greatest potential for change, but also the greatest challenge is at the population level - to create an environment where 'healthy choices are easy choices'. However, most developments in the industrialised food sector over the past 3 decades have been in the opposite direction. We have a food supply that has become increasingly 'obesogenic': energy-dense, nutrient-poor foods rich in sugars, fats and salt and frequently low in protein are widely and continuously available and portion sizes have increased significantly. These are the foods that are most intensively promoted. Such processed foods are much more cost-effective in calories/$ than those foods being promoted for healthy eating – and are part of the explanation for the social gradients in both obesity and diabetes. Prevention of type 2 diabetes at the population level cannot be achieved without major transformation of the current food environment.

Surgery and Pharmacotherapy Intervention for Obesity

Joseph Proietto

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The failure rate of obesity treatment is very high (NH&MRC 2003) and recent research has shown that the most likely reason is the vigorous defense of body weight involving persistent changes in hunger controlling hormones and in metabolic rate. Because of these biological changes it follows that pharmacotherapy after weight loss is both logical and necessary. When pharmacotherapy is contraindicated or not effective, bariatric surgery may be necessary. What is available?

In Australia there are currently two drugs that have TGA approval for use in weight control; Phentermine (Duromine) and Orlistat (Xenical). Recently the FDA in the USA has approved a combination of phentermine and topiramate (Qnexa) and Locaserin. Under investigation are Liraglutide (Victoza); a combination of leptin and amylin and a combination of bupropion and naltrexone (Contrave). In addition there are other products in earlier stages of development. The role and scientific evidence for the effectiveness and safety of these products will be discussed.

Three bariatric procedures are performed in Australia: adjustable gastric banding, sleeve gastrectomy and Roux-en-Y bypass. Each procedure has its strengths and weaknesses. The mechanisms of action, the risks and benefits and the role of the procedures will be discussed.
Sex specific differences in placental glucocorticoid sensitivity in pathological pregnancies

Vicki L Clifton, Nicolette A Hodyl, Michael J Stark, Zarqa Saif, Annette Osei-Kumah

1. Robinson Institute, University of Adelaide, Elizabeth Vale, SA, Australia

The human male and female fetus exhibit different strategies for survival to an adverse maternal environment. In pregnancies complicated by asthma we have identified that females reduce growth while males institute strategies to continue to grow. If a secondary event arises such as an asthma exacerbation, the female fetus does not change its smaller growth trajectory and survives to term. Conversely the male fetus is more at risk of growth restriction, preterm delivery or stillbirth in the presence of an exacerbation. These data suggest females adjust growth in response to an adverse maternal environment to survive any further reductions in oxygen or nutrients while males continue to grow normally at risk of a poor outcome if another adverse event occurs. We hypothesise that this sexually dimorphic response is the result of differences in sensitivity of the placenta to glucocorticoids which have downstream effects on pathways associated with growth. Our current research focuses on identifying the mechanism by which glucocorticoids confer a sex specific difference. Female placentae modulate intracellular cortisol concentrations by adjusting cortisol metabolism in response to maternal glucocorticoid concentrations relative to male placentae. We have identified sex differences in placental glucocorticoid receptor (GR) expression, function and regulation in human placentae of both term and preterm pregnancies. GR gene expression may be regulated by differential microRNA expression between the sexes. Different isoforms of the GR and their localisation in the cytoplasm and the nucleus vary between the sexes. In combination these findings suggest the male fetus institutes a state of glucocorticoid resistance in order to continue to grow in an adverse maternal environment via differential expression and localisation of the GR while the female fetus tightly regulates glucocorticoid responses via modulating cortisol metabolism and GR isoform interactions.

Effects of androgen deprivation therapy on body composition and bone health - 2 year follow-up of men attending a dedicated Men’s Health service.

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3. Endocrinology, Royal Melbourne Hospital, Parkville, VIC, Australia
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Androgen deprivation therapy (ADT), an effective treatment for prostate cancer, has been associated with accelerated bone loss, visceral fat gain, and insulin resistance.

Aim: To evaluate bone and metabolic outcomes in men with non-metastatic prostate cancer receiving ADT.

Methods: A prospective cohort study of men receiving long-term ADT (2007-2011) was performed. All men attended the Austin Health Men’s Health Clinic (MHC) and underwent standardised assessments according to current guidelines. Baseline data at ADT commencement was presented at ESA 2011 (abstract #240). Men were followed for 2 years and paired data was analysed using Student’s t-test.

<table>
<thead>
<tr>
<th>Co-morbidity</th>
<th>Number</th>
<th>Mean Baseline</th>
<th>Mean 2 year</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>91</td>
<td>86.26</td>
<td>86.57</td>
<td>ns</td>
</tr>
<tr>
<td>Body Mass Index (BMI kg/m²)</td>
<td>91</td>
<td>29.44</td>
<td>29.89</td>
<td>0.009</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>44</td>
<td>106.57</td>
<td>110.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>90</td>
<td>138.66</td>
<td>132.49</td>
<td>0.03</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>90</td>
<td>76.70</td>
<td>72.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting cholesterol (mmol/L)</td>
<td>90</td>
<td>5.07</td>
<td>4.73</td>
<td>0.002</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>90</td>
<td>1.58</td>
<td>1.42</td>
<td>0.028</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>84</td>
<td>3.04</td>
<td>2.78</td>
<td>0.022</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>84</td>
<td>1.20</td>
<td>1.24</td>
<td>ns</td>
</tr>
<tr>
<td>Proportion on statin</td>
<td>90</td>
<td>35.6%</td>
<td>58.9%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proportion with diabetes</td>
<td>95</td>
<td>22.1%</td>
<td>29.5%</td>
<td>0.007</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>84</td>
<td>6.24</td>
<td>6.11</td>
<td>ns</td>
</tr>
<tr>
<td>HbA1c (%) overall</td>
<td>65</td>
<td>6.27</td>
<td>6.29</td>
<td>ns</td>
</tr>
<tr>
<td>HbA1c (%) excluding diabetes</td>
<td>48</td>
<td>5.81</td>
<td>5.96</td>
<td>0.012</td>
</tr>
<tr>
<td>Lumbar Spine Bone Mineral Density (BMD) (g/cm²)</td>
<td>74</td>
<td>1.2068</td>
<td>1.1911</td>
<td>ns</td>
</tr>
<tr>
<td>Total Hip BMD (g/cm²)</td>
<td>74</td>
<td>0.9942</td>
<td>0.9758</td>
<td>0.001</td>
</tr>
<tr>
<td>Vitamin D level (nmol/L)</td>
<td>88</td>
<td>64.8182</td>
<td>74.5682</td>
<td>0.004</td>
</tr>
</tbody>
</table>
Results: 126 men were eligible for 2-year follow up. 95 men had data available for analysis (16 failed to attend, 6 deceased, 6 moved away from local area, 3 ADT ceased prior to 2 year follow up). Comparison of comorbidities at baseline and 2 years are presented in tables 1 and 2 (stratified according to treatment for the comorbidity at 2 years).

Non-diabetic patients had a significant rise in HbA1c associated with an increased prevalence of type 2 diabetes at 2 years. Despite increases in BMI and waist circumference, patients had a lowering of blood pressure, and improvement in lipid profile.

Total hip BMD declined despite increases in vitamin D to optimal levels. BMD was maintained in those who received anti-osteoporosis therapies, whereas a decline was seen in those untreated over 2 years.

Conclusion: ADT is associated with adverse effects on body composition and bone health. Active treatment of cardiovascular risk factors and osteoporosis according to current guidelines is effective in minimising cardiovascular risk and maintaining bone density. Larger studies are needed to determine effects on cardiovascular outcomes and fracture prevention.


Reference ranges and determinants of testosterone, dihydrotestosterone and estradiol levels measured using liquid chromatography-tandem mass spectrometry in a population-based cohort of older men.

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Context
In men, testosterone (T) levels decline with increasing age, while the prevalence of ill-health increases. Controversy exists over the appropriate threshold for classifying T as low rather than normal in older men. The relevance of assessing dihydrotestosterone (DHT) and estradiol (E2) to define hormonal status in this context remains unclear.

Objective
We assessed distributions and associations of T, DHT and E2 in men aged ≥70 years, and established reference ranges for these in healthy older men.

Participants
Community-dwelling men aged 70-89 years resident in Perth, Western Australia.

Main outcome measures
Demographic, medical and physical characteristics of the cohort were collated. Plasma T, DHT and E2 were assayed using liquid chromatography-tandem mass spectrometry in early morning samples from 3,690 men.

Results
In the cohort as a whole, mean±SD for T was 13.1±4.9 nmol/L, DHT 1.4±0.7 nmol/L and E2 73.4±29.1 pmol/L. Increasing age, higher body mass index, waist:hip ratio, dyslipidemia, diabetes and higher luteinising hormone (LH) were independently associated with T and DHT in the lowest quartile. Increasing age, diabetes and higher LH were associated with lower E2. In a reference group of 394 men aged 74.2±3.8 years reporting excellent or very good health with no history of smoking, diabetes, cardiovascular disease (CVD), cancer, depression or dementia, the 2.5th percentile for T was 6.4 nmol/L, DHT 0.49 nmol/L and E2 28 pmol/L. Applying these cut-offs to all 3,690 men, those with low T or low DHT had increased odds ratio (OR) for frailty, diabetes and CVD. Men with both low T and low DHT had higher OR for these outcomes.

Conclusions
The 2.5th percentile in a reference group of healthy older men provides age-appropriate thresholds for defining low T, DHT and E2. Additional studies are needed to test their potential applicability and clinical utility in older men.
Male and Female fertility are reduced in SLIRP knockout mice

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Publish consent withheld

Role of metabolic pathways in the regulation of oestrogen biosynthesis in obesity and breast cancer.

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The risk of postmenopausal breast cancer increases two-fold in obesity, and the majority of postmenopausal and obesity-related breast cancers are oestrogen-dependent. The adipose tissue is the major source of oestrogens in postmenopausal women and oestrogen production within breast adipose stromal cells (ASCs) drives tumour growth. Our laboratory’s work has focused on understanding how dysregulated metabolism, as is seen in obesity and cancer, increases oestrogen production within the breast. In this regard, we have studied a number of metabolic pathways, including those which modulate the activity of CRTC2 and HIF1α, for their role in regulating a key regulator of oestrogen biosynthesis, aromatase.

AMPK is a master regulator of energy homeostasis and is activated by upstream kinase LKB1. Our work has demonstrated that LKB1/AMPK are negative regulators of aromatase expression in ASCs by sequestering the CREB-coactivator CRTC2 in the cytoplasm. Inflammatory factor PGE₂ (produced in obesity and by tumour cells) and the adipokine leptin inhibit LKB1/AMPK. As a result, CRTC2 enters the nucleus, binds to the proximal promoter P11 of aromatase via cAMP response elements (CRE) and increases its expression. Conversely, adiponectin, produced by lean adipose tissue, stimulates LKB1/AMPK and inhibits the PGE₂-mediated expression of aromatase. We have recently identified a putative hypoxia response element which overlaps the proximal CRE of aromatase P11. HIF1α has an established role in the vascularisation of tumours and is emerging as a key mediator of metabolic responses in cancer. We now demonstrate that HIF1α binds directly to aromatase promoter PII and acts cooperatively with CREB to stimulate aromatase expression. HIF1α is increased in tumour-associated ASCs a phenomenon mediated, at least in part, by PGE₂.

Taken together, our work suggests that dysregulated metabolism is not only a characteristic of adipocytes or the tumourous epithelium, but also of the breast adipose stroma, and that aromatase expression is tightly regulated by these metabolic pathways in obesity and breast cancer. As a result, these studies have led to the exploration of therapeutics which target metabolic pathways for their use in the treatment of obesity-related postmenopausal breast cancer.

Targeting Hsp90-dependent functional maturation of the androgen receptor in human prostate cancer

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The molecular chaperone heat shock protein 90 (Hsp90) is an important target for cancer therapy as it is required for the correct maturation and function of its various client proteins, many of which are known oncoproteins. In prostate cancer, targeting Hsp90 is particularly attractive as the androgen receptor (AR), the key mediator of prostate cancer cell growth and survival, depends on Hsp90 for its ligand-binding capacity and stability. Despite promising results in pre-clinical studies, to date Hsp90 inhibitors including 17-allylamino-17-demethoxygeldanamycin (17-AAG) have demonstrated limited efficacy in clinical trials for advanced prostate cancer. While this is in part due to drug formulation issues, we propose that a treatment strategy that simultaneously targets multiple aspects of AR action will more effectively eliminate AR-dependent prostate cancer cells than single agent treatment strategies. In this study, we markedly enhanced the efficacy of 17-AAG by combining lower doses of this agent with either an antiandrogen (bicalutamide) or a histone deacetylase inhibitor (vorinostat), both of which are inhibitors of AR function and/or expression. Interestingly, comparison of gene expression profiles altered by each combination indicated that these treatments did not markedly enhance abrogation of androgen signalling compared with individual agents. Rather, the combination treatments regulated expression of unique gene sets that were enriched for cell cycle, apoptosis, MAPK and insulin signalling. To further enhance the efficacy of a combinational approach involving Hsp90 inhibitors, we investigated two new generation Hsp90 inhibitors, NVP-AUY922 and the orally available NVP-HSP90. We found that both agents were significantly more potent with regards to modulation of Hsp90 client proteins, inhibition of cell proliferation and induction of cell death than 17-AAG in prostate cancer cell lines. To assess the combinational approach in human disease, we have developed
a unique approach to study human prostate cancer where tumours are cultured as explants, with maintenance of tissue integrity, cell proliferation and androgen signalling. Using this strategy, we observed that human prostate tumour tissue responds to the novel synthetic Hsp90 inhibitors, but not 17-AAG, with a marked reduction in proliferation. Collectively, these findings suggest that these novel Hsp90 inhibitors warrant further clinical investigation in prostate cancer. We therefore propose to conduct a pharmacodynamic study to assess the biological effects of AUY922, when administered to patients with localized prostate cancer prior to surgery.

Evidence of molecular efficacy for AUY922 in this study will underpin subsequent clinical trials for this agent in prostate cancer.

Cross talk of the androgen receptor and DNA damage pathways: molecular and translational prostate cancer relevance

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⁹Prostate cancers (PCa) are exclusively dependent on the action of the androgen receptor (AR) for cell survival and proliferation, and there is a significant need to develop new means for targeting recurrent AR activity in both locally advanced and castration-resistant PCa(1, 2). PARP1 (Poly ADP-ribose polymerase 1) is an enzyme that modifies a subset of nuclear proteins by poly (ADP-ribose)-ylation, and is known to play a critical role in base excision repair(3). This function of PARP1 has been cultivated as a therapeutic target for tumors tumors that harbor alterations of specific DNA repair pathways(4, 5). Multiple enzymatic inhibitors of PARP1 function are in clinical trial; while little dose limiting toxicity has been observed, suppressing PARP1-mediated DNA damage repair in BRCA1/2 deficient tumors leads to synthetic lethality and heightened clinical response to chemotherapy. Recently, it has been revealed that PARP1 has a second major cellular function on chromatin as a transcriptional coregulator, capable of modulating chromatin structure and selected transcription factor activity(6-8).

New observations in our laboratory point toward PARP1 inhibitors as a means to simultaneously dampen AR activity and sensitize PCa cells to genotoxic insult. This premise is based on three major arms of investigation. First, abrogation of PARP1 activity results in sensitization of both androgen deprivation-therapy (ADT) naïve and castration-resistant PCa cells to ionizing radiation, thus indicating that PARP1 activity plays a significant role in the cellular response to radiotherapy. Second, PARP1 activity was foretold to be increased as a function of tumor progression in model systems of human disease, suggesting that gain of PARP1 activity may promote resistance to combined ADT and radiotherapy. Third, robust molecular analyses indicate that PARP1 is recruited to sites of AR activity on chromatin, and therein serves as a requisite cofactor for AR activity. The dependence of AR on PARP1 activity is conserved in cells that failed hormone therapy, thus indicating that the requirement for PARP1 is maintained or enhanced during the process of tumor progression. Together, these data strongly support a model wherein the dual functions of PARP1 in controlling AR activity and the response to radiotherapy can be leveraged to improve treatment of locally advanced prostate cancer.

Dynamics of pancreatic islet basement membrane proteins during isolation and transplantation

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2. Department of Immunology, The John Curtin School of Medical Research, The Australian National University, Canberra, ACT, Australia
3. Discipline of Obstetrics and Gynaecology, The University of Adelaide, Adelaide, SA, Australia

Although advances in immunosuppression have improved outcomes in clinical transplantation of islets for the treatment of insulin-dependent diabetes, numerous significant hurdles remain. Among these, the changes to islet architecture produced during harvest of cells impair their survival and function early after transplantation. Therefore we investigated changes in the composition of islet basement membrane (BM) proteins before harvest, in culture and after transplantation beneath the kidney capsule to better understand their roles in islet integrity and function following transplantation. Using immunofluorescence and specific antibodies against BM proteins laminins, collagen type IV, nidogen-2 and perlecan, we (i) compared expression of proteins in intact mouse (CBA and C57BL/6) islets with that after dissociation by collagenase, in standard culture and following transplantation (3 - 10 days); (ii) ascertained the potential for BM restoration in islets in vitro and following iso- and allograft transplantation; and (iii) identified a potential mechanism of BM recovery. Islet vasculature was assessed with an endothelial marker, PECAM-1, and flow cytometry was used to investigate the expression of BM matrix proteins in isolated islet beta cells. Collagenase digestion markedly decreased islet BM, which did not recover over 4 days in vitro culture. After transplantation, there was a transient absence of a peri-islet BM and intra-islet vascular endothelium, suggesting a period of increased risk to islet cells. Although complete restoration of the islet BM occurred after isointransplantation, allotransplantation resulted only in incomplete recovery. Complete re-vascularisation of islets occurred in isografts, however allografts lacked intra-islet capillaries, consistent with an association between islet vascularisation and the restoration of the BM architecture. Flow cytometry indicated that beta cells were not the source of BM proteins. Furthermore, in contrast to isografts, the allografts had increased peri-islet vasculature and dilatation, and reduced PECAM-1 staining. Peri-islet capillaries often contained nucleated DAPI-positive cells; consistent with the delivery of mononuclear cells to the engrafted islets. Absence of BM barrier function may increase islet susceptibility to mononuclear cell invasion during rejection. Furthermore, we identified critical roles for vascular endothelial cell migration and subendothelial BM remodelling in the reassembly of the peri-islet BM, and in the restoration of the intra-islet vasculature. We postulate that prolonged matrix detachment from both peri- and intra- islet BMs negatively impacts on the survival of islet beta cells, and supplements mononuclear cell mediated destruction of islet allografts in the absence of adequate anti-rejection therapy. These findings strongly support the critical need for the islet BMs to be preserved during islet isolation.

Placental Restriction Alters Insulin Actions and microRNAs Expression in Insulin Sensitive Tissues of Adult Offspring in the Rat

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Intrauterine growth restriction (IUGR) increases the risk of developing type 2 diabetes, in part through insulin resistance, which emerges after enhanced insulin sensitivity early in life. Why this occurs is unknown and we hypothesized that IUGR alters expression and actions of microRNA. MicroRNA is a small non-coding RNA that can co-ordinately regulate many molecules and pathways, in insulin sensitive tissues of offspring. Placental restriction (PR) and IUGR was induced in rats by bilateral uterine vessel ligation, at day 18 of pregnancy to restrict fetal growth. Insulin secretion and sensitivity assessed in vivo in adult offspring (at 3 and 6 months of age). Expression of selected insulin signalling and related molecules in liver, skeletal muscle and omental fat in older offspring was analysed by qRT-PCR. MicroRNA expression was analysed by Exiqon miRCURY arrays v11 and qRT-PCR. Predicted targets were identified by miRecords database then subjected to Ingenuity Pathway Analysis.

PR induces insulin deficiency in young adult male offspring, which persists, with later onset in females. PR increases insulin sensitivity in young adult female offspring which also persists, with no effect in males. In older offspring, PR reduces hepatic expression of insr, p110b and Stc2a2 and skeletal muscle expression of insr and p110b in males; and reduces hepatic expression of p110b but increases that of p85α in females, with no changes in skeletal muscle. PR increased microRNA expression in insulin sensitive tissues in older offspring only (liver: miR-126, miR-199b; skeletal muscle: mo-451) and in fat of males only (miR-16, 18a, 19b, 20b, 21, 106a, 142-3p). Their predicted targets include insulin signalling molecules and other molecules regulating metabolism, and functions including lipid metabolism, molecular transport and small molecule biochemistry.

PR alters expression of microRNAs in insulin sensitive tissues in older offspring, which may contribute to changes in insulin signalling, impaired lipid and metabolic control.
Effects of metformin treatment of gestational diabetes on offspring glucose tolerance and body composition

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Background: Exposure to diabetes in utero results in an increased risk of insulin resistance, diabetes and obesity in offspring. Determining the long term safety and efficacy of drugs to treat gestational diabetes (GDM) thus has important implications on the metabolic health of our population. There is renewed interest in metformin for the treatment of GDM. In the randomised MiG trial, metformin treatment of GDM resulted in similar outcomes to insulin and decreased maternal weight gain. There are no data on long term effects on the offspring beyond 2 years.

Aims: The effects of metformin treatment of GDM on the long term metabolic phenotype of male offspring were studied in a mouse model.

Methods: We studied β-ARNT mice that have a β-cell specific deletion of ARNT and are a model of mild GDM. β-ARNT females were mated with floxed-control males, and vice versa to obtain diabetic and non diabetic pregnancies respectively. Litters are ~50% β-ARNT and ~50% floxed-control. β-ARNT females were either given drinking water +/- metformin 20mg/kg for the entire pregnancy, floxed control dams were given vehicle. Male offspring were assessed by DEXA, serial weights, dynamic testing of glucose metabolism and indirect calorimetry.

Results: Maternal glucose tolerance was similar in metformin and vehicle treated dams, thus isolating the effects of metformin on offspring. β-ARNT offspring from GDM pregnancies had significantly impaired glucose tolerance at 10 weeks of age compared to β-ARNT offspring from normoglycemic pregnancies. This effect was completely prevented in metformin treated GDM pregnancies. In floxed control offspring, GDM pregnancy was associated with reduced body fat percentage at 12 weeks.

Conclusions: Metformin treatment of GDM in pregnancy has significant beneficial effects on offspring glucose tolerance.

Maternal folic acid supplementation in pregnant rats improves glucose tolerance in adult offspring

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Background: Folic acid supplementation (FAS) during pregnancy is recommended to prevent neural tube defects in offspring. Women can be exposed to up to 3 times the recommended dosage of folic acid however and that this may impact on other health outcomes in offspring, with maternal folate status linked to impaired insulin action and increased adiposity in children [1]. Maternal FAS (MFAS) in rats alters lipid metabolism in progeny [2], but outcomes for glucose control and insulin action are unknown.

Methods: Female Wistar rats were fed a control (C; 2 mg kg−1) (n=22) or a FAS diet (FAS; 6 mg kg−1) (n=23) from two weeks prior to mating until delivery. Glucose tolerance (intraperitoneal, IPGT), insulin secretion (during IPGT) and insulin tolerance (ITT) were measured in offspring (males = 12; females =12 for each treatment group) at 3 and 6 months of age. Insulin action (disposition) was calculated as insulin secretion adjusted for sensitivity.

Results: MFAS altered glucose tolerance differently in males and females with age (TRT x SEX x AGE; p=0.02): improving glucose tolerance in adult offspring (p=0.036), the aged (p=0.035) and in young females (p=0.033), but increasing fasting plasma glucose in aged offspring (p=0.001). MFAS increased insulin sensitivity in aged adults only (p=0.032). MFAS reduced insulin secretion in adult offspring (p=0.004) and aged (p=0.04) and young adults (p=0.019). MFAS reduced insulin disposition in adult offspring (p=0.042) and in young adults (p=0.047), particularly males (p=0.01).

Conclusions: MFAS impairs fasting glycaemia, but improves glucose tolerance, in adult offspring, partly via increased insulin sensitivity and despite reduced insulin disposition or action, particularly in males. Therefore MFAS persistently modifies function of insulin sensitive tissues and the endocrine pancreas in offspring to affect their glucose control. Whether MFAS offspring exhibit further deterioration of fasting glycaemia with ageing and the mechanisms responsible, warrant investigation.


Lipotoxicity in Pancreatic β-cells and the Protective Effects of Adiponectin in vitro

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Type 2 diabetes is associated with increased plasma FFA levels and reduced adiponectin levels. Pancreatic β-cells are particularly susceptible to the effects of lipotoxicity and adiponectin replacement therapy is a promising alternative for treatment of type 2 diabetes. However, the precise mechanism(s) by which chronic exposure to palmitate represses insulin gene expression in pancreatic β cells remains largely unknown. Here we show that both globular adiponectin and full-length adiponectin enhance β-cell viability and reduce cell death by apoptosis in insulinoma INS-1 β cells exposed chronically to high glucose levels. Moreover, these effects were also observed in INS-1 cells exposed chronically to high glucose levels with 500 μM palmitate when treated with globular adiponectin, but not when treated with full-length adiponectin. Serine palmitoyl transferase (SPT) inhibitor myriocin (10 μM) inhibits cellular ceramide accumulation and attenuates palmitate-induced repression of C/EBPβ and insulin gene expression, and exogenous c2-ceramide (50 μM) induced expression of C/EBPβ and repressed expression of insulin in INS-1 cells and isolated pancreatic rat islets. Our study provides strong evidence for a protective effect of adiponectin in pancreatic β cells during states of glucotoxicity and lipotoxicity. In addition, our findings indicate that ceramide C/EBPβ-dependently mediates the repressive effect of palmitate on activity of the insulin promoter. We are currently investigating the protective effects of adiponectin to elucidate the underlying molecular mechanism(s) of ceramide-mediated induction of C/EBPβ expression and repression of insulin expression.


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Maternal Folic Acid Supplementation Induces Differential Changes in the Hepatic Transcriptome of Young Adult Male Progeny

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Maternal folic acid supplementation (MFAS) is promoted as a preventative measure against neural tube defects in the newborn. With folate known to participate in epigenetic processes such as the methylation of DNA and histones, which can regulate expression of coding genes and non-coding RNA genes, MFAS may have unforeseen consequences on the hepatic transcriptome of young adult male progeny, thus this aspect was examined.

Female Wistar rats were fed one of two diets: Control (n=8, 2mg folic acid/kg) or Folic Acid Supplemented (n=8, 6mg folic acid/kg), from two weeks before mating until delivery. Male offspring were weaned onto a standard chow diet, killed with the liver collected on postnatal day 90. Global transcriptional profiling was performed with Affymetrix Rat Gene 1.0ST Array. Data were normalised with RMA plugin, and analysed on BRB Arraytools using class comparison. Canonical pathways and molecular networks modulated by the differentially expressed genes were identified with Ingenuity Pathway Analysis.

MFAS caused differential expression of twenty two genes in the liver of young adult male offspring, with 10 being upregulated (1.19 to 1.76 fold), including acyl-CoA synthetise medium-chain family member 3 (Acsm3), interleukin 17 receptor B (Il17rb); and 12 being down regulated (0.43 to 0.85), including aldo-keto reductase family 1, member B7 (Akr1b7) . The upregulated genes were associated with a network in cellular growth and were enriched in seven canonical pathways including notch signalling. The downregulated genes were associated with three networks involved with cellular assembly and organisation, as well as six canonical pathways that participate in the metabolism of galactose, fructose, mannose, and pyruvate.

Maternal folic acid supplementation alters hepatic expression of genes involved in carbohydrate and lipid metabolism and may affect their regulation in young adult male offspring, which may account for their reportedly improved lipidemia and glucose tolerance.


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Increased placental nutrient transporter expression at mid-gestation after maternal growth hormone treatment in pigs: A placental mechanism for increased fetal growth

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Background: Growth hormone (GH) is important in maternal adaptation to pregnancy. In the pig, maternal GH treatment throughout early-mid pregnancy increases fetal growth, despite constraining effects of adolescent and primiparous pregnancy, high litter size and restricted maternal nutrition. GH cannot cross the placenta, suggesting that placental responses may contribute to its fetal growth-promoting effects. The facilitated glucose transporter GLUT1 and the system A amino acid transporter SNAT2 are important regulators of fetal growth, and the aim of this study was to investigate whether maternal GH treatment increased their expression in the pig.
Methods: Fetuses and placentas were collected at d 50 of gestation (term ~115 d) from multiparous (sows) and primiparous pregnant pigs (gilts) treated with GH (~15 μg.kg⁻¹.d⁻¹) or vehicle from d 25-50 of gestation (n=7-8 per group) for assessment of weight and size, structural correlates of function and expression of GLUT1 and SNAT2 nutrient transporters.

Results: Maternal GH treatment increased fetal growth (10%, P=0.018), and did not alter placental size or structure. GLUT1 was localised to endothelial cells and trophoblast, especially in the basal membrane, whereas SNAT2 was expressed in trophoblast, predominantly basally, and endothelial cells and amnion of the mid-gestation porcine placenta. Maternal GH treatment increased protein expression of GLUT1 (+35%, P=0.037) in trophoblast and on its basal membrane (+44%, P=0.011), and increased SNAT2 protein expression in the basal (+44%, P=0.001), but not the apical, trophoblast cytoplasm. Conclusions: Our findings suggest that maternal GH treatment increases fetal growth, in part, by enhancing placental nutrient transporter protein expression and hence fetal nutrient supply and may have potential to ameliorate intrauterine growth restriction.


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Mitochondrial uncoupling protein 2 in the placenta may protect the preterm infant from increased reactive oxygen species following chorioamnionitis

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In human pregnancy, a delicate balance exists between the production of reactive oxygen species (ROS) and anti-oxidant defences that protect the human placenta and fetus. The mitochondrial respiratory chain is the major source of ROS in most mammalian cells. Mitochondrial uncoupling protein 2 (UCP2) plays a critical role in the control of mitochondrial ROS production, and has also been implicated in regulating immune activity. Given that we have recently reported sex-specific alterations in placental ROS production in preterm neonates in response to antenatal betamethasone exposure, we aimed to examine placental UCP2 expression to elucidate mitochondrial processes that potentially contribute to poor perinatal outcomes in preterm infants. UCP2 mRNA expression was assessed in the placenta of very preterm (24-28 weeks; n=23), preterm (29-36 weeks; n=19) and term (37-41 weeks; n=11) neonates. Placental ROS production was assessed by measures of lipid peroxidation and nitrative stress in placental tissue homogenates. Arterial and venous cord blood TNFα levels were determined using ELISA. Histological chorioamnionitis was recorded from placental pathology reports following all preterm deliveries. Antenatal steroid administration, birth weight, infant sex and mode of delivery were recorded. Placental UCP2 expression increased significantly with gestation (p=0.015) and was unaffected by infant sex or steroid exposure. Placental UCP2 expression was significantly reduced in small for gestational age (SGA) infants, irrespective of prematurity (p<0.05) and was decreased in pregnancies with chorioamnionitis (p=0.006). UCP2 expression inversely correlated with arterial cord blood TNFα levels (r=−0.41), but had no relationship with measures of lipid peroxidation or nitrative stress. Exposure of the developing fetus to increased placental ROS is thought to contribute to the development of morbidities commonly associated with preterm birth. Our current data adds mechanistic support to this theory, with reduced placental UCP2 expression associated with chorioamnionitis, maternal inflammation (evidenced by increased maternal TNFα levels) and SGA deliveries in preterm neonates.

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A panel of IGF system mRNA transcripts in the maternal blood are dysregulated in abnormal fetal growth

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Publish consent withheld

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Getting off beat: Circadian rhythm changes during pregnancy

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Physiological rhythms entrained by the circadian clock are present in virtually all organs including those of the reproductive system. In mammals, circadian timing is driven by a ‘master clock’ in the suprachiasmatic nucleus that influences peripheral tissue clocks via endocrine, autonomic and behavioural cues. The molecular clock machinery comprises a network of ‘clock’ genes, namely Clock, Bmal1, Period (Per) and Cryptochrome (Cry). These genes generate endogenous oscillations that drive rhythmic expression of downstream genes and thus physiological and behavioural processes. Importantly, disturbances in clock gene expression are implicated in a range of pathologies including cancer and obesity.
Pregnancy is arguably the most physiologically challenging state that an organism encounters across the life cycle. Accordingly, major metabolic adaptations are required to maintain maternal homeostasis whilst concurrently providing for the growing fetus. Interestingly, key changes in hepatic clock gene expression in rodent pregnancy (suppression of Bmal1, Clock and Per rhythms) are generally opposite to those that occur in obesity (amplification of Bmal1, Per and Cry gene rhythms). This suggests that obesity may counter the normal pregnancy-induced changes in hepatic clock gene rhythms and associated changes in downstream genes and thus maternal adaptations.

The recent recognition that clock genes are expressed in the placenta, together with observations linking circadian disruption with compromised placental function, suggests that circadian variation may be an important component of the normal placental phenotype. While there is good evidence for rhythmic expression of several genes in the rodent placenta, the conventional transcriptional-translational feedback loops of the clock machinery appear less robust and coordinated. Further study is needed to elucidate the function of the placental clock genes across gestation and among different species, particularly those in which greater circadian development occurs in utero. Such studies will likely provide important insights into placental physiology and pathology.

### Why is shiftwork bad for your health?

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1 in 5 Australian workers operate outside the “normal” hours of 8am to 6pm, are forced to sleep during the day, often in noisy environments and have reduced sleep time and poor quality sleep. Some of the greatest environmental disasters, 3-Mile Island, Chernobyl and the Exxon Valdez wreck all happened during night shifts. Fatigue clearly is a major OHS issue for industries using shiftworkers. There is also a more personal side to the impact of shiftwork. In recent years evidence has emerged that shiftwork may be a hidden contributor to the development of some important chronic diseases. For example shiftworkers are at an increased risk of developing diabetes, cardiovascular disease and becoming obese, as well as developing certain cancers. And the longer you do shiftwork, the higher the risk of disease.

Humans are diurnal and our physiological systems have evolved accordingly. For example in the hypothalamus the suprachiasmatic nucleus (SCN) operates as a “clock” powered by a suite of clock genes and is entrained to the environment by light perception via the retina. This “central master clock” coordinates brain function (e.g. the timing of sleep) as well as clock gene expression and specific, rhythmic, “functional” genes in the liver, muscle, adipose, pancreas, etc. Importantly shiftworkers, like day-workers, are exposed to light during the day during their commute or in their bedrooms. As a consequence, they rarely re-adjust their central SCN rhythmicity to their nocturnal work schedules and continue to secrete melatonin at night while working. Emerging evidence implicates the conflict between central and peripheral clocks as a contributor to pathological changes in organ function, such as insulin resistance.

Shiftwork is not going away and is in fact expected to increase in Australia with the mining boom. There is an urgent need to understand what is happening to the health of shiftworkers and to develop strategies to minimise the risk that they are exposed to, either by designing better rosters, better lighting systems, identifying those at particularly high risk of developing chronic disease or by developing drugs to reset the cellular timing system.

### Cardiometabolic effects of Obstructive Sleep Apnea: A Real Deal?

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Obstructive sleep apnea (OSA) is a common disorder that affects up to 25% of adult men. Untreated severe OSA increases the risk of all cause and cardiovascular mortality based on cohort studies. The increased risk could be due to obesity and its complications such as increased visceral abdominal fat, insulin resistance and increased liver fat which are central components of the metabolic syndrome.

Current evidence regarding the impact of cardiovascular mortality attributable to OAS induced metabolic dysregulation is unclear however. There is accumulating evidence that intermittent hypoxia and sleep fragmentation may contribute to the OSA-obesity-metabolic syndrome in both animal and human studies.

In spite of the potential independent role of OSA in the contribution towards metabolic syndrome, a healthy diet, weight loss are equally if not more important than continuous positive airway pressure (CPAP) in the management of OSA.

### Absence of 11b-HSD2 specifically within the fetal brain alter adult ‘depressive’ behaviour.

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Maternal stress and consequent fetal glucocorticoid overexposure increases offspring susceptibility to neuropsychiatric disorders. Fetal brain exposure to glucocorticoids is regulated by 11b-hydroxysteroid dehydrogenase type 2 (11b-HSD2) which inactivates glucocorticoids in the placenta and fetal brain. 11b-HSD2 offspring generated by heterozygous matings exhibit altered placental function, decreased birth weight, delayed neurodevelopment and increased anxiety and depressive-like behaviour as adults. This raises the question as to whether it is placental or fetal brain 11b-HSD2 that underpins programmed
outcomes? A knockout of 11b-HSD2 specifically within the brain during development was created by crossing NestinCre mice with floxed 11b-HSD2 mice to produce 11b-HSD2<sup>Cre/Fl</sup> (Con) and NestinCre.11b-HSD2<sup>Cre</sup> (CentralHSD2KO) mice. 11b-HSD2 activity in fetal tissue and placenta was measured, neurodevelopmental landmarks assessed and adult behaviour characterised alongside measurement of HPA axis, serotonin, dopamine and their metabolites and central gene expression. Brain-specific reduction in 11b-HSD2 activity was confirmed in the fetal heads of CentralHSD2KO mice. Birth weight and markers of neurodevelopment were unaltered. Anxiety was assessed in unstr essed and acutely stressed adult offspring and was unaltered. However depressive-like behaviour, as assessed by the tail suspension test, was increased in mice with brain-specific deletion of 11b-HSD2 with CentralHSD2KO spending a greater percentage of time hanging in comparison to the Con mice (68 and 53% respectively, P<0.05). Depressive-like behaviour was also exhibited in the novelty-induced hypophagia study with the CentralHSD2KO mice having a reduced latency to feed in comparison to the Con mice (53±8s and 28±4s respectively, P<0.01). Accompanying this altered behaviour was reduced expression of the serotonin receptor SHT-1a in the hippocampus of CentralHSD2KO mice in comparison to Con (15.0±1.1 grains/cell and 19.3±1.5 grains/cell respectively, P<0.01). Our data suggest that fetal brain 11b-HSD2 impacts specifically on depressive-like behaviours, but that broader anxiety-related and neurodevelopmental effects are likely to relate to indirect effects of 11b-HSD2 in the placenta.

**Targeted deletion of the glucocorticoid receptor gene in different cell types of the developing mouse lung reveals an essential role for glucocorticoid signalling in respiratory mesenchyme**

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Glucocorticoid signalling is essential for lung maturation where they stimulate alveolar cell differentiation, changes in lung structure, reabsorption of lung liquid and lung surfactant production. Although synthetic glucocorticoids are used to improve lung maturation in very preterm babies, their molecular and cellular mechanisms of action are poorly understood. Deletion of the glucocorticoid receptor (GR) gene by gene targeting in all cells of the mouse causes retarded lung development and perinatal death. To investigate the critical cells and compartments for glucocorticoid activity in the developing lung we have used the Cre recombinase/floxed recombination system to delete the GR gene in lung epithelial cells (an inducible SPC-2Cre), mesenchymal fibroblasts (Dermo-1 Cre) and endothelial cells (Tie-2 Cre). The viability of GR/Tie-2-Cre and GR/SPC-Cre mice at birth was not affected, but in contrast only 8% of GR/Dermo-1-Cre mice survived birth. Deletion of the GR was confirmed using immunostaining with a GR-specific antibody Histological analysis of GR/Dermo-1-Cre mice showed a condens ed lung phenotype and an increased tissue to airspace ratio, similar to a total-GR-null mouse. Finally, immunostaining with Ki67 at El8.5 showed greatly increased numbers of proliferating mesenchymal cells in mesenchyme-GR null mice. These results clearly show that glucocorticoids have an essential mesenchymal role in the developing lung to promote appropriate lung maturation prior to birth, whereas action in epithelial cells are not critical during the perinatal period.

**In vivo evidence for a novel pathway of vitamin D3 metabolism initiated by CYP11A1**


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It is well recognised that vitamin D3 is activated by sequential hydroxylation at C25 and C1 to produce 1α,25-dihydroxyvitamin D3 (1,25(OH)₂D₃). We have previously reported that purified CYP11A1 can sequentially metabolize vitamin D3 to a number of hydroxy-metabolites with the major ones being 20-hydroxyvitamin D3 (20(OH)D3) and 20,23-dihydroxyvitamin D3 (20,23(OH)₂D₃), suggestive of an alternative pathway of vitamin D metabolism. We have also shown that these metabolites are biologically active on a range of cells causing inhibition of proliferation and inflammation, and stimulation of differentiation, but unlike 1,25(OH)₂D₃, do not raise calcium levels in rats and mice. We now show that this alternative novel pathway for vitamin D3 activation occurs in vivo. 20(OH)D3, 20,23(OH)₂D₃ and more minor metabolites including 22-hydroxyvitamin D3 (22(OH)D3), 20,22-dihydroxyvitamin D3 (20,22(OH)₂D₃) and 17,20,23-trihydroxyvitamin D3 (17,20,23(OH)₃D₃) were produced by human placental and mammalian adrenal fragments incubated ex vivo with vitamin D3. Use of inhibitors and studies performed with isolated mitochondria indicated that these products were from CYP11A1 action. 1α-Hydroxy derivatives of 20(OH)D3 and 20,23(OH)₂D₃ were also detected in the placenta indicating that the CYP11A1-derived products can be acted on by CYP2B7, as previously demonstrated with the purified enzyme. In epidermal keratinocytes, which express CYP11A1, we observed the new pathway with higher proportions of 22(OH)D3 and 20,22(OH)₂D₃ being produced than in the adrenal or placenta. Importantly, we detected endogenous production of 20(OH)D3, 22(OH)D3, 20,23(OH)₂D₃, 20,22(OH)₂D₃ and 17,20,23(OH)₃D₃ by immortalized human keratinocytes demonstrating that the pathway occurs in the absence of high concentrations of exogenous vitamin D3. As final support that this new pathway occurs in vivo we detected of the predominant metabolite, 20(OH)D3, in human serum. Thus we provide in vivo evidence for a novel pathway of vitamin D3 activation initiated by CYP11A1 with the product profile showing organ/cell type specificity and being modified by CYP2B7 activity.
A New Regulatory Surface Identified in the Helix 1 to Helix 3 Loop of the Glucocorticoid Receptor Ligand-Binding Domain Allows Modulation by FKBP51 and FKBP52 Cochaperones

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The hormone-binding competent glucocorticoid receptor (GR) is found in complex with a heat shock-binding protein 90 (Hsp90) dimer, p23 and one member of a group of cochaperones termed immunophilins. Selective incorporation of the immunophilins FKBP52 or FKBP51 into the mature GR heterocomplex results in an upregulation or inhibition of receptor action, respectively. The mechanism by which immunophilins act upon the GR ligand-binding domain (LBD) is unknown. Decreased cortisol sensitivity in the guinea pig has been attributed to five residue changes within the helix 1 to helix 3 (H1-H3) loop of the guinea pig GR LBD. It has been hypothesised that this loop might serve as a contact point for FKBP52 and/or FKBP51 with the receptor. Using mouse embryo fibroblast (MEF) knockout models, a comparison of hormone-dependent transcriptional activity between human GR and a receptor containing the guinea pig GR mutations was performed. Our study revealed that loss of FKBP52 negated the differential hormone sensitivity between human and guinea pig GR suggesting that the amino acid changes in the H1-H3 loop favour an interaction with FKBP51 over FKBP52. This was further demonstrated in a transcriptional assay in MEF FKBP51 knockout cells where FKBP51 overexpression resulted in significant attenuation of receptor activity in GR containing the guinea pig mutations. We conclude that the loop provides an additional modulatory surface for GR regulation by the FKBP cochaperones. The H1-H3 loop may impact allosterically on proximally located LBD residues that contribute to GR Hsp90-dependent stability, nuclear translocation and hormone-induced conformational changes, features that are crucial for immunophilin-mediated modulation of GR.

Protein-protein interactions control multi-hydroxylation reactions for oestrogen synthesis by P450aromatase (CYP19)

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Cytochrome P450aromatase is a membrane bound enzyme in vertebrates that catalyses the synthesis of estrogens from androgens. This reaction involves electron delivery from NADPH via cytochrome P450 reductase (CPR). Little is known about how the P450arom performs the aromatase reaction or how it associates with CPR in the endoplasmic reticulum (ER). In humans, there is only one P450arom, however pigs have three isozymes. The catalytic efficiency of one of these, the porcine gonadal P450arom, is much lower than the human and the porcine placental isozymes despite the high amino acid sequence homologies.

We have used in vivo and in vitro techniques to study the interaction of P450arom proteins and lipid membranes. FRET (Forster Resonance Energy Transfer) studies explored the protein-protein interactions in the ER. A QCM (quartz crystal microbalance) was used to measure the mass of protein(s) binding to a lipid membrane and in association with the Western blot data the stoichiometry for P450arom and CPR was determined. QCM also provides information about the conformational and structural organisation of proteins in the lipid membrane. In silico calculations were also used to further probe the human placental and porcine gonadal P450arom.

Our FRET studies showed that the human P450arom forms dimers in vivo. The QCM showed tight binding of all recombinant P450arom enzymes examined to the lipid membranes. The reconstituted P450arom:CPR complexes exhibited high catalytic rates. However, the human P450arom ‘associated’ very differently with the lipid membrane than the porcine gonadal P450arom. The rate of porcine P450arom binding was most influenced by the amount of CPR present. Thus, despite their high homology the structural organisation of each P450arom within the membrane differs. The solvation energy of the respective human and porcine gonadal P450arom dimer also differs, indicating that dimerization may influence the mechanism of P450arom function.

Species-Specific Differences in the Expression and Activity of BMP15: Implications for Ovulation and Fertility

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Oocyte-derived bone morphogenetic protein-15 (BMP15) is a regulator of folliculogenesis, via actions on granulosa cell growth and differentiation. Studies in sheep have also shown that BMP15 is a primary determinant of ovulation rate within species. What is less clear is whether BMP15 could contribute to the marked differences in ovulation rate between species? Like other TGF-β superfamily ligands, BMP15 is synthesised as a precursor with the N-terminal prodomain mediating the folding and dimerisation of the C-terminal mature domain. Dimeric precursors are cleaved by proprotein convertases and BMP15 is secreted from the oocyte non-covalently associated with its prodomain.

In this study, we found that hBMP15 was expressed at much higher levels than oBMP15, whereas mBMP15 was not expressed. Sequence analysis of the region of the prodomain (α-helix) that mediates synthesis of TGF-β ligands identified significant differences between species. Substituting non-conserved hBMP15 residues across this region into m- or oBMP15 resulted in a significant increase in growth factor expression. Thus, specific adaptations in the m- and oBMP15 prodomains limit mature BMP15 expression.

Following purification, hBMP15 potently stimulates a luciferase response in COV-434 granulosa cells. BMP15 activity is dependent upon its ability to assemble a signalling complex of type I (ALK6) and type II (BMPRIA) receptors. Within the putative type I receptor binding site of BMP15, significant species differences are observed (Arg329/Asp330 in human; Pro329/Tyr330 in mouse; His329/Tyr330 in sheep). Substituting Arg329/Asp330 of hBMP15 for the corresponding m- or oBMP15 residues reduced bioactivity 5- and 40-fold respectively, identifying these residues as mediators of high affinity interactions with ALK6.

Collectively, we have identified specific residues in the pro- and mature domains of hBMP15 that enhance growth factor expression and activity. These adaptations may contribute to the variation observed in ovulation rate and fecundity between mammals.

Targeting activin to counteract muscle wasting and cachexia
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Muscle wasting is observed during ageing and disuse, but is also associated with diseases, including muscular dystrophy, sepsis, renal failure, AIDS, diabetes and cancer. In this latter group of conditions, muscle loss is the most debilitating aspect of the syndrome of cachexia, a condition characterised by pronounced weight loss, muscle weakness, anaemia, insulin resistance, and extreme fatigue. Recent evidence suggests that signalling via the activin type II receptor (ActRIIB) plays a dominant role in the aetiology of cachexia. In multiple cancer cachexia models, pharmacological blockade of the ActRIIB pathway not only prevented further muscle wasting, but restored previous muscle loss. ActRIIB mediates the signalling of a subset of transforming growth factor-β (TGF-β) ligands, including myostatin, activin A, activin B and GDF-11. To show a causal link between increased activin signalling and muscle wasting, we utilised adeno-associated viral vectors (AAV) to express activin A in the right tibialis anterior (TA) muscles of C57BL/6 mice. At the lowest viral dose (104 viral genomes), activin A caused a rapid and sustained decrease in muscle mass, which was characterised by inactivation of protein synthesis and activation of protein degradation pathways. At higher viral doses (2x105 viral genomes), the mass of the treated TA muscle decreased by 60%, circulating activin A levels rose 12-fold and significant decreases were observed in total body, liver and testes masses. These highly catabolic effects of activin A identify it as a likely mediator of cancer cachexia. To this end, we have recently developed the first specific activin A antagonist and shown in vivo that it is capable of inhibiting activin-induced muscle wasting.

Protein Arginine Methyltransferase 6 function in breast cancer and association with clinical outcomes: understanding epigenetic regulation.
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Protein arginine methyltransferase-6 (PRMT6), a transcriptional cofactor: (i) methylates histones and transcription factors, (ii) influences alternative splicing of mRNA and (iii) regulates steroid-dependent transcription, however, little is known about its function and cellular targets in breast cancer. To identify novel PRMT6-dependent pathways, we used a combination of siRNA and exon-specific microarray profiling in MCF-7 breast cancer cells in vitro. Validation of PRMT6 gene expression targets was examined in breast cancer cell lines, human normal breast tissue and primary human breast tumours by quantitative real-time PCR. This approach, demonstrated PRMT6 knockdown significantly affected: (i) the transcription of 159 genes, and (ii) alternative splicing of 449 genes. Importantly, the levels of PRMT6 mRNA were significantly decreased in breast cancer, relative to normal breast tissue. The PRMT6-dependent transcriptional and alternative splicing targets identified in vitro, were validated in human breast tumours. We then evaluated the PRMT6 transcriptionally regulated gene signature in the context of
clinical outcome associations in three independent breast cancer datasets. We generated a PRMT6-dependent gene expression signature that provides an indication of PRMT6 function (and by inference the level of PRMT6 expression) in breast cancer cells. Interrogation of the cancer datasets with a high PRMT6 gene expression signature demonstrated that PRMT6 dysfunction (i.e. a low level of PRMT6 expression) is associated with better overall relapse-free and distant metastasis-free survival in the ER+ breast cancer subgroup (i.e. we observed a significant inverse correlation between the PRMT6 gene expression levels and PRMT6 dependent signature scores). These results suggest that dysregulation of PRMT6-dependent signalling may be involved in breast cancer pathophysiology and the molecular consequences identifying a unique and informative biomarker profile.

Intratumoral androgen production and actions in Triple Negative Breast Cancer-correlation with tumor cell proliferation and clinical outcome.

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Publish consent withheld

Effects of Ligand Activation of Peroxisome Proliferator-Activated Receptor γ in Granulosa Cell Tumours

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Granulosa cell tumours (GCT) of the ovary are rare, hormonally-active neoplasms characterised by an indolent course and late relapse. Using a panel of GCT and two GCT-derived cell lines, our investigations have established that the critical pro-survival NF-κB signalling pathway is activated in GCT, and that inhibition of this pathway promotes apoptosis1. Peroxisome proliferator-activated receptor-gamma (PPARγ), a transcription factor that impedes proliferation and promotes terminal differentiation, is highly expressed in GCT and thus presents a potential therapeutic target. Overexpression of PPARγ in GCT suggests resistance to the actions of PPARγ. We have found that this is due to transrepression by NF-κB. We have shown previously that abrogation of NF-κB signalling in GCT-derived cell lines enables PPARγ agonists to initiate apoptosis. Intriguingly, a key NF-κB-induced protein, the X-linked inhibitor of apoptosis protein (XIAP), is also highly expressed in GCT and blocks apoptosis. As XIAP inhibits key portions of the apoptotic pathways, it is an attractive therapeutic target. We hypothesise that combined targeting of PPARγ and XIAP presents a potential novel therapeutic strategy for the treatment of GCT. We investigated whether XIAP inhibition would sensitize GCT cells to PPARγ-mediated apoptosis. Treatment of GCT-derived KGN cells for 24 hours with either 20μM troglitazone (PPARγ agonist), 25μM embelin (XIAP inhibitor) or 500nM CmpA (a small molecule Smac mimetic that specifically antagonises XIAP) alone does not induce apoptosis. However, when activation of PPARγ was combined with inactivation of XIAP, we observed a significant decrease in cell proliferation and viability, characterised by a significant increase in apoptosis after 8 hours. Similar results were observed for another GCT-derived cell line, COV434. We conclude that, while the use of PPARγ agonists may have potential for treating GCT, a combination of therapies involving the abrogation of XIAP may be of greater efficacy.


Kallikrein proteases promote paclitaxel resistance and progression in ovarian cancer

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High levels of tumour kallikrein-related peptidase 4 (KLK4) and KLK7 are associated with a poor outcome for women with serous epithelial ovarian cancer (EOC), for which peritoneal dissemination and chemoresistance are key events (1). As accumulation of fluid in the peritoneal cavity, ascites, is a common feature of EOC, we wished to determine the role of KLKs in EOC dissemination and chemoresistance in this microenvironment. We examined KLK4- and KLK7-transfected SKOV3 EOC cells in 3-dimensional (3D) suspension culture to mimic the ascites fluid. KLK4-SKOV3 cells formed multicellular aggregates (MCAs) as seen in ascites, as did SKOV3 cells treated with active KLK4. MCA formation was reduced by treatment with a KLK4 blocking antibody, or the selective sunflower trypsin KLK4 inhibitor (SFTI-FCQR). SFTI-FCQR also reversed paclitaxel
resistance of KLK4-MCAs. KLK4-MCAs formed larger cancer cell foci in mesothelial cell monolayers than those formed by vector and native SKOV3 cells, suggesting KLK4-MCAs are highly invasive in the peritoneal microenvironment. In patient samples, a high level of KLK4 is expressed by ascitic EOC cells compared to matched primary tumour cells, further supporting a role for KLK4 in the ascitic microenvironment. Interestingly, KLK4 transfected SKOV3 cells expressed high levels of urokinase plasminogen activator (uPA), particularly in 3D-suspension, and high levels of both KLK4 and uPA were observed in cells from patient ascites. On the other hand, KLK7-transfected SKOV3 cells also formed large compact MCAs and were resistant to paclitaxel, but high levels of α5β1 integrin were observed (2), suggesting different signaling pathways induced by these KLKs. In summary, our data suggest that KLK4 inhibition in conjunction with paclitaxel treatment may improve the outcome for women with high KLK4 levels in EOC. Key factors in different signaling pathways induced by KLK4 and KLK7 may also be tested for therapeutic potential for this cancer.


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**BRAF mutation in papillary thyroid cancer**

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Introduction: Mutations in the BRAF oncogene have been linked to papillary thyroid cancer (PTC) in various populations, giving it potential as a tumourigenic marker. The most common mutation is V600E which leads to activation of MAPK and uncontrolled cellular growth. PCR based methods are used to detect the BRAF mutation but their detection sensitivity and accuracy has not been examined. This study compares the utility of three methods of detection of the V600E mutation in a Sydney PTC cohort.

Methods DNA was extracted from histologically confirmed cancer and non cancer thyroid tissue from 17 patients who had a thyroidectomy at RPA Hospital. Evidence for the BRAF mutation was examined in all samples using the BRAF ACE kit (1) and melt curve analysis by Light Cycler(R) 480 (2) and results were confirmed by direct sequencing. HT29 cells, containing the BRAF mutation were used as positive control and for determination of assay sensitivity.

Results: The assays used between 10 and 30ng of DNA and had similar sensitivities (~70%). By direct sequencing 11 patients were BRAF positive but only 10 were detected by either Melt curve analysis or BRAF ACE kit. There were no mutations found in the normal tissues by any method. Compared with Melt curve analysis the BRAF ACE kit is labour intensive (2 vs 0.5 days) and uses the largest amount of sample (30 vs 10ng DNA).

Conclusion: Similar to other studies, the incidence of BRAF mutation in this cohort is 65%. At present, direct sequencing remains the most reliable method for detection of BRAF mutation. However melt curve analysis has potential utility and may be readily adaptable as a routine test. As clinical decisions are now being made on samples obtained by fine needle biopsy the development of reliable and sensitive techniques using smaller amounts of DNA are essential.


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**Stem cells, estrogens and local hormone action in prostate health and disease.**

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Endocrinology is the study of hormones secreted by glands of the endocrine system directly into the blood, but researchers in this field were the first to discover and identify the importance of cell-cell signalling in tissues and organs. This has been a primary focus of my work over 25 years, and we have learnt how perturbations of the normal biology of cell-cell interactions in endocrine organs, causes disease. In organs that are subject to master control by systemic hormones, proving and then interrogating these local mechanisms, required nifty techniques that deconstructed and then reconstructed the cellular components. This was done with two cell types but has now extended to multiple cellular interactions. We have used tissue recombination, to study stromal-epithelial signalling, and 3D cell cultures, to mimic more accurately the structural organisation of tissues and cell polarity. As therapies require the targeting of specific cell types, there is a need to identify and show action on select sub-populations of cells (e.g. stem/progenitor cells, cancer stem cells or drug resistant cell types), increasing the difficulty and complexity of reconstructing the normal or abnormal tissue/organ. Studies using human cells and tissues are imperative for preclinical work, which increases the challenge.

The problems have been overcome with the development of sophisticated in vitro and in vivo systems to investigate regulatory mechanisms in the human prostate gland during health and disease. We have proved that estrogens (as well as androgens) promote beneficial and adverse effects in the human prostate. In healthy organs, estrogens prevent disease, acting in concert...
DNA binding cofactors contribute to plasticity of progesterone receptor cistromes

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The ovarian hormone progesterone regulates a diverse range of normal female reproductive functions and is essential for normal mammary gland development. However, it is now well established that progesterone analogues in hormone replacement therapy increase breast cancer risk, and there is evidence that endogenous progesterone exposure also influences breast cancer development. The mechanisms driving that increased risk and the factors confining that influence to mammary epithelial cells are incompletely understood. Progesterone effects are mediated via the nuclear progesterone receptor (PR), a DNA binding transcriptional regulator. Where examined, the transcriptional responses to progesterone in different normal and malignant cell types are quite distinct, suggesting that differing patterns of PR genomic interaction may underlie this cell type specific response. We used genome wide PR ChIP sequencing to compare PR cistromes in two cell lines: T-47D breast cancer cells and MCF-10A immortalized normal breast cells stably expressing PR (AB32). Comparing the PR cistromes in the two cell lines revealed a remarkably low overlap in PR binding, which was reflected in a similarly low overlap in transcriptional response. Analysis of PR binding regions for enriched motifs revealed the pioneer factor, FOXA1, as a cofactor in a subset of binding events in T-47D cells. In AB32 cells, which lack FOXA1, AP-1 and NF1 transcription factors were identified as likely PR cofactors in these cells. Introduction of FOXA1 into AB32 cells profoundly altered progesterone response in these cells, demonstrating the potential for cell type specific factors to influence PR action. Our data suggest that cell-specificity of PR binding is determined by the coordinated effects of key binding cofactors and that altered availability of these cofactors may result in aberrant progesterone regulation in breast cancer. Our current work focuses on the ligand and PR isoform-specificity of this phenomenon.

Cure rates after single dose radioactive iodine treatment for thyrotoxicosis are lower for Maori than Caucasians

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Introduction: Graves’ disease (GD) and toxic multinodular goitre (TMNG) are the most common causes of thyrotoxicosis presenting to an endocrinology service. In New Zealand (NZ) the usual definitive therapy is radioactive iodine (I131). Not all patients are cured by a single dose of I131. Features reported to decrease the effectiveness of I131 include male gender, young age and a large gland (1-4). Our clinical impression is that Maori patients are also more resistant to I131 treatment. This has not previously been reported.

Method: Retrospective review of I131 for thyrotoxicosis at Waikato Hospital, NZ during the 3 year period prior to 1 December 2010.

Results: A total of 326 doses of I131 were given to 285 patients. Follow up data were available on 283 patients. Median follow-up was 858.5 days (range 30-1525). 83.4% were female. Mean age was 53.12 years (± 14.96) years. Maori comprised 32% of the I131 group and Caucasians 55%. The diagnosis was GD for 61.1% and TMNG for 34.3%. Most patients (98.2%) received a fixed dose of 555mBq I131. At last follow up cure had been achieved in 72.1%. There was no difference in cure rates according to gender (p=0.1212) or diagnosis (GD 70.5% vs TMNG 73.2%; p=0.6377). Younger patients (<50 years) were less likely to achieve cure as compared to ≥50 years (63.7% vs 78.6% respectively, p=0.0059). Maori patients were less likely to achieve cure than Caucasian patients (60.4% vs 77.1% respectively, p=0.0058). Maori were also more likely to be younger (49.80 ± 11.29 years vs 55.84 ± 16.04, p=0.0018) and more likely to have a TMNG (47.3% vs 31.2% p=0.0124).

Discussion: A fixed dose of I131 successfully cured hyperthyroidism in 72% of patients treated. In the population studied, age <50 years and Maori ethnicity predicted a lower rate of cure from a single dose of I131 therapy.

Explo...tumorigenesis in 3D cell culture system

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Background: Endometrial cancer (EC) is the most common malignancy of the female genital tract in developed countries. The majority of cases could be divided into two different subtypes. Type I oestrogen-dependent tumours are mostly associated with hyperestrogenism. Tumours with this type usually sustain the expression of oestrogen (ERs) and progesterone (PRs) receptors as found in normal endometrium and therefore generally have a good prognosis. In contrast, type II oestrogen-independent tumours are less common, however more aggressive and lethal1. While oestrogen is believed to be a major promoter of tumour growth in the initial stages of EC, little is known of its role in the progression to malignant and metastatic disease. In this study, we reconstructed different phenotypes of EC in a three-dimensional (3D) cell culture system to interrogate the molecular basis underpinning oestrogen-relative modulations during endometrial tumorigenesis.

Methods: Two human endometrial adenocarcinoma cell lines, Ishikawa (type I-oestrogen-dependent) and KLE (type II-oestrogen-independent) were studied. First, an in vitro 3D monotypic culture system was established by culturing endometrial epithelial cells on top of a reconstituted basement membrane (rBM). Subsequently, cell morphology, cell polarity and quantification of cell proliferation and cell death in these 3D structures were analyzed by immunofluorescence/confocal microscopy. Expression profiles of ERs and PRs in these two cell lines were further characterized through RT-PCR and immunoblot analysis. 2D monolayer cultures served as controls.

Results: Our preliminary data showed that the well-differentiated Ishikawa cells and the poorly-differentiated KLE cells manifested distinguished phenotypes that mimic the parental tumours in vivo. The two cell lines exhibited differential expression patterns of ERs and PRs as well as strikingly distinctive hormonal responses when cultured in 3D compared to in 2D. Detailed analysis is still under investigation.

Conclusion: Our in vitro 3D rBM cultures well recapitulate the native tissue organisation and cellular functions of endometrial epithelia in tumours, which provide a more physiologically relevant model system for studying oestrogenic roles in endometrial neoplastic evolution, and potentially translate experimental results into patient cares.

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Follicle-stimulating hormone stimulates the tumourigenic behavior of breast cancer cells in vitro.

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Breast cancer is the most commonly diagnosed cancer in women with one in nine Australian women diagnosed before the age of 85. Many of the known risk factors for developing breast cancer are associated with increased life time exposure to hormones, predominantly oestrogens. Follicle-stimulating hormone (FSH) may also have a role in breast cancer. FSH is frequently used in artificial reproductive technologies to stimulate folliculogenesis and oocyte development, and several epidemiological studies have reported an elevated breast cancer risk in some women who have undergone in vitro fertilization [1]. Furthermore, elevated levels of serum FSH have been correlated with a more aggressive breast tumour phenotype in premenopausal women [2]. Here, we report the expression of the FSH receptor (FSHR) and the in vitro tumourigenic effects of FSH at numerous concentrations. Using Real Time RT-PCR and Western immunoblotting we report the expression of FSHR in clinical breast cancers and oestrogen-receptor negative (ER-) and positive (ER+) breast cancer and fibroblastic breast disease cell lines and also a dose-dependent regulation of FSHR in response to FSH. Using a Real Time Cell Analyzer (Roche Applied Science) we demonstrate that FSH stimulates the migration of both ER- breast cancer cells and cells derived from fibroblastic breast disease and also the invasion of ER- breast cancer cells. Finally, for the first time, we demonstrate that FSH stimulates the expression and activity of matrix metalloproteinases 2 and 9, ECM-degrading proteases that are strongly associated with tumour invasion and metastasis of breast cancer. This study provides novel evidence for a role in the progression and development of breast cancer. Although further studies are required, these findings may indicate that the use of FSH for the treatment of infertility could stimulate the tumourigenic activity of pre-existing breast cancer and fibroblastic breast cells.


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De novo steroidogenesis in prostate cancer cells is increased by insulin-like growth factor II.
Insulin-like growth factor II (IGF II) has been postulated to increase growth and aggressiveness of many cancers, including prostate cancer (CaP). We investigated the role of IGF II in de novo steroidogenesis in prostate cancer cells, a major pathway for reactivation of androgen pathways and CaP progression. IGF II but not IGF II receptor mRNA expression was increased in patient samples during progression to castrate resistant prostate cancer (CRPC), as was immunoreactivity to insulin receptor (INSR) and IGF I receptor (IGF-IR) antibodies. Treatment of androgen receptor (AR) positive CaP cell lines LNCaP and 22Rv1 with IGF II for 48hr resulted in increased expression of steroidogenic enzymes at the protein and mRNA levels, including steroid acute regulatory protein (SIAR), cytochrome p450 family member (CYP)17A1, aldol-keto reductase family member (AKR)3B, and hydroxysteroid dehydrogenase (HSD)17B3. IGF II treatment resulted in increased steady state steroid levels and increased de novo steroidogenesis and increased AR activation as demonstrated by PSA mRNA induction. Inhibition of IGF-IR / INSR signalling axis attenuated the effects of IGF II on steroid hormone synthesis. We present a potential mechanism for prostatic IGF II contributing to CaP progression by inducing steroidogenesis, and that the IGF II signalling and related pathways present attractive targets for CaP therapy.

Expression of Somatostatin within the Hypothalamus throughout the Alteration in Pulsatile Growth Hormone Secretion in the Early-Adult Mouse

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The release and synthesis of growth hormone (GH) from somatotrophs located within the anterior pituitary gland is stimulated by GH releasing hormone (GHRH) and inhibited by somatostatin (SRIF) from corresponding neurons in the hypothalamus. We previously characterized the age-associated decline in pulsatile GH secretion in mice, and observed a rapid decline in total and pulsatile GH secretion between 12 and 16 weeks of age. While it has been suggested that the age-associated decline in GH secretion may be a consequence of reduced GHRH cell numbers (and consequently GH secretion), it remains unclear whether SRIF contributes to this change.

Using in situ hybridisation and morphometric methods, we mapped the distribution of SRIF within the mouse brain at 4, 8 and 16 weeks of age. These ages correspond to early pubertal, early adulthood and adulthood, respectively. Given that SRIF inhibits the secretion of GH, we anticipated that age-associated changes in pulsatile GH secretion might occur in conjunction with age-related changes in the level of hypothalamic SRIF expression. Consistent with previous observations in rats, SRIF perikarya are located along the rostro-caudal extent of the periventricular nucleus (PeV), arcuate nucleus (ARC) and other hypothalamic regions including suprachiasmatic, ventromedial (VMH) and dorsomedial (DMH) nuclei. Additional measures combining retrograde tracing (intraperitoneal fluorogold injection) further clarify the neuroendocrine mechanisms of SRIF in regulating GH secretion within the ARC specific to the mouse. Expression patterns of SRIF in early pubertal, early adult and adult mice clarify the potential role of SRIF in regulating GH secretion in the mouse, and provide new insights regarding the role of somatostatin in regulating age-associated changes in pulsatile GH secretion.

This work was supported by The Australian National Health and Medical Research Council and The University of Queensland. Hwee Yim Angeline Tan is a recipient of the International Postgraduate Research Scholarship.
Waking cortisol, resilience, but not 5-HTTLPR polymorphisms, predict depression.
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Publish consent withheld

Sex Differences in the Role of cAMP Response Element Binding Protein in Gonadotropin-Releasing Hormone Neurons

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Although it is widely accepted that steroid hormones, like estradiol (E2) can generate a sexually dimorphic response in neurons, little is known about the sexually differentiated signaling pathways that are influenced by them. Our previous studies have demonstrated that rapid non-genomic E2 actions on cAMP response element binding protein (CREB) are sexually differentiated in gonadotropin-releasing hormone (GnRH) neurons. However, the physiological importance of this sexual dimorphism still remains elusive. In this study, we have used GnRH neuron-specific CREB deleted mutant mice (GnRH-CREB KO) with or without global cAMP response element modulator (CREM) deletion to investigate the estrogen negative feedback on GnRH neurons in both sexes. In control mice of both sexes, gonadectomy increased plasma luteinizing hormone (LH) levels and these were then suppressed by acute E2 treatment. In female GnRH-CREB KO mice, basal levels of LH and the post-ovariectomy increment in LH were normal but the E2 induced rapid suppression of LH was significantly less effective. In contrast, in male GnRH-CREB KO mice, although basal LH concentrations and the response to E2 were normal, the post-gonadectomy increment in LH was significantly increased. These results demonstrate that CREB operates differently in GnRH neurons in females and males, and that CREB has important role in estrogen negative feedback in both sexes.

Matrix metalloproteinase (MMP) may be required for gonocyte transformation in postnatal testicular tubules

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Background and Aim: Cryptorchidism may cause infertility by failed transformation of neonatal gonocytes into adult dark spermatagonia (AD-S), the putative stem cells for spermatogenesis (1-3). Gonocytes migrate centrifugally to the tubular basement membrane to become AD-S. Regulation of this transformation remains unknown. We aimed to investigate neonatal rodent testis MMP production to see whether MMPs loosen extracellular matrix between Sertoli cells to facilitate gonocyte movement.

Methods: Sprague-Dawley rat testes (n=4-6 per group) were collected at embryonic day 19 (E19), postnatal (P) days P0 (birth), P2, P4, P6, P8 and P10 and fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for immunohistochemistry. Immunofluorescent confocal images were captured for presence of MT1-MMP, MMP2, TIMP2, MVH, AMH and AR in paraffin embedded tissue sections. MVH-positive gonocytes in cross-sections of testicular tubules in confocal images were counted and analysed. Testicular proteins were analysed by immunoblotting.

Results: MT1-MMP was strongly present in gonocytes at E19, then decreased, whereas it increased in testicular somatic cells from P0 to P10. Testicular protein levels of MT1-MMP, MMP2 and AR were constant from E19 to P10. AMH protein sharply decreased after P2, while TIMP2 gradually increased from E19 to P10. Number of MVH+ germ cells decreased from 13 per tubular cross-section at E19 to 1 at P6, then started to increase from P8 to P10. Gonocytes migrated to basement membrane at P2-6 where spermatagonia stem cells are localized.

Conclusion: MT1-MMP, MMP2 and TIMP2 were present in testis from E19 to P10 during gonocyte migration and transformation into spermatogenic stem cells. Increased knowledge about germ cell development may aid efforts to improve fertility in cryptorchidism.

References:

Vitamin D insufficiency is prevalent but does not influence glycaemia in pregnancy
Characterisation of the bioactivity of the naturally occurring Inhibin Proα-peptide.

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Inhibin A and activin A reciprocally regulate follicle stimulating hormone synthesis by the pituitary. Similar to other TGF-β proteins, inhibin A and activin A are synthesised as large precursor molecules, comprising N-terminal pro- and C-terminal mature domains. The pro-inhibin heterodimer comprises α- and βα-subunit chains, whereas pro-activin is a homodimer of two ββ chains. We have previously shown that key residues in an α-helix (α1) within the prodomain promote the folding and dimerisation of pro-inhibin A and activin A. Following dimerisation, the inhibin α- and β-subunit prodomains are enzymatically cleaved from the mature domains at consensus RXRR sites (site1). The inhibin α-subunit is a unique TGF-β ligand, comprising a second cleavage site (site2) within its prodomain. Using site-directed mutagenesis we showed that silencing of site2 in the inhibin α-subunit prodomain, abrogated the synthesis of inhibin A. Thus, processing at site2 limits the bioavailability of inhibin. Cleavage at site2 in the inhibin α-subunit prodomain releases a 43-amino acid Proo-peptide, comprising the α1-helix. We aimed to determine the influence of this peptide on inhibin and activin bioactivity. Ligand blot analysis and solid-phase binding assays indicated that the proo-peptide binds specifically to mature inhibin, and was unable to bind activin. Furthermore, the Proo-peptide was capable of suppressing inhibin A bioactivity in primary rat pituitary cells. To ascertain the contribution of Proo in naturally occurring inhibin forms, we silenced the cleavage sites in the inhibin α-subunit and β-subunits using in vitro mutagenesis to promote the production of Proo-inhibin forms. Interestingly, we found that the Proo-inhibin forms had suppressed immunoreactivity in inhibin ELISAs. Activation of the Proo-containing inhibin forms using dissociating agents rescued the immunoreactivity. The present results indicate that the naturally occurring inhibin Proo-peptide is an inhibin-specific binding protein that limits the immuno- and bioactivity of inhibin forms.

Postnatal mRNA Expression of Ten Bone Morphogenetic Protein Family Members in the Developmental Mouse Testis

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In the male reproductive system transforming growth factor-β superfamily members including bone morphogenetic proteins (BMPs) and their receptors have been shown to regulate steroid production and male germ cell development vital for fertility. Many studies however have restricted their research to normal or cancerous cell lines derived from testicular cells, however data derived has not correlated well to results published from mice testis tissues and/or testicular cells directly. Therefore, in this study we clarify the expression patterns of BMPs in the testis directly to achieve a greater understanding of these vital genes and gain insight into their probable functions.
To determine the relative expression patterns of Bmp2, Bmp3, Bmp3b, Bmp4, Bmp5, Bmp6, Bmp7, Bmp8a, Bmp8b and Bmp15 in mouse testis across development we obtained fresh tissues at 2, 4, 6 and 8 weeks and performed total ribonucleic acid (RNA) extractions. RNA quantification followed by reverse transcription quantitative polymerase chain reaction (RT-qPCR). Real time gene amplification results were analysed using beta-actin as the control. All BMPs screened were expressed in the postnatal mouse testis at all developmental stages. With the exception of Bmp5 all genes screened were expressed at a higher level at 2 weeks then during adulthood. Bmp5 expression was least at 2 weeks and only increased its expression by 6 weeks of age. Bmp3 and Bmp15 were the lowest expressed genes while Bmp4, Bmp5, Bmp6, Bmp7, Bmp8a and Bmp8b were expressed in greater amount throughout development. Patterns of gene expression illustrated provide useful clues as to which BMP genes have predominance at particular developmental stages of germ cell development.

Non-DNA binding-dependent androgen receptor pathway in fat

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Our androgen receptor knockout (AR^{ΔZF2}) mouse model has an in-frame deletion of exon 3 of the AR gene which abolishes DNA binding-dependent AR actions, but retains non-DNA binding-dependent actions. AR^{ΔZF2} male mice have increased subcutaneous and renal fat mass compared to wildtype (WT) males, but decreased total body mass. This phenotype differs from AR null mouse models which have a late onset obese phenotype, associated with increased fat mass and increased total body mass^{2-3}. To further investigate the non-DNA binding-dependent pathways in fat, we performed orchidectomy in AR^{ΔZF2} males.
male mice aged 7 weeks, to remove all endogenous androgens, and then treated mice with non-aromatizable dihydrotestosterone (DHT) or control implants for 10 weeks. Differences between control orchidectomised and DHT-treated orchidectomised AR<sup>ΔZF2</sup> males must arise through non-DNA binding-dependent AR pathways. We validated our orchidectomy surgery by showing that in WT orchidectomised males, seminal vesicles completely regressed, and kidney mass was decreased by 33% compared to sham-operated males (p<0.001). DHT treatment of orchidectomised males increased kidney mass to 13% above sham (p<0.001), suggesting a slightly supraphysiological androgen delivery. There was a mean difference of 13% in subcutaneous fat and 18% in renal fat in DHT-treated orchidectomised AR<sup>ΔZF2</sup> males compared to AR<sup>ΔZF2</sup> orchidectomised control males (n=20/group), but these were not significant and the study was underpowered to determine if this difference was significant. The IL-6 gene, known to be repressed by the non-DNA binding-dependent AR pathway<sup>1</sup> was not different between DHT-treated orchidectomised AR<sup>ΔZF2</sup> males and AR<sup>ΔZF2</sup> orchidectomised control males (n=12/group). However, Western analyses in subcutaneous fat showed that ERK phosphorylation was increased by 87% in DHT-treated orchidectomised AR<sup>ΔZF2</sup> males compared to orchidectomised control males (n=11/group). This data demonstrates a molecular role of non-DNA binding-dependent AR signalling in subcutaneous fat.


### Placental mitochondrial dysfunction in pregnancies complicated by asthma


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Asthma is a common chronic respiratory condition complicating around 12% of pregnancies in Australia. Neonates born to mothers with asthma often display reduced growth or intrauterine growth restriction if mothers have an asthma exacerbation during pregnancy. This most frequently occurs when mothers do not use inhaled corticosteroids to treat their asthma. The mechanisms contributing to poor fetal growth in pregnancies complicated by asthma are unclear, however mitochondrial dysfunction has recently been proposed to play a role. For example, in an in vitro model of rat embryo culture, inhibition of mitochondrial function decreased embryo development, gene expression and once implanted, fetal and placental growth were altered. The aim of this study was to characterize the expression of key mitochondrial genes in placentae from pregnancies complicated by asthma according to inhaled corticosteroid use and from healthy control pregnancies. Placentae were collected within 45 minutes of delivery and snap frozen for RNA and protein analysis. Asthma was associated with an increase in placental malate dehydrogenase-2 (MDH2) expression relative to controls irrespective of inhaled corticosteroid use. The abundance of placental Catechol-O-methyltransferase (COMT) and Coproporphyrinogen oxidase (CPOX) was increased with maternal asthma when no inhaled corticosteroids were used however were reduced to levels equivalent to controls when inhaled corticosteroids were used during pregnancy. The alterations in these genes can induce reactive oxygen species (ROS) production in the cell. The normalization of placental CPOX and COMT expression with inhaled corticosteroids may protect the placenta from increased ROS production, ultimately allowing the fetus to grow normally in presence of maternal asthma. The identification of mitochondrial dysfunction in asthmatic pregnancies may aid in understanding pathways leading to altered fetal growth, and therefore lead to the identification of novel targets for intervention during pathological pregnancies.

### Effect of cortisol and lipopolysaccharide on micro RNA and glucocorticoid receptor expression


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**Introduction:** Micro RNAs (miRs) are non-coding small RNAs and act as important post-transcriptional regulators of gene expression by altering the abundance or translational efficiency of mRNAs. We have identified sex differences in placental miRs expression in the presence of maternal asthma which target genes involved in cellular growth, differentiation and glucocorticoid receptor signalling. We have also previously shown that in the presence of maternal asthma both male and female fetuses exhibit differential growth response to similar concentrations of cortisol. This may be related to the differential gene regulatory mechanisms initiated by males and females in utero for growth and survival. We hypothesise that this could be mediated by and glucocorticoid receptor (GR) expression. The aim of this study was to 1. Examine the expression of miRs (372, 519b, 519c, 534, 210, 211, and 144) that target the GR and 2. Identify miR regulation of GR expression in an inflammatory environment.

**Method:** The trophoblast cell line BeWo was cultured and treated with either LPS (10ng/ml), cortisol (1uM) or both. Cells were also treated with media alone and vehicle control. Cells were harvested at 2 and 24 hours and total RNA and protein was extracted. Real time PCR was used to determine the mRNA levels of miRs and GR. Western blot was used to determine GR protein expression.

**Results:** All miRs examined were detected in BeWo cells except mir-543 and 519b. There was differential effect of cortisol and LPS on miR expression. Notably, there was significant decrease in mir-144 expression at 24hrs in the presence of LPS and cortisol but no change in GR mRNA and increased protein expression. On the contrary, cortisol alone did not change the expression of mir-144 but significantly increased GR mRNA and protein.
Conclusion: The results of this study suggest the expression of miRs can be affected by cortisol and in an inflammatory environment but the regulation of the GR mRNA could be mediated not only by mir-144 but other miRs present in the cells that target the GR and this needs to be confirmed.

Toll-Like Receptor activation in preterm neonates: the effect of gestational age and antenatal betamethasone therapy

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Some but not all preterm neonates develop inflammatory related conditions in the neonatal period, including sepsis and nocosomial infections. The reasons for this differential response is unclear, but may be related to individual responses to antenatal glucocorticoid therapy in utero. Glucocorticoids exhibit potent anti-inflammatory properties and therefore may suppress neonatal innate immune function leading to the development of these common morbidities. In order to explore this, we characterised the ability for neonatal monocellular cells, separated from term and preterm cord blood, to respond to a variety of pathogens which activate different toll like receptors. Data was analysed with respect to gestational age and time since antenatal betamethasone exposure. Cytokine production in response to in vitro stimulation by TLR agonists (Peptidoglycan TLR2; Poly I:C TLR3; Lipopolysaccharide TLR4; Imiquimod TLR7; CpG oligonucleotide TLR9) was measured by ELISA. Preliminary results indicate that cord blood monocellular cells from preterm neonates failed to respond to immunological challenge more frequently than term neonates, (p<0.05). Time between birth and antenatal betamethasone exposure did not explain the differences in response rate. This study suggests that preterm neonates may be more prone to inflammatory conditions in the neonatal period due to a fundamental immaturity in their innate immune response, leaving them unable to recognise and therefore respond to pathogens appropriately. Studies are continuing in our laboratory to identify antecedents and elucidate further the mechanisms leading to an immature innate immune response.

The satiety adipokine Nesfatin-1/NUCB2 is expressed in human term trophoblast and placental mesenchymal stem cells and is increased during oxidative and ER stress

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Nesfatin-1 is an 82 amino acid anorectic adipokine derived from the nucleobindin2 precursor protein (NUCB2). Via interaction with melanocortin 3/4 receptors in the hypothalamus, nesfatin-1 inhibits food intake and increases blood pressure in rodents. In humans nesfatin-1/NUCB2 is widely expressed, however, its expression in gestational tissues has not been previously reported. Here we demonstrate that nesfatin-1/NUCB2 protein is expressed in the highly-secretory syncytiotrophoblast of human term placenta and in the trophoblast cell lines JEG-3 and BeWo. Isolated mesenchymal stem cells from the chorionic membrane (CM-MSC) and chorionic villi (CV-MSC) also express NUCB2 at the mRNA level. Endoplasmic reticulum (ER) stress has recently been implicated in placental pathologies which can result in pre-eclampsia and intrauterine growth restriction. The NUCB2 promoter region contains cis-elements for transcriptional activation by ATF6, a transcription factor involved in the unfolded protein response. Using the chemical ER stressor tunicamycin and spliced XBP-1 as a marker of ER stress, we demonstrate that ER stress increases NUCB2 expression in JEG-3 and BeWo cells and in CM-MSC and CV-MSC. 24 hour treatment with non-cytotoxic doses of tunicamycin (0.9µg/ml) increases NUCB2 mRNA expression by 2.2 (+/-0.6) fold in the JEG-3 cell line and by 2.5 (+/-0.5) fold in the BeWo cell line. Both CM-MSC and CV-MSC were more sensitive to tunicamycin than were the choriocarcinoma cell lines and therefore a lower stressor dose was required. Tunicamycin (0.9µg/ml) - induced ER stress resulted in a 2.7 (+/-0.8) and 1.9 (+/-0.9) fold increase in NUCB2 transcription in CM-MSC and CV-MSC respectively. Collectively, these data indicate that NUCB2 is an ER-stress responsive gene expressed in term placenta and placental cell lines and MSC. Future work will investigate if nesfatin-1/NUCB2 is increased in tissues from pre-eclamptic pregnancies and other placental pathology where ER stress is evident.

Overweight, obesity and central obesity in women with polycystic ovary syndrome: A systematic review and meta-analysis

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Background: Polycystic ovary syndrome (PCOS) is closely associated with obesity but the prevalence of obesity varies between published studies. The objective of this research was to describe the prevalence of overweight and obesity, obesity and central obesity in women with and without PCOS, and to assess the confounding effect of ethnicity, geographical regions and the diagnostic criteria of PCOS on the prevalence.

Methods: MEDLINE, EMBASE, CINAHL, Cochrane Central Register of Controlled Trials (CENTRAL), and PSYCINFO were searched for studies reporting prevalence of overweight, obesity or central obesity in women with and without PCOS. Data
were presented as prevalence (%) and risk ratio (RR) [95% confidence interval (CI)]. Random-effect models were used to calculate pooled RR.

Results: This systematic review included 106 studies while the meta-analysis included 35 studies (15129 women). Women with PCOS had increased prevalence of overweight [RR (95% CI) 1.95 (1.52, 2.50)], obesity [2.77 (1.88, 4.10)] and central obesity [1.73 (1.31, 2.30)] compared to women without PCOS. Caucasian women with PCOS had a greater increase in obesity prevalence than Asian women with PCOS compared to women without PCOS, [10.79 (5.36, 21.70) versus 2.31 (1.33, 4.00), P<0.001 between subgroups].

Conclusions: Women with PCOS had a greater risk of overweight, obesity and central obesity. Although our findings support a positive association between obesity and PCOS, our conclusions are limited by the significant heterogeneity between studies and further studies are now required to determine the source of this heterogeneity. Clinical management of PCOS should include prevention and management of overweight and obesity.

Effects of in vivo hexarelin treatment on contractility, [Ca2+]i, membrane potential and Ito current in ventricular myocytes from streptozotocin-induced diabetic rats

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Publish consent withheld

Evaluation of post hypophysectomy ACTH sufficiency: early and week 6 overnight metyrapone test compared with short synacthen test and insulin hypoglycaemia test.

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Assessment of ACTH-sufficiency after pituitary surgery is complicated, with a profusion of tests and disagreement about which is 'best'. We performed a prospective study to compare the overnight metyrapone test performed at day 5 and week 6 post pituitary surgery, the short synacthen test (SST) at week 6 and the insulin tolerance test (ITT) at week 7 in the evaluation of post hypophysectomy ACTH sufficiency. 23 patients were enrolled between 2009 and 2011. Patients with Cushing's Disease were excluded. Standardised glucocorticoid replacement was given peri operatively. Glucocorticoid replacement was ceased if day 3 and 4 cortisol were >400nmol/L and the metyrapone test was normal (serum 11-deoxy cortisol >200nmol/L by HPLC) at day 6, otherwise 20mg hydrocortisone/day was continued. Short synacthen, overnight metyrapone and insulin tolerance tests were performed in all patients at 6-7 weeks unless contraindicated.

No adverse events were experienced. 17 patients had ITT performed. 1 patient had insufficient stimulus on day 5 metyrapone test and 1 failed to attend for week 6 metyrapone test. Overall 7 patients had sufficient ACTH and 4 had insufficient ACTH on all tests and 5 had discordant data. Mean stimulated ACTH (87.6ng/L and 134ng/L) and 11-deoxycortisol (284nmol/Land 460nmol/L) appeared lower at day 5 compared to week 6 data. There were fewer false positives in the day 5 than 6 week test, when compared with the ITT peak cortisol 500nmol/L as gold standard. The average 9hr post metyrapone cortisol values were similar (82nmol/L and 90.6nmol/L for day 5 and week 6 test respectively). Overall the sensitivity and specificity (for ACTH sufficiency) were 87.5% and 86% respectively for the day 5 metyrapone test and 100% and 43% respectively for the week 6 metyrapone test compared with ACTH as gold standard . The sensitivity and specificity for the SST were 100% and 71% respectively compared with the ITT as gold standard and 86% and 100% respectively compared with the 6 week metyrapone test as gold standard. Day 3 or 4 cortisol >400nmol/L had a positive predictive value of 70% and 100% compared with ITT and 6 week metyrapone test respectively.

We conclude that: a) the Metyrapone test appears safe, b) peri operative HPA suppression may influence early but not late Metyrapone tests, and c) further data are required to establish which test provides the best combination of ease of administration, safety, cost and performance.

Does weight affect serum cortisol levels?

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Introduction: Some literature describes reduced serum cortisol levels in obesity however this is not a well recognized phenomenon and the number of subjects studied to date has been small. Low cortisol levels generate concern over adrenal reserve and necessitate further investigations such as Synacthen stimulation test which carries its own risk. Aim -This study assessed relationship between serum cortisol levels, weight and body mass index (BMI).

Method – Subjects attending community pathology having assessment of serum cortisol at their GP request were offered participation in this study (n=71) Participants provided information for a questionnaire including details of medications, height and weight. Exclusions include those with history of adrenal/pituitary disease or medications altering cortisol. BMI was calculated from participant provided data. Cortisol was assayed (Siemens Centaur XP) and results converted to Multiple of Medians (MoM) (to control for time of collection) using previously derived data (n=13,953). BMI and weight were correlated with
Interferences in testosterone assays - can LCMS be affected??

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Aim: To report possible interference in testosterone on immunoassay and LCMS (Liquid Chromatography/Mass Spectrometry)

Background: Many laboratories use testosterone immunoassays routinely with more laborious and time consuming LC/MS assays reserved for confirmation of abnormal results and to exclude interferences.

Method: A 30 year old woman with acne, referred for endocrine review had elevated testosterone (8.7 nmol/L). Range < 2.0 nmol/L on routine testosterone immunoassay. Her only medication was ethinyloestradiol/dienogest (Valette®). On examination there was mild acne but no virilisation. Abdominal and pelvic examinations were normal with unremarkable pelvic ultrasound and abdominal CT. The laboratory advised immunoassay testosterone was confirmed and that the sample had also been analysed by LC/MS at an independent laboratory with significantly elevated testosterone results (>474.4 nmol/L). Without LC/MS fractionation of the original serum sample, results were not considered diagnostic.

Conclusion: There are clearly differences in measured cortisol response to synacthen stimulation between various automated platforms. We recommend adopting 30 minute serum cortisol cut-off derived from 165 healthy volunteers by El-Farhan et al: 81% patients would “pass” with Abbott (>430.4 nmol/L), Centaur (>498.7 nmol/L), and E170 (>573.5 nmol/L >524.4 nmol/L for males and females respectively). Using >474.4 nmol/L on Immulite, 85% patients would have adequate response. This study suggests those with increased BMI (> 25) have a lower distribution of serum cortisol and Cortisol MoM compared to lower BMI subjects and raises issues about interpretation of serum cortisol in obese patients.

Cortisol Conundrum: Caution on 550 nmol/L cut-off for Synacthen Stimulation Tests. Time to adopt method-specific diagnostic cut-offs!

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Synacthen stimulation test (SST) is a dynamic assessment of adrenal insufficiency and the 30-minute serum cortisol of >550 nmol/L is widely accepted as a diagnostic cut-off for adequate synacthen response. However, recent studies have demonstrated variability in serum cortisol concentrations measured by different automated immunoassay platforms.

At PathWest QEII, serum cortisol has been analysed on the Abbott Architect since September 2008. The running average 30-minute stimulated cortisol has fallen significantly, from 670 nmol/L to 540 nmol/L. There has been no notable drift on the Centaur.

All SSTs performed since January 2012 were analysed on Abbott Architect, Roche E170, Siemens Centaur and Immulite automated platforms. To date, there are 110 SST with complete data including 26 patients (24%) with a history of pituitary tumour or surgery. The median 30-minute serum cortisol in nmol/L was 540 on Abbott, 666 on Immulite, 599 on Centaur and 706 on E170. Using >550 nmol/L diagnostic cut-off, 45% patients would pass on Abbott, 77% on Immulite, 64% on Centaur and 80% on E170.

Using the suggested method-specific 30-minute serum cortisol cut-off derived from 165 healthy volunteers by El-Farhan et al: 81% patients would “pass” with Abbott (>430.4 nmol/L), Centaur (>498.7 nmol/L), and E170 (>573.5 nmol/L >524.4 nmol/L for males and females respectively). Using >474.4 nmol/L on Immulite, 85% patients would have adequate response.

Conclusion: There are clearly differences in measured cortisol response to synacthen stimulation between various immunoassay platforms. We recommend adopting 30-minute serum cortisol of >430 nmol/L for defining adequate synacthen response when Abbott Architect method is used.

Cortisol and cortisol MoMs.

Results – A total 67 subjects, 41 females (mean age 39.7), 26 males (mean age 50.6), with BMI levels ranging from 16.6 - 46.4 were included. A left hand shift (to lower levels) in the distribution of Cortisol and Cortisol MoM was found in those with higher BMI. Cortisol results (p <0.01) and Cortisol MoM (p <0.01) were lower in both those with BMI > 25 compared with those with BMI < 25.

Conclusion – This study demonstrated a left hand shift (to lower levels) in the distribution of Cortisol and Cortisol MoM in obese subjects with a BMI > 25. This study suggests those with increased BMI (> 25) have a lower distribution of serum Cortisol and Cortisol MoM compared to lower BMI subjects and raises issues about interpretation of serum cortisol in obese patients.


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Results: 9 days after cessation of therapy testosterone was 1.2 nmol/l with SHBG 194 nmol/L. Follow up blood after 1 month confirmed testosterone 1.6 nmol/L and with further decrease in SHBG to 47 nmol/L consistent with discontinuation of OCP. Review of the structure of dienogest confirmed a similar structure to testosterone with a carbon-nitrogen triple bond. 

Conclusion: This case raises the possibility of interference with both immunoassay and LC/MS testosterone methods. Clinical review, careful assessment and discussion with laboratory experts may help clarify abnormal testosterone results. The similar structure of dienogest to testosterone suggests that metabolites created in vivo may be interfering with both immunoassay and LC/MS in this patient however further confirmatory studies would be appropriate.


The Rise and Rise of Vitamin D Testing in Australia: Lessons for other nations and an international need to establish test guidelines

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Objective To examine the utilization of 25OHD testing in each state of Australia to determine the cost impact and value and to establish an evidence base enabling the development of 25OHD testing guidelines.

Background Despite rising awareness of 25OHD deficiency and its consequences, there are no national comprehensive reports of pathology test utilization for 25OHD.

Design Longitudinal analysis of all 25OHD pathology tests in Australia.

Setting Primary and Tertiary Care

Measurements Rate of tests per 100000 individuals and benefit for 25OHD, FBC and bone densitometry by state and quarter between 2000 and 2010. Number of 25OHD tests by provider by state and month between 2006 and 2010.

Results 25OHD testing increased 94 fold from 2000 to 2011. Rate varied by state whereby the most southern state represented the highest increase and northern state the lowest increase. In contrast, the rate of a universal test such as FBC remained relatively stable increasing 2.5 fold. Of concern, a 0.5 fold increase in bone densitometry was seen. GPs performed 80% of tests and specialists 20%. Approximately 50% of tests were subsequent with some individuals having up to 79 tests between 2006 and 2010.

Conclusions The rate of 25OHD testing rose dramatically. Consequences of such findings are widespread in terms of quality of care and cost. The marked variation in frequency of 25OHD testing indicates large sums of money are being expended. Further research is required to determine the cost benefit of such expenditure. Our data indicate that adoption of simple guidelines may improve efficiency and effectiveness of 25OHD testing.

Development of a Sensitive Enzyme-linked Immunosorbent Assay (ELISA) for the Determination of Pulsatile Luteinizing Hormone Secretion in Mice

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The stimulation of luteinizing hormone (LH) from the anterior pituitary gland is regulated through the activation of hypothalamic gonadotrophin releasing hormone (GnRH) neurons. Assessment of pulsatile LH secretion in transgenic mouse models will significantly advance efforts to assess key factors involved in the initiation of pubertal development. Due to blood volume constraints, repeated measurement of circulating levels of LH in mice remains highly challenging. We developed a simple technique for the detection of pulsatile levels of LH in freely moving mice. This was achieved through the development of an Enzyme-linked Immunosorbent Assay (ELISA) for detection of mouse LH in small quantities (2 μl) of whole blood. The specificity and accuracy of this assay was validated following guidelines established by the International Union of Pure and Applied Chemistry (IUPAC). We incorporated an established method for tail-clip blood sample collection to determine circulating levels of LH secretion in 36 whole blood samples collected consecutively over a period of 6 hours. A standard curve was generated following serial dilution of a known amount of mouse LH, and demonstrates accurate detection of LH in the range of 0.0018 to 1.00 ng/ml. The accuracy of LH detection across this range was assessed by spike recovery analysis in whole blood and plasma samples. We observed, on average, 83.75±4.32% and 81.96±3.19% recovery for whole blood and plasma samples, respectively. The upper and lower detection limits of this assay in 50 μl of whole blood (diluted at 1:30) were 30 and 0.057 ng/ml, respectively. Assessment of repeated blood samples collected from postpubertal male C57Bi6J mice demonstrate peak secretion periods of LH and inter-pulse stable baseline secretion periods. This method provides a sensitive and accurate tool for the assessment of pulsatile LH secretion in mice, and will allow for the reliable assessment of pulsatile LH secretion in transgenic mouse lines.

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Validation and application of an ultra-sensitive liquid chromatography (LC)-tandem mass spectrometry (MS) assay to measure androgens and estrogen in human urine.

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We developed an ultra-sensitive and specific LC tandem MS method to simultaneously determine testosterone (T), estradiol (E2), and dehydroepiandrosterone (DHEA) in extracts of human urine without derivatization. To demonstrate the applicability of this method, we analyzed urine androgens and estrogen changes in adolescents during puberty.

Using this method, adolescent males (n=27, 13-16 years old) had lower E2 (1.98±0.22 vs 4.93±0.89 ng/ml, P<0.05) and higher T (26.22±1.95 vs 8.23±1.00 ng/ml, P<0.05) excretion compared to females (n=20, aged 13-16 years old) but the genders did not differ in urine DHEA excretion (22.41±1.49 vs 27.47±4.17 ng/ml, P=0.21).

We successfully developed and validated an ultra-sensitive and specific LC-MS method for valid measurement of endogenous sex steroids (T, E2, DHEA) simultaneously in human urine.

Disease Profile of Patients Attending At Endocrine Clinic in tertiary care hospital of Eastern Nepal

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Background: Endocrine diseases have variety of manifestation affecting many systems of the human body. Endocrine diseases like diabetes, hyper/hypothyroidism, hirsutism, hyperprolactinemia are risk factor for many non-comunicable diseases.

Objectives: The aim of this study was to find out the pattern of endocrine diseases of patients attending at outpatient at endocrine clinic in BPKIH.

Materials & Methods: We took all the patients coming to endocrine OPD once a week from January 2010 to December 2010.

Results: Among 271 patients selected according to defined criteria during a year, 172 were female and 99 male. Majority of them (25.8%) were in age group of 41-51 years. About 36.9% were obese and 25.8%, 18.1%, 12.5% and 6.6% were normal weight, overweight, morbid obese and underweight respectively. In endocrine disease pattern; 59.4% had diabetes mellitus, 27.7% hypothyroidism and 8.1% hyperthyroidism. Goiter, (Infertility & hyperprolactinemia), (Autoimmune thyroiditis & osteomalacia) were found in 1.8%, 0.7%, and 0.4% respectively. 12.2% had stage I hypertension and 8.5% stage II hypertension.

Conclusion: In conclusion we found majority of patients were type 2 DM followed by hypothyroidism and hyperthyroidism. We also found obesity in most of the patients. So appropriate medical treatment, life style interventions and motivation therapies are needed for proper mangament of these endocrine clinic patients.

Management Of Primary Hyperparathyroidism At Eastern Health

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Introduction

Primary hyperparathyroidism (PHPT) is the most common cause of hypercalcemia. In Western countries, the clinical profile of primary hyperparathyroidism has shifted from a symptomatic disease characterised by hypercalcemic symptoms, nephrolithiasis, overt bone disease and neuromuscular symptoms to a disease of asymptomatic hypercalcemia detected on routine biochemical screening. Majority of asymptomatic patients do not have disease progression, as evidenced by stable biochemical measures and BMD for up to a decade of observation. This then raises the question of if, and when, these
asymptomatic patients should undergo surgery. According to the 3rd International Workshop on the Management of Asymptomatic Primary Hyperparathyroidism, surgery is recommended for those under 50 years old, serum calcium >0.25mmol/L above upper limit of normal, reduced creatinine clearance <60ml/min, T-score< 2.5 at any site, previous fragility fracture and in individuals whom surveillance is not feasible.

**Aims**

To determine if patients with primary hyperparathyroidism were appropriately managed with parathyroid surgery according to international guidelines.

**Objectives**

1. To determine the percentage of patients who underwent parathyroidectomies for PHPT between 01/01/2010 – 01/10/2011 at Box Hill Hospital and Maroondah Hospital.
2. To assess if there is evidence that accepted clinical and biochemical criteria were being used to select for parathyroidectomy.
3. To determine the success rate and complications related to parathyroid surgery at these 2 hospital sites.
4. To assess whether there are predictors of increased risk of post-surgical complications.

**Methods**

Retrospective audit of all patients with primary hyperparathyroidism presenting to Box Hill and Maroondah Hospital between 01/01/2010 and 01/10/2011.

**Results**

45.94% patients underwent parathyroid surgery in the 21 month study period. Patients who underwent surgery were significantly younger (64.57 years old vs 80.45, P<0.01) and had higher serum calcium levels (3.05 vs 2.90, P=0.01). Successful localization studies were more common in the surgical group but this was not statistically significant. Older age, pre-op calcium and Vitamin D levels, pre-op bisphosphonate use were shown to be not statistically significant in increasing rates of post-op hypocalcaemia. Success rate, defined as normalisation of serum calcium was achieved in 30 out of 34 (88.2%) patients. Post-operative complications include transient hypocalcaemia requiring calcium supplementation (29.5%) and persistent hypercalcaemia post operation (11.8%). Only one third of our patients had DEXA scans as part of their work up.

**Conclusion**

Our study suggests that younger age and positive results of localization studies may have determined the decision to proceed to surgery rather than disease severity or complications. Patients with PHPT who meet the criteria for surgery should be referred for parathyroidectomy given the high success rate and low complication risk. Patients should have a bone mineral density performed as part of their work up as cross sectional studies show an increased rate of fractures among people with PHPT. Furthermore, surveillance with BMD every 1-2 years is recommended for patients who do not undergo parathyroidectomies.

**Identification of subjects with glucocorticoid-induced hyperglycaemia while on high dose glucocorticoids as part of chemotherapy protocol - Cross Sectional study**

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**Background**

Glucocorticoids are employed as part of various anti-cancer therapy protocols. Glucocorticoids have a variety of actions that lead to hyperglycemia or an exacerbation of pre-existing diabetes. Patients with diabetes mellitus or glucose intolerance exhibit higher blood glucose levels while taking glucocorticoids. In addition, new-onset hyperglycaemia or, rarely, a non-ketotic hyperosmolar state or diabetic ketoacidosis develop without warning in patients with early subclinical diabetes or glucose intolerance [5,7, 8].

Hyperglycaemia increases length of stay in hospitalised patients by putting patients at higher risk of complications, and adverse outcomes [12, 13].

**Study Goal**

Investigation of prevalence of glucocorticoid induced impaired glycaemic control, and analysis of the relation of predictive history/physical exam and development of hyperglycaemia (BGL>11.1mmol/L) and dysglycaemia (BGL>7.8mmol/L) [14].

**Methods**

53 Nondiabetic patients in oncology ward, Box Hill Hospital on high dose steroids* as part of anti-cancer therapy protocol were studied over 5 months period. Patients were screened for history/physical exam identifiable risk factors and blood glucose levels were monitored daily at 4pm for 2-5 consecutive days while receiving steroids.

*High dose steroid identified as Prednisolone ≥ 10mg, and any dose of Dexamethasone.

**Results**

BGL rise was observed in significant number of patients studied (15% BGL≥11.1mmol/L, and 71.60% BGL ≥ 7.8mmol/L). Average BGL was higher by 1.25mmol/L in patients receiving corticosteroid doses ≥ 400mg of Hydrocortisone (P value=0.039); however there was no statistically significant linear relation between corticosteroid dose, and BGL level.

Average BGL was also higher in patients with BMI≥25 by 1.42mmol/L (P value=0.038) and there was a significant relation between BMI and hyperglycaemia (R² = 0.12, Pvalue=0.018).

**Conclusion**

As prevalence of hyperglycaemia was significant, routine monitoring of blood glucose level in patients receiving high dose glucocorticoids with anti cancer treatment seems required, and patients with BMI≥ 25 and higher doses of steroids are at increased risk.
Testosterone Levels in Men with Minimal Trauma Fracture

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Introduction: Androgen deficiency is a risk factor for osteoporosis in men. However, illness and acute fracture may lead to a reduction in circulating testosterone.

Methods: To explore this possibility, we conducted a case-control study of 240 men presenting to the Emergency Department (ED) with a radiologically confirmed minimal trauma fracture (MTF), and of 75 controls.

Results: Compared to controls, cases had lower total testosterone (TT, 7.1 ± 13.2 nmol/L, p < 0.0001) and calculated free testosterone (cFT, 113 ± 172 pmol/L, p < 0.01). Cases were older (74 vs 68 years, p < 0.001), had lower lumbar spine T-score (-0.6 vs 0.0, p = 0.04), femoral neck T-score (-1.7 vs -1.1, p < 0.0001), and renal function (eGFR 78 vs 82 ml/min, p = 0.02). There was no difference in BMI (27.4 vs 27.9 kg/m²), and 25OH-vitamin D (58 vs 64 nmol/L), p = n.s.. Lower TT remained associated with a higher fracture risk after adjustment for differences between groups including age and bone mineral density (OR 1.21 p = 0.0001). Of the cases, the 142 admitted to the hospital had lower TT than the 98 discharged from the ED (4.6 vs 10.3 nmol/L, p < 0.0001), and lower cFT (78 vs 151 pmol, p < 0.0001). There was also a difference in TT between cases discharged from ED and controls (10.3 vs 13.2 nmol/L, p < 0.0001); but not in cFT (151 vs 172 pmol/L, p = n.s.). In the 34 cases with follow-up testosterone (median of 4 months after the initial testosterone), follow-up TT was 8.5 vs 5.1 nmol/L, and cFT was 127 vs 81 pmol/L, both significantly (p < 0.001) higher compared to the initial testosterone.

Conclusions: The diagnosis of hypogonadism and appropriate commencement of androgen replacement in men is challenging. Neither should it be based on measurements following minimal trauma where, at least in part, deficits in serum testosterone may be effects of an acute, fracture-associated, stress response.

The frequency of osteoporosis testing in females aged 45 to 74 did not rise despite a marked increase in testing for vitamin D deficiency: a ten year observational study.

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Publish consent withheld

Retrospective study: Management of the initial hypoglycaemic event in hospitalised patients and its impact.

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Objectives: To review the inpatient management of hypoglycaemia and its impact.

Design: Retrospective single centre study.

Patients: We analysed all documented hypoglycaemic (BSL < 3.5mmol/L) events in an acute inpatient setting between November 2011 and January 2012 at a major teaching hospital. We retrospectively reviewed the management of each episode.

Main Outcome Measures: Evaluated any association with length of stay, recurrence, preventative action taken, type of Diabetes, hypoglycaemic agent used and impact if occurred after hours.

Results: Total of 100 patients were evaluated. Of them, 57 had 1 or more recurrences, 19 were notified to the doctor after the initial episode, 42 had preventative action taken, such as modifying the dose of hypoglycaemic agent that led to the event, 47 were given complex carbohydrates after initial treatment and 62 had their initial event after-hours. If a doctor was notified of the initial hypoglycaemic event, the odds of recurrence was 30% greater (p=0.031). When a doctor was notified, preventative action was taken in 73.7% compared to 8.6% if not (p=0.002), but taking preventative action alone was not associated with the
number of recurrences. Patients with recurrent episodes of hypoglycaemia were also associated with longer length of stay, 13.5 days vs. 7.5 days (p=0.001). Recurrences were not associated with age, being on insulin, type of Diabetes and the time of occurrence.

Conclusions: Hypoglycaemia is common in inpatients and not often reported to the treating doctors. This leads to recurrent episodes and is associated with a longer length of stay in hospital.

Free and Total Cortisol Measured by Immunoassay and Mass-spectrometry Following ACTH$_{1-24}$ Stimulation in the Assessment of Pituitary Patients

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Background: Plasma cortisol measured by immunoassay after ACTH$_{1-24}$ stimulation is used to assess the hypothalamic-pituitary-adrenal (HPA) axis. Liquid chromatography-mass-spectrometry (LCMS) has greater analytical specificity than immunoassay and equilibrium dialysis allows measurement of free cortisol.

Aim: To investigate the diagnostic accuracy of total and free cortisol measured by LCMS in the low and high dose ACTH$_{1-24}$ stimulation test in pituitary patients.

Methods: We studied 60 controls (34 female, age 61±12 years, BMI 27.7±5.6 kg/m2) and 21 patients with pituitary disease (14 female, age 57±15 years, BMI 30.4±6.6 kg/m2) in whom HPA sufficiency (n=8) or deficiency (n=13) had been defined by insulin tolerance test (peak cortisol cut-off 550 nmol/L) or morning cortisol (deficient ≤100 nmol/L, sufficient ≥500 nmol/L). Subjects attended on two occasions for 1µg ACTH$_{1-24}$ intravenous and 250µg ACTH$_{1-24}$ intramuscular ACTH$_{1-24}$ stimulation testing. Total and free (following equilibrium dialysis) plasma cortisol concentrations were measured by in-house LCMS assay and total plasma cortisol by immunoassay (Elecsys 2010, Roche Diagnostics). The pituitary patients’ HPA axes were assessed using the lower limits of the 95% confidence intervals derived from the controls.

Results: The cortisol concentration at 30 minutes during the 1µg ACTH$_{1-24}$ stimulation test and at 30 and 60 minutes during the 250µg ACTH$_{1-24}$ stimulation tests were similarly concordant with previous HPA axis assessment. Measurements of total cortisol by immunoassay were concordant with previous HPA axis assessment in 19/21 and 20/21 patients using the 1µg and 250µg ACTH$_{1-24}$ tests respectively. Immunoassay cortisol measurement had a negative bias with increasing concentrations relative to LCMS. The sensitivities of total and free cortisol by LCMS in diagnosing HPA status were similar to those derived from the immunoassay (Table).

Conclusion: In pituitary patients HPA axis assessment by total and free plasma cortisol measured by LCMS after ACTH$_{1-24}$ stimulation have similar diagnostic accuracy to total cortisol by immunoassay.

Acknowledgement: This study was supported by a grant from the Faculty of Health Science, Flinders University.

The MAPrc Women’s Mental Health Clinic

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The Monash Alfred Psychiatry Research Centre Women’s Mental Health Clinic is a tertiary referral, second opinion clinic for women with mental illness. This multidisciplinary clinic has both endocrinological and psychiatry input and focuses on the biological and hormonal aspects of mental illness in women. Common presentations include perimenopausal depression and cyclical exacerbations of mental illness such as schizophrenia. Rarer presentations include cases of Triple X Syndrome and undiagnosed Sheehan’s Syndrome. Treatments include standard psychotropic medications, hormonal therapies and newer experimental therapies in refractory cases. The poster includes data of the number and types of cases seen as well as brief case studies.

Comparison of Outcomes after Laparoscopic vs Posterior Retroperitoneoscopic Adrenalectomy

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Objectives: To compare peri- and post-operative outcomes of our first ten cases of posterior retroperitoneoscopic adrenalectomy (PRA), with our previous ten cases of laparoscopic adrenalectomy (LA).

Methods: A retrospective chart review and telephone interviews were conducted with twenty patients who underwent adrenalectomy by the same surgeon. Analgesia use, operative time, length of stay, complications, and return to activity were recorded.

Results: Data was collected for ten PRAs and ten LAs. Age, BMI, and tumour size, were similar between the two groups. There were no conversions to open surgery, transfusions or deaths. There was one post-operative pneumonia and one incisional hernia in the LA group, and no major complications in the PRA group. PRA patients had a shorter length of stay (median 1 vs 2 days p <0.05) and a faster return to normal activity (median 4.5 vs 33 days, p <0.05) compared to LA patients. All patients were given paracetamol routinely, and opioids upon request. The mean inpatient, post-operative opioid use was lower in patients undergoing PRA compared to LA (1.25 vs 21.0 milligrams of intravenous morphine equivalent, p = 0.37). The median days on opioids was less for PRA patients compared to LA patients (0.5 vs 9. days, p <0.05).

Conclusion: This case series supports previous reports that PRA results in reduced post-operative analgesia use, reduced length of hospital stay and shorter period until return to regular activity when compared to the standard laparoscopic approach.


Amiodarone-induced thyrotoxicosis – do anti-thyroid drugs alone provide adequate treatment?

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Introduction: Amiodarone-induced thyrotoxicosis (AIT) occurs in 3-12% of patients receiving amiodarone. There is a poor evidence base for treatment and limited data in an Australian population.

Objective: To compare the efficacy of antithyroid drugs (ATD) alone versus ATD and prednisone (ATD+PRED) in the treatment of AIT.

Methods: The pharmacological management of AIT using ATD alone versus ATD+PRED was reviewed in 25 patients, with the outcome being time to euthyroidism or thyroidectomy.

Results: There was a trend for baseline free thyroxine (fT4) level to be different between the treatment groups: ATD+PRED (n=9) 63.0 ± 11.0 pmol/l, ATD (n=11) 42.9 ± 8.0 pmol/l, no treatment (NIL, n=5) 25.0 ± 12.9 pmol/l, P=0.054. Of those treated initially with ATD, 6/11 (55%) required the addition of PRED due to inadequate response (n=5) or adverse event to ATD (n=1). Baseline fT4 was significantly higher in those ultimately treated with ATD+PRED (58.8 ± 8.3 pmol/l) compared to those treated with ATD or NIL (28.3 ± 3.1 pmol/l), P<0.01. In patients with fT4 <30 pmol/l, 75% (6/8) achieved euthyroidism without antithyroid drugs alone.

Discussion: These data indicate that in patients with AIT, those with higher fT4 levels generally require glucocorticoids. The lack of difference in time to achieve euthyroidism between the groups is probably due to the greater disease severity in the group treated with ATD+PRED. Mild disease (fT4 <30pmol/l) can be successfully treated with ATD alone. Thyroidectomy in those responding poorly to medical therapy resulted in a uniformly good outcome.

An adrenal adenoma co-secreting cortisol and aldosterone associated with both a bronchial adenocarcinoma and a papillary thyroid carcinoma.

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A 46-year-old woman with a left upper-lobe bronchial adenocarcinoma was referred for evaluation of adrenal masses and a right-sided thyroid mass identified incidentally during staging of her bronchial lesion via PET scan. Abdominal CT identified the adrenal masses: 1.3 x 1 x 1 cm (15 Hounsfield units) and 2.5 x 1.5 x 1.5 cm (40 Hounsfield units) in the left and right adrenals, respectively. The right adrenal mass was PET positive.

Born in China, she had a significant family history of malignancy, atypical of any cancer syndrome. Furthermore, she has a 9-year history of treatment-resistant hypertension (despite being on 5 anti-hypertensive agents) associated with hypokalemia. Although not overtly Cushingoid, she had difficulty sleeping and weight gain. Biochemical assessment revealed elevated aldosterone with suppressed renin levels, elevated urinary and salivary cortisol levels, and suppressed ACTH levels. Cortisol levels failed to suppress on 2 day low and high dose oral dexamethasone suppression tests. Thyroid ultrasound with FNA was suspicious for malignancy. Post-laparotomy, she underwent a unilateral right-sided adrenalectomy, revealing 2 adenomas - 22 mm and 7 mm, respectively. Post-adrenalectomy, both the hyperaldosteronism and the hypercortisolism resolved, markedly improving her blood pressure. However, she required glucocorticoid replacement and remained persistently hypokalemic. Nine months post-adrenalectomy, she still requires glucocorticoid and potassium replacement. Her blood pressure is well controlled on 3 antihypertensives. She subsequently underwent a hemiroidectomy, revealing a localised 9 mm BRAF mutation-positive papillary thyroid carcinoma.

This is the first documented case of bilateral biochemically-active adrenal adenomas associated with papillary thyroid, and bronchial carcinomas. Although recognised, adenal adenomas co-secreting cortisol and aldosterone are relatively uncommon. The basis of the on-going features of mineralocorticoid excess is currently being investigated. This unique cluster of uncommon tumours coupled with her family history, suggests a novel genetic cancer syndrome. The possibility of BRAF mutation expression in the other tumours is being explored.

### Hypoadrenalism and Acute Neurological disturbances

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Introduction: A 36-year-old lady presented obtunded to another hospital. She had bilateral pleural effusions and ascites and underwent laparotomy. She was known to have premature ovarian failure of eight years duration. No pathology was demonstrated at operation and post operatively she required ventilation and ionotropic support. Subsequent investigation demonstrated hypothyroidism and hypoadrenalism as the cause of illness.

Follow up: She was transferred to our institution for further investigation when she developed a seizure disorder in association with ongoing weakness necessitating ventilation. Extra-pontine myelolysis was demonstrated on MRI of the brain. She had not been hyponatraemic.

Conclusions: The association between hypoadrenalism and the acute neurological disturbance will be discussed.


### Induction of spermatogenesis in the setting of panhypopituitarism and prior chemotherapy

**Santhi Chalasani, Veena Jayadev**

Gonadotropin therapy is highly effective at inducing spermatogenesis and fertility in gonadotropin-deficient men. The introduction of IVF and ICSI has transformed the treatment of male infertility by requiring a minimal number of sperm to achieve pregnancy. Various techniques for testicular sperm retrieval are available including testicular sperm extraction or microdissection, as well as percutaneous and microsurgical epididymal sperm aspiration. This case highlights the complexities of induction of spermatogenesis, sperm extraction techniques, and methods of inducing antegrade ejaculation after local surgery.

A 36-year-old man was diagnosed with a testicular germ cell tumour in 2003. He underwent a left orchidectomy, para-aortic lymph node resection, and chemotherapy. In 2006 he represented with a history of headaches and double vision. A diagnosis of macroprolactinoma was confirmed on biochemistry (prolactin: 46,000 mIU/L) and MRI pituitary (2.5 x 1.5 x 1.5 cm tumour). The remainder of his pituitary profile showed evidence of testosterone, cortisol and thyroxine deficiency. He was managed with Cabergoline 750mcg weekly, Thyroxine 100mcg daily, and Testosterone undecanoate 1000mg every 12 weeks. He had good biochemical response (prolactin: 596 mIU/L), and pituitary MRI in March 2010 showed no residual tumour.

In March 2011 he presented desiring fertility. His 33-year-old nulliparous wife described regular menstrual cycles. He described retrograde ejaculation since his surgery. His Testosterone therapy was ceased. Baseline bloods showed a testosterone of 6.8 nmol/L, LH of 2.6 IU/L, FSH of 4.0 IU/L, prolactin of 333 mIU/L, TSH of 1.8 mIU/L, and free T4 of 16pmol/L. Analysis of a post-ejaculatory urinary study showed no sperm. The patient was commenced on recombinant HCG 125 mcg twice weekly, and FSH 150 units three times weekly with urinary alkalisation. Subsequent analyses of post-ejaculatory urine samples revealed occasional non-motile sperm. Treatment with pseudoephedrine did not induce antegrade ejaculation. He was referred for epididymal biopsy.


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Autoimmune thyroid disease following alemtuzumab therapy for multiple sclerosis

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The humanised anti-CD52 monoclonal antibody Alemtuzumab induces an immune mediated depletion of CD4+ T cells, and has been shown to be superior to interferon β-1a in treating relapsing-remitting multiple sclerosis (RRMS), with reduction in relapse rates and improved disability scores. However, a significant proportion of patients develop new autoimmunity as a result of treatment, and the thyroid gland appears to be particularly susceptible.

A 38 year-old woman with RRMS was treated with two courses of Alemtuzumab 12 months apart. Pre treatment thyroid function tests were normal and auto-antibodies were not detected. 12 months after the 2nd course of Alemtuzumab she developed symptoms of hyperthyroidism, and thyroid function tests showed TSH <0.02 mIU/L, FT4 41.0 pmol/L, T3 15.4 pmol/L. TSH receptor antibody was elevated at 8 U/L. Thyroid technetium scan revealed diffusely increased tracer trapping consistent with Graves’ Disease. She was commenced on Carbimazole therapy.

Novel autoimmune thyroid disease is reported in up to a third of RRMS patients treated with Alemtuzumab. Graves’ Disease is most common, with cases of thyroiditis and hypothyroidism also being reported. The majority of cases are detected 12-36 months after the initial treatment dose of Alemtuzumab, indicating that the development of autoimmunity is timed with immunological reconstitution. The pathogenesis of reconstitution Graves’ Disease remains unclear, but is likely related to preferential expansion of autoreactive T cells, and high circulating baseline IL-21 levels are thought to be a predisposing factor.

As there is accumulating evidence of its superior efficacy compared to interferon β-1a, Alemtuzumab is predicted to become standard treatment for RRMS in the future. Therefore, there is likely to be an increased incidence of autoimmune thyroid disease in this population. This case highlights the need to develop standardised screening protocols to ensure prompt referral and implementation of treatment in this patient group.


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Kallmann syndrome and schizencephaly: concurrent neuron migration defects.

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The pathogenesis of Kallmann syndrome lies in faulty migration of olfactory sensory neurons and gonadotrophin-releasing neurons from the olfactory placode through the cribriform plate into the olfactory bulb and hypothalamus, respectively. Genetic mutations of anosmin-1, FGF8, FGR1, PROK2, PROKR2, WDR11 and CHD7 genes cause neuron migration failure, hypogonadotropic hypogonadism and variable anosmia. We describe a patient with dual neuron migration abnormality. Literature review suggests this is the first reported case of dual neuron migration abnormalities in a patient with Kallmann syndrome. Given the common pathogenesis of these disorders, we propose to perform whole-brain MRI scanning in a series of new diagnoses of Kallmann syndrome, rather than limiting scans to pituitary and olfactory areas, in
an attempt to characterise any concurrent neuron migration abnormalities that may represent an unknown associated feature of the disorder.

Gestational hypercalcaemia in familial hypocalciuric hypercalcaemia

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FHH is typically a benign condition. Little is known about its course and management during pregnancy. Two sisters with presumed FHH were followed prospectively through their pregnancies for clinical course and biochemical parameters. Their kindred were retrospectively examined across four generations for available information. Genetic testing for inactivating mutations of the calcium sensing receptor (CaSR) was performed in two women and in other members where possible.

The index case demonstrated incidental hypercalcaemia (corrected calcium 3.03mM), low normal PTH (1.3pM), 25-hydroxyvitamin D insufficiency (34nM) and fractional calcium excretion of 1.7% during pregnancy. Her pregnant sister also had incidental hypercalcaemia (corrected calcium 2.9mM), normal phosphate (0.86mM), low normal PTH (3.1pM), 25-OH vitamin D insufficiency (34nM) and fractional calcium excretion of 0.9%. Both were heterozygous for the R220W missense mutation of the CaSR in addition to the R990G and A986S polymorphisms. A third pregnant member of the kindred had persistent hypercalcaemia despite previous parathyroidectomy. Biochemistry at 36 weeks gestation showed corrected calcium of 2.61mM, PTH of 2.3pM and 25-OH vitamin D of 53nM. All three had uncomplicated, term pregnancies without neonatal complications.

Asymptomatic hypercalcaemia was the typical presentation in this kindred. Corrected calcium levels ranged between 2.86 – 3.02 mM and PTH levels ranged from low to high normal (3.1 – 10.6 pM). Fractional excretion of calcium in this kindred ranged from 0.6 – 1.7%. Three members had previously undergone parathyroidectomy and two exhibited persistent post-operative hypercalcaemia.

FHH was described in this kindred based on the inheritance patterns, clinical courses and biochemical parameters, and confirmed on genetic testing. Our prospective observation suggests that FHH runs a benign course in pregnancy. The tools available for the diagnosis of FHH include urinary calcium excretion and gene testing. It is important to differentiate FHH from primary hyperparathyroidism to prevent unnecessary parathyroid surgery. Variations in the inherited mutations of the calcium sensing receptor may explain the diversity of serum calcium levels and specific clinical phenotypes.

A rare case of hypercalcaemia with unexpected dual pathology consisting of a sarcoid granuloma within a parathyroid adenoma.

Anne Trinh, Vivian Grill
Publish consent withheld

1. Balasanthiran A, Sandler B, Amonoo-Kuofi K et al. Sarcoid granulomas in the parathyroid gland – a case of dual pathology: hypercalcaemia due to a parathyroid adenoma and co-existent sarcoidosis with granulomas located within the parathyroid adenoma and thyroid gland. Endocrine Journal 2010, 57(7):603-607

Bilateral adrenal haemorrhage and adrenal crisis in the context of antiphospholipid antibody syndrome.

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A 36 year old gentleman presented with 24 hours of central abdominal pain radiating to the left upper quadrant and subsequent vomiting. Past medical history was significant for hypertension, dyslipidaemia, IgA nephritis at age 18, and a deep vein thrombosis in the context of dengue fever at age 34. He was intermittently compliant with warfarin and INR monitoring. Blood pressure was 150/90, heart rate 80bpm. Examination revealed generalized abdominal tenderness. Urine dipstick was positive for blood and protein. WCC was 14.5 x 10⁹/L, electrolytes and renal function were normal, INR was 1.6, lactate 4.4. CT scan of the abdomen revealed a dilated stomach. A nasogastric tube was inserted and the patient was made nil by mouth to await gastroscopy. He soon became febrile, thrombocytopenic and coagulopathic, and the left flank was tender on examination. Antibiotics were commenced for presumed sepsis. Repeat CT scanning demonstrated bilateral adrenal swelling and haemorrhage. On day six haemoglobin dropped from 14.4g/dL to 8.5g/dL. A 24-hour urine collection quantified protein excretion at 345mg.
On day nine he became hypotensive at 70/40mmHg. Serum cortisol was <6nmol/L and serum sodium was 130mmol/L. Intravenous hydrocortisone and IV fluids were commenced. On day 13 a lupus anticoagulant screen was positive. On day 18, after increasing abdominal pain, the patient underwent a laparoscopic appendicectomy. Histology was normal. He made a full recovery and is stable on oral cortisone acetate and fludrocortisone, with lifelong anticoagulation as treatment for the antiphospholipid antibody syndrome. Adrenal insufficiency is the presenting complaint in 36% of cases of APS [antiphospholipid antibody syndrome]1 and is the most common endocrine manifestation of APS2. The pathogenesis is believed to be adrenal vein thrombosis and haemorrhagic infarction3. In this patient, the haemorrhage may have been caused by thrombosis in the setting of subtherapeutic anticoagulation, or haemorrhage due to anticoagulation. Other known causes of bilateral adrenal haemorrhage were excluded in this patient, such as tuberculosis, trauma and administration of Synacthen; heparin-induced thrombocytopenia screen was negative. Bilateral adrenal haemorrhage may also occur in sepsis. No focus of infection was identified in this patient.


A rising trend in search requests for vitamin D using Google corresponds to increased test orders for 25-hydroxyvitamin D by practitioners in Australia

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Publish consent withheld

Corticosteroid-responsive hypercalcemia due to a pthrp-secreting adamantinoma in a patient with severe graves disease

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A 32-year-old man presented with thyrotoxicosis and associated polyuria and polydipsia. His past history included hypertension, treated with indapamide, atenolol and felodipine, and osteofibrous dysplasia of the right tibia with recurrent fractures, diagnosed at 4 years of age. He had a moderate diffuse goitre, mild proptosis and marked peripheral signs of hyperthyroidism; his right lower leg was grossly enlarged and deformed. Pathology tests confirmed severe Graves’ disease (free T3 35.5, TSH <0.1, TRAb >200) and marked hypercalcaemia (Ca 3.40). He declined admission and was treated with carbimazole 20mg tds. After two weeks he was moderately thyrotoxic with Ca 3.18, ionized Ca 1.44, PTH < 0.5, 25-vit D 92; carbimazole was increased to 25mg tds and prednisolone 25mg mane added. After a further 4 weeks he was mildly thyrotoxic and normocalcaemic (Ca 2.33, ionized Ca 1.24, PTH 93).

He re-presented several months later with a recurrence of severe thyrotoxicosis and hypercalcaemia, having ceased his previous drug treatment. He was recommenced on carbimazole and prednisolone, and after stabilization received 1-31treatment. Carbimazole and prednisolone were weaned, but he required further courses of corticosteroids for worsening ophthalmopathy and for IgA nephropathy. Between courses of prednisolone his serum Ca was high-normal and PTH low-normal. Additional investigations for non-PTH-mediated causes of hypercalcaemia were unhelpful except for PTHRP 3.6 (RR 0.0 – 1.3), MRI of his right tibia reported extensive fibrous dysplasia and possible malignancy. He underwent open tibial biopsies, which reported adamantinoma. A right above-knee amputation was performed; histopathology confirmed adamantinoma. He has since remained normocalcaemic, with an undetectable PTHRP level. He later progressed to ESRF and receives maintenance haemodialysis. This is the first report of PTHRP secretion by an adamantinoma, although there are three case reports of hypercalcaemia in patients with metastatic adamantinoma3,5, including one with increased urinary cAMP2. Interpretation of this patient’s intermittent hypercalcaemia was complicated by his thyrotoxicosis, diuretic therapy and renal disease. Adamantinoma recurrences can occur after many years; PTHRP may be a useful marker of recurrence, and perhaps of malignant transformation in osteofibrous dysplasia. Corticosteroids may be effective for the control of hypercalcaemia in patients with adamantinoma.

Reversal of rapid bone loss in iatrogenic hyperthyroidism by bisphosphonate treatment and withdrawal of triiodothyronine

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A 54-year-old woman was seen in June 2004 for osteoporosis management. She had been estrogen-deficient for five years and her mother had severe osteoporosis. In November 2002 she was prescribed thyroid hormone supplementation, initially thyroxine (T4) and then tri-iodothyronine (T3). Her dose of T3 was progressively increased, so that by early 2004 she was taking 90 µg twice daily. She developed palpitations, sweating, heat sensitivity, muscle weakness, fatigue, anxiety and agitation.

In April 2004 the T3 was ceased and she was referred to an endocrinologist. Her previous symptoms resolved and she became clinically and biochemically euthyroid. It was found that she had had normal serum levels of free T4, free T3 and TSH before commencement of thyroid hormone treatment. She had negative thyroid antibodies and no family history of thyroid disease. The bone mineral density (BMD) of her lumbar spine (LS) and femoral neck (FN) has been measured on six occasions before, during and after T3 treatment, using the same (Norland) DEXA scanner. During a 14 month period on T3 treatment, there was a 12.5% decrease in her vertebral BMD and a 10.6% decrease in her femoral neck BMD, resulting in severe vertebral osteopenia and established FN osteoporosis. She sustained a non-traumatic rib fracture in early 2004 and a Colles’ fracture in a fall in 2007. Her osteoporosis was treated by alendronate (70mg weekly) and vitamin D3 (2000 I.U. daily). Subsequently her vertebral BMD improved by 20.5% and her femoral neck BMD by 6.1% over the next six years. She then ceased alendronate, at which time her T scores were -3.00 LS Tand -2.06 FN (Geelong).

Her alkaline phosphatase (ALP) was normal at baseline and rose during T3 treatment, reaching a peak of 188; isoenzymes confirmed a predominantly bone source. She did not develop hypercalcemia. Her ALP level returned to normal several months after T3 was ceased, and her urine deoxypyridinoline/creatinine ratio and serum osteocalcin were also normal then. Thyrotoxicosis is associated with increased bone turnover and negative calcium balance. This patient illustrates the marked adverse effects, and reversibility, of T3 excess on BMD and fracture risk.


A Case Report Of Suprasellar Mass Lesion Associated With Panhypopituitarism And Diabetes Insipidus

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Introduction: Mass in the suprasellar region could be due to a variety of causes including those of a neoplastic, vascular, congenital, infective or inflammatory origin. We report a case of suprasellar mass of likely metastatic origin which was associated with panhypopituitarism and central diabetes insipidus.

Case description: We present the case of a 72 year old gentleman who had recurrent presentations over 3 months with nausea and vomiting, associated with polyuria and significant postural drop. Biochemical testing confirmed panhypopituitarism with predominant severe secondary adrenal insufficiency, and central diabetes insipidus. MRI brain showed a 17mm suprasellar mass. Further investigations revealed a 10mm lesion in the right lung apex with hilar and mediastinal lymphadenopathy on CT chest. Poorly differentiated metastatic adenocarcinoma was confirmed from mediastinoscopy and lymph node biopsy. PET scan confirmed a lung primary with secondary mediastinal lymphadenopathy, but raised the possibility of the suprasellar lesion being a meningioma rather than a metastatic lesion. However the patient had rapid deterioration in his clinical condition with increasing confusion, headaches and behavioural changes. Although he was unfit for brain biopsy, the suprasellar mass was thought to be metastatic due to significant increase in size on repeat MRI associated with clinical deterioration. He has received palliative cranial radiotherapy, but has been deemed unfit for chemotherapy.

Discussion: Suprasellar metastasis is very uncommon (1-2% of all sellar masses) and often has a delayed diagnosis. Common presentations are with headache, visual field deficits, diabetes insipidus and anterior hypopituitarism. Surgery is often with a transphenoidal approach. Radiotherapy has been used to control tumour extension either postoperatively or instead of surgery. Chemotherapy has also been used as adjuvant. Prognosis is generally poor with a mean survival of 6 months.

References:

Diagnostic dilemmas in hyperandrogenism and pregnancy
Septo-Optic Dysplasia and Liver Cirrhosis – more than just a coincidence?

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Introduction: Septo-optic dysplasia (SOD) is a rare syndrome complex characterized by midline forebrain defects, optic nerve hypoplasia, and hypothalamic dysfunction. SOD is usually associated with short stature secondary to growth hormone deficiency. We present an unusual case of SOD with liver cirrhosis and severe insulin resistance at presentation leading to early diabetes and preserved growth.

Case: A seven and half year old boy presented with hypotension, hypercapnic respiratory failure and bilateral pneumonia, on background of bilateral blindness due to optic nerve dysplasia (which was known since 6 months of age). His height was 137 cm (> 97th centile), weight was 59 kg (> 97th centile). Investigations revealed panhypopituitarism with diabetes insipidus and MRI changes were consistent with septooptic dysplasia. He had raised liver enzymes, hepatosplenomegaly with coarse liver echotexture and portal hypertension. Metabolic liver disease screen was normal. Liver biopsy confirmed micronodular cirrhosis with severe steatosis and negative iron, PAS staining. Soon after, he was diagnosed with type 2 diabetes mellitus and severe insulin resistance. Progressive weight gain has complicated the management. However he achieved an adult height of 180 cm despite growth hormone (GH) deficiency. He did not receive GH therapy. He has been managed at our clinic for panhypopituitarism replacement therapy and type 2 diabetes for the last 12 years.

Discussion: Congenital hypopituitarism is associated with neonatal cholestasis, but liver cirrhosis in childhood is rare. The link between hypopituitarism and hepatic dysfunction will be discussed. Hypothalamic involvement can directly influence insulin resistance independent of weight gain. Possible relationship of hypothalamic pathology with insulin resistance and early onset diabetes will be explored. Hyperinsulinemia can also promote growth despite growth hormone deficiency which explains the normal height achieved.

Key references:

Life threatening Pseudohypoaldosteronism

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K, a morphologically normal female baby was born at term to unrelated parents, and presented at six days of age with life threatening shock (Lactate 7.8mmol/L), hyperkalaemia (11.5mmol/L) with runs of VT, and hyponatraemia (120mM). Spot urine showed urine Na 95 mmol/L and Urine K 3 mmol/L despite hyponatraemia. Urine metabolic profile, renal ultrasound and echocardiogram were normal. The working diagnosis was isolated defect in mineralocorticoid biosynthesis or pseudohypoaldosteronism type 1.

Subsequent investigations revealed a Cortisol of 1407 nmol/L, renin (1253 mIU/L) and aldosterone (>30,000 & 47,000 pmol/L).
Her adopted father had been on an ACEI for hypertension since 19 years of age. Initial treatment included normal saline boluses, bicarbonate infusion, calcium gluconate and insulin-dextrose infusions and resorinum for hyperkalemia and shock which resulted in normalisation of her fluid volume and electrolytes. IV antibiotics were used until sepsis was excluded - blood cultures were sterile. High dose fluoroquinolose was trialed for several days without effect. K was commenced on normal saline infusion for slow correction of her sodium deficit. She was subsequently commenced on oral sodium chloride (20%), sodium bicarbonate and a low potassium and high sodium formula and was discharged home at 4 weeks age.

Molecular confirmation of a diagnosis of recessive PHA1 was received, demonstrating compound heterozygous mutations in the SCNN1A gene. K is now 18 months old and is tracking well in her growth and development (Weight 10th centile; height 25th centile), although has had recent weight loss, vomiting and delayed gastric emptying. She is on a low potassium formula and salt replacement via PEG feeds as she has food aversion which is being managed. She is currently doing well with a total Na replacement of 11mmol/kg/day (2/3 as Na citrate and 1/3 as NaCl). She is on regular resorinum 0.3mg bd guided by potassium levels, domperidone 2mg bd, erythromycin 20mg bd and ferroliquid 3ml daily. She has had a chronic cough since age 1 month which improved with macrolides and prokinetic agents as well as gastrojejunal feeds indicating gastroesophageal reflux may be contributing. Her CT chest showed bronchial wall thickening and mucus impaction in the posterior segment of the right upper lobe with patchy consolidation.

Pseudohypoaldosteronism type 1 is a rare genetic disorder caused by mutations in one of the the amilode-sensitive sodium channel - ENaC (1)(2). ENaC is decreased in autosomal recessive pseudohypoaldosteronism type1, resulting in aldosterone resistance in multiple organs. In contrast, autosomal dominant PHA1 is due to loss of function mutations of the NR3C2 gene which encodes for the mineralocorticoid receptor, (1)(3) and this may be isolated to the kidney which generally results in a milder phenotype that often improves with time.

Issues:
1) Management of growth with vomiting, delayed gastric emptying, food aversion
2) Risk of future chest infections
3) Planning for future pregnancies, genetic counselling
4) Further investigation of hypertension with father with hypertension from age 19, paternal grandmother with hypertension and now older sibling with hypertension
5) Role of carbexoxolone therapy


Adrenal Infiltration presenting as Hypoadrenalism

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Introduction: A previously well 77 year old gentleman presented with syncope and dizziness in January 2012. He was found to have hyponatraemia and hyperkalaemia. As part of a work up for renal failure, a chain of investigations led towards a CT abdomen with contrast being performed. This led to the discovery of bilaterally enlarged adrenals and splenomegaly. He was noted to have thyrombocytopenia, and a subsequent bone marrow biopsy in February 2012 revealed a low grade lymphoma.

Follow up: He was subsequently readmitted in May 2012 with fevers and abdominal pain. CT Adrenals revealed increased size of the bilateral adrenal masses, right side 61mm and left side 94 mm, of irregular margins and heterogeneous appearances. He underwent adrenal biopsy, which subsequently revealed large B-cell lymphoma on biopsy. He is presently undergoing chemotherapy.

Conclusions: Less than 100 cases of confirmed adrenal insufficiency due to local metastases have been published (1). For any tumour to cause adrenal insufficiency, it has to destroy 90% of the glandular tissue in both adrenal glands (2). Although adrenal metastases are well known to occur in most metastatic solid organ malignancies, adrenal insufficiency resulting from this lymphomatous infiltration has been rarely described (3).

References:

A case of life-threatening hypercalcaemia – a parathyroid ‘storm’

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A 41 year old Samoan gentleman presented with three days of abdominal pain. On examination he was dehydrated and had a fixed, hard left-sided neck swelling.

Investigations:
- Bloods: cCa 5.14mmol/L (iCa 2.57mmol/L), Cr 439μmol/L, eGFR 13, PTH 302.3pmol/L (1.6-6.9)
- ECG: shortened QTc (377ms) and changes mimicking ST elevation
- Imaging: Subperiosteal resorption of the acromio-clavicular bones, brown tumours of the pelvis.
- Neck ultrasound demonstrated a large hypervascular mass within the left thyroid lobe.

The patient was initially managed with intravenous hydration, frusemide and calcitonin. Bisphosphonate therapy was deferred due to severe renal failure. The calcium level continued to rise, peaking at 6.1mmol/L, 36 hours after presentation. Whilst preparing for haemofiltration the patient had three successive cardiac arrests (ventricular fibrillation). After 12 hours of continuous filtration the calcium remained at 5.49mmol/L. SestaMIBI scanning revealed an avid lesion in the left thyroid lobe upper pole. An emergency parathyroidectomy was performed 48 hours from his admission after four further cardiac arrests.

A large parathyroid nodular mass (70g) was removed intraoperatively. Histopathology was suggestive of a parathyroid carcinoma with thick fibrous bands throughout and a high mitotic rate.

Post-operatively, the PTH level dropped to 11.6pmol/L. The calcium level gradually decreased requiring calcium and calcitriol supplementation on discharge from hospital.

Parathyroid carcinoma is a rare malignancy accounting for approximately 1% of patients with primary hyperparathyroidism. Patients are typically symptomatic at presentation, often with the renal and skeletal, and rarely cardiac complications of severe hypercalcaemia. There are no pathognomonic features of parathyroid carcinoma on histopathology. A set of morphologic criteria and immunohistochemistry (such as Ki-67 and cyclin D1, and loss of parafibromin) may assist the diagnosis. Morbidity and mortality is secondary to intractable hypercalcaemia.

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A Case of Severe Hypercalcaemia Due to Primary Hyperparathyroidism Presenting In Third Trimester Pregnancy

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A Case of Severe Hypercalcaemia Due to Primary Hyperparathyroidism Presenting In Third Trimester Pregnancy

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Publish consent withheld


Fuel on Fire

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Mr. MN is a 43y.o. man with McCune-Albright Syndrome (MAS) with acromegaly, craniofacial fibrous dysplasia (FD) and radiological evidence of bilateral optic canal compression who highlights the challenge of management of this rare condition. He has had a number of debulkings and nasal drillout procedures since 2000 and continues to have significant facial deformity with R epiphora and global dystopia, as well as complete occlusion of his R nasal passage. He has previously received zoledronic acid in 1999 in an attempt to slow bone turnover resulting in severe pain. Due to this previous adverse reaction, chronic ethmoid osteomyelitis, and a lack of bone pain and fractures, he is not for further bisphosphonate therapy. Acromegaly was diagnosed on a recent admission with facial cellulitis and a 14 X 15 X 18mm right-sided pituitary mass was identified on MRI.
Given the extent of his FD, surgical management is not feasible and due to concerns about malignant transformation, we are reluctant to pursue radiotherapy. He has continued on somatostatin analog therapy for the past six months with some symptomatic response however continues to have significantly elevated GH, IGF-1 and bone turnover markers. Of concern is the presence of bilateral optic canal compression in the setting of growth hormone excess, a known risk factor for optic neuropathy and blindness. This case demonstrates the difficulty in managing patients with the combination of acromegaly and craniofacial fibrous dysplasia. The principles of therapy for acromegaly in MAS, role of bisphosphonates, risks of malignant transformation, and options for somatostatin analog resistance will be highlighted.


A Case of Carcinoid Heart Disease

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Publish consent withheld

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